RESTING METABOLIC RATE AND DIET-INDUCED THERMOGENESIS

studies in humans
on individual differences
and on the impact of nutritional
and non-nutritional factors

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J.A. Weststrate

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op dinsdag 2 mei 1989
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

Aan mijn moeder

Financial support by Unilever Research Laboratory for the publication of this thesis is gratefully acknowledged.

Stellingen

1. Het ontbreken van gegevens over de reproduceerbaarheid van metingen van het thermogeen effect van voeding betekent niet, dat de reproduceerbaarheid van deze metingen goed is.
(dit proefschrift)

2. De hoogte van de ruststofwisseling wordt bij obese vrouwen mede bepaald door de lichaamsvetverdeling.
(dit proefschrift)

3. De hoogte van het thermogeen effect van voeding is bij vrouwen niet gerelateerd aan het lichaamsvetgehalte en de lichaamsvetverdeling.
(dit proefschrift)

4. De hoogte van het thermogeen effect van voeding hangt mede af van factoren buiten de voeding.
(dit proefschrift)

5. De bevinding dat bij proefdieren onder normale omstandigheden het grootste deel van koolhydraten na vertering wordt omgezet in vet, mag niet naar de mens worden geëxtrapoleerd.
(o.a. dit proefschrift)
- Björntorp P, Sjöström L. Carbohydrate storage in man: speculations and some quantitative considerations. Metabolism 1978;27:1853-65.
- Acheson KJ, Flatt JP, Jéquier E. Glycogen synthesis versus lipogenesis after a 500 g carbohydrate meal in man. Metabolism 1982;31:1234-40.

6. Uit het familiaal voorkomen van bepaalde kenmerken, zoals een verhoogd vetgehalte in het lichaam, mag niet geconcludeerd worden dat vooral genetische factoren hiervoor verantwoordelijk zijn.
- Garn SM et al. Living together as a factor in family-line resemblances. Hum Biol 1979;51:565-87.

7. Observationeel onderzoek naar de mate waarin variatie in een kenmerk geassocieerd is met erfelijke - of omgevingsfactoren mag op logische gronden niet causaal geïnterpreteerd worden.
- Kempthorne O. Logical, epistemological and statistical aspects of nature-nurture data interpretation. Biometrics 1978;34:1-23.

8. Het dogma in de voedingswetenschap dat een voedingsmiddel nooit als 'gezond' of 'ongezond' aangemerkt kan worden, staat een werkelijk effectieve voedingsvoorlichting in de weg.

9. Sommige 'light' producten bevatten nog steeds veel energie. Minder energierijke varianten van gangbare producten zouden daarom alleen de kwalificatie 'light' mogen krijgen, wanneer zij tenminste 25 procent minder energie bevatten dan hun 'normale' tegenhanger, én in absolute zin een energiedichtheid hebben in het watervrije product, die niet hoger is dan de energiedichtheid van een gedroogd voedselpakket, zoals samengesteld volgens het Advies Richtlijnen Goede Voeding van de Nederlandse Voedingsraad.

10. Een vermindering van het aandeel van vet in de energievoorziening van de gemiddelde Nederlandse voeding ten gunste van dat van koolhydraten zal, naast een daling op populatieniveau van het serumcholesterolgehalte, ook leiden tot een afname van het gemiddelde lichaamsgewicht.

11. De gewoonte in het academische curriculum de eerste jaren van de studie vooral te gebruiken voor het leren van in het verleden geproduceerde kennis en de laatste jaren voor de productie van nieuwe kennis, leidt tot een laag totaal studierendement.

12. Er valt de eerstkomende jaren een grotere bijdrage te verwachten aan de preventie van overgewicht van het op de markt brengen van een smakelijke, verzadiging-gevende, laag-energetische snack dan van congressen over overgewicht.

13. Bij de toekenning van onderzoeksgelden aan universitaire vakgroepen door derden dient de originaliteit van de vraagstelling sterker te wegen dan de afwezigheid van een onderzoeklijn.

14. De mogelijkheden om een beter voedingspatroon in Nederland ingang te doen vinden, worden belemmerd door het verbod 'gezondheidsclaims' toe te kennen aan producten, die zijn samengesteld volgens het Advies Richtlijnen Goede Voeding van de Voedingsraad.

15. Hoe meer aandacht in de media voor gezondheidsproblemen, des te zieker men zich voelt.
zie ook: Barsky AJ. The paradox of health. N Engl J Med 1988;318:414-8.

16. Berichtgeving in de media over de aanwezigheid van kankerverwekkende stoffen in voedingsmiddelen dient gepaard te gaan met een evaluatie van het gezondheidsrisico in relatie tot het gezondheidsrisico van meer alledaagse activiteiten, zoals autorijden, matig alkoholgebruik of de consumptie van pindakaas.
zie ook: Wilson R, Crouch EAC. Risk assessment and comparisons: an introduction. Science 1987;236:267-70.

17. De veelgehoorde opmerking van ouderen dat vroeger 'alles' beter was, moet vooral van toepassing worden geacht op het functioneren van hun geheugen.

18. De verstrekking van een OV-jaarkaart aan studenten zal de bereikbaarheid van het hoger onderwijs niet vergroten.

Proefschrift J.A. Weststrate
Resting metabolic rate and diet-induced thermogenesis:
studies in humans on individual differences and on the
impact of nutritional and non-nutritional factors
Wageningen, 2 mei 1989.

Contents

Voorwoord

Voor u ligt een proefschrift met een aantal studies over de energiewisseling bij de mens. Een deel van de onderzoekingen betreft de energiewisseling van mensen met overgewicht. Dergelijk onderzoek spreekt velen aan. Een groot deel van de bevolking voert een moeizaam gevecht tegen een overvloed aan calorieën. 'Dikmakers' dienen zich op veel plaatsen verleidelijk aan ter leniging van de 'lekkere' trek. Menigeen is niet opgewassen tegen al die calorische verlokkingen. Als gevolg hiervan worden nogal wat mensen te dik. Vaak, echter, menen overgewichtige mensen dat zij door een 'efficiëntere' energiewisseling dik zijn geworden. Deze mensen zouden, bij wijze van spreken, dik worden alleen al bij het zien van een bord eten. Verrassend genoeg is er in wetenschappelijke zin nog weinig bekend over verschillen tussen mensen in de efficiëntie van de energiewisseling. Een antwoord op de vraag, of er personen zijn, die een verhoogde kans hebben op overgewicht als gevolg van een 'efficiëntere' energiewisseling is van belang; niet alleen ter mogelijke geruststelling van diegenen, die het gevecht tegen overgewicht als een verloren zaak beschouwen, maar vooral bij de behandeling en preventie van overgewicht. Alleen wetenschappelijk onderzoek kan over deze zaak uitsluitsel geven. Het is de hoop van de onderzoeker dat de onderzoekingen van dit proefschrift hieraan een bijdrage zullen leveren.

Een proefschrift dient het bewijs te leveren dat de promovendus zelfstandig origineel onderzoek kan verrichten en hierover kan rapporteren. Met name rapportage in daartoe geëigende vaktijdschriften is belangrijk. Dit betekent, dat proefschriften veelal betaan uit een bundeling van artikelen met een inleiding en een algemene discussie die de bevindingen in een passend kader plaatsen. Dit proefschrift vormt hierop geen uitzondering. Het beschrijft verschillende onderzoekingen met uiteenlopende vraagstellingen.

Het onderzoek in dit proefschrift is tot stand gekomen dankzij de hulp van velen. Traditiegetrouw wil ik dit voorwoord gebruiken om de mensen te danken, die mij bij het onderzoek hebben geholpen.

Allereerst wil ik mijn promotor Professor Dr. J.G.A.J. Hautvast hartelijk

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Jaap Seidell wil ik danken voor de vele gezellige uren die wij doorbrachten met het bedenken van nieuwe baanbrekende onderzoeken, waarvoor helaas het geld ontbrak.
I am indebted to Professor Dr. E. Jéquier and Dr. Y. Schutz from the "Institute de Physiologie" of the University of Lausanne in Switserland for introducing me to the 'ins and outs' of the measurements of diet-induced thermogenesis.
De Directie van Unilever Research Laboratorium in Vlaardingen ben ik erkentelijk voor de tijd die zij mij verleenden om dit proefschrift af te ronden. Mijn collega's van de sectie Fysiologie van Unilever Research wil ik danken voor het begrip dat zij de afgelopen maanden toonden voor mijn nogal onregelmatige werktijden.
Aan het onderzoek is door 14 doktoraal studenten meegewerkt, die ik graag persoonlijk wil bedanken voor de plezierige samenwerking en hun onmisbare hulp: Greet Vansant, Cathaline den Besten, Zandrie Hofman, Alida Wapsta, Jos Steenbergen, Peter Weys, Eric Poortvliet, Jacqueline Dekker, Miriam Stoel, Liliane Begheyn, Karin van der Kooy, Ingrid Wunnink, Leoniek Robroch en Theo Dopheide.
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Abstract

RESTING METABOLIC RATE AND DIET-INDUCED THERMOGENESIS: studies in humans on individual differences and on the impact of nutritional and non-nutritional factors

THESIS, DEPARTMENT OF HUMAN NUTRITION, WAGENINGEN AGRICULTURAL UNIVERSITY, WAGENINGEN, THE NETHERLANDS, 2 MAY 1989

Jan A. Weststrate

This thesis contains studies on resting metabolic rate (RMR) and diet-induced thermogenesis (DIT) of humans using indirect calorimetry (ventilated hood system) to assess energy expenditure.
A literature survey of aspects of human energy exchange and of problems of energy balance is included.
At first, methodological studies on the measurement of RMR, DIT and fuel utilization are presented. Measurements of RMR and respiratory quotient showed good reproducibility, in contrast to those of DIT and fuel utilization rates.
Secondly, the nature and magnitude of inter-individual differences in RMR and DIT were assessed. No significant difference in DIT was found between non-obese women and obese women with a wide range in body fat distribution. Men had a significantly higher DIT compared to women. Obese women with a non-abdominal type of body fat distribution had reduced RMR's in comparison with non-obese men, non-obese women and obese women with an abdominal type of body fat distribution.
Thirdly, the impact of nutritional and non-nutritional factors on RMR and DIT was studied. No significant diurnal variation in RMR and DIT was found. Psychological stress did not affect RMR, but potentiated DIT. Unduly heavy exercise was found to have a significant after-effect on RMR and DIT, whereas moderate to heavy exercise did not have a systematic after-effect on RMR. Ethanol (20 g) induced a significant thermic effect, but did not affect DIT. Differences in palatability among otherwise identical mixed meals or sucrose solutions did not produce differences in DIT. Short-term carbohydrate overfeeding increased DIT, but not RMR. Carbohydrate overfeeding attenuated the after-effect on RMR of unduly heavy exercise.
This thesis shows that inter-individual differences in RMR and DIT exist, even after statistically accounting for differences among individuals in age, body weight and body composition. This thesis shows also that, in addition to nutritional factors, non-nutritional factors affect the postprandial rise in energy expenditure.

Chapter 1.
General Introduction

1.1. INTRODUCTION

This thesis deals with the study of human energy exchange. Energy exchange involves the relationships between energy intake and energy expenditure and includes the processes for storage and utilization of energy containing compounds. In this thesis the energy expenditure component of human energy exchange is studied, in particular, the rate of energy expenditure at rest in fasting conditions and at rest after feeding. These components of energy expenditure are referred to as resting metabolic rate and diet-induced thermogenesis, respectively. Together they account for 70-85 percent of total energy expenditure in the average adult individual. Because of their quantitative importance for total energy expenditure, the study of resting metabolic rate and diet-induced thermogenesis is essential for gaining insight in the determinants of inter-individual variations in overall energy requirements. The questions how much food humans need and the factors that determine their needs are basic to the science of nutrition. This thesis investigates methodological aspects of studies on resting metabolic rate and diet-induced thermogenesis; it describes the size and nature of inter-individual differences in the rate of resting energy expenditure, and assesses the impact of various nutritional and non-nutritional factors on resting metabolic rate and diet-induced thermogenesis.

1.2. BACKGROUND OF THESIS

In the last two decades interest in the study of human energy exchange gained momentum. This was, in particular, the result of an increased prevalence of problems of energy balance, both in the developed countries as in developing countries (1-4). In developed countries the prevalence of overweight and

obesity attained levels that caused concern among physicians and nutritionists. The widespread occurrence in developing countries of energy undernutrition with its clinical sequelae received worldwide attention. Obesity and energy undernutrition are two sides of the same coin. Both are the clinical manifestations of a prolonged disturbance of energy balance. In the former, energy intake has exceeded energy expenditure and in the latter the opposite process has occurred. In itself it is not clear why these problems would cause a revival of the study of human energy exchange. The first Law of Thermodynamics offers a simple solution to both problems. The obese should decrease their energy intake and the undernourished should increase food intake. Obviously the problem is far more complicated.

Interest for studying energy exchange in relation to obesity arose at the suggestion that the obese would have lower rates of energy expenditure and, consequently, lower energy requirements in comparison with the non-obese (5-7). Such lower rates of energy expenditure would be of pathogenetic significance. In addition, they could offer an explanation for the frequently observed weight relapse in the obese after weight loss (8). The hypothesis of lowered energy requirements in the obese was in agreement with observations on the relevance of energy expenditure for genetic-based differences in body composition among various animal species (9,10).

Interest for studying energy exchange in relation to undernutrition stemmed from observations in developing countries that individuals with surprisingly low energy intakes were reported to be in remarkably good health (11). It was suggested that these individuals had adapted their rate of energy expenditure to the relatively low energy intakes (11). Assessing the determinants and magnitude of this type of adaptation is considered to be highly relevant for a critical appraisal of the food availability situation in a developing country (11,12).

Thus, the study of energy exchange has two sides also. In developed countries it may offer clues to the etiology of problems of energy overnutrition and in developing countries it may elucidate the nature and quantify the magnitude of a supposed biological adaptation phenomenon with significant food policy implications.

Energy exchange involves two components, the first is energy intake and the second is the use of food energy by metabolism for a variety of bodily functions and for physical activity, ie, energy expenditure. The current theory on energy requirements states that it is the rate of energy expenditure

that fixes the level of energy intake at which energy requirements are met (13). This implies that differences in energy requirements among individuals with similar degrees of physical activity should be reflected primarily in differences in resting metabolic rate or diet-induced thermogenesis. The question rises whether inter-individual differences exist in these components of energy expenditure. The answer to this question is affirmative with respect to resting metabolic rate (14-18). It is well known that differences occur among individuals in resting metabolic rate and that these differences are depended on inter-individual variation in body weight and body composition (14-18). Persons with greater amounts of metabolically active cells will have higher resting metabolic rates compared to persons with smaller amounts of metabolically active cells (14-18). Whether inter-individual differences in diet-induced thermogenesis exist and the factors that influence such differences are questions that currently can not be answered with sufficient certainty.

In the study of human energy exchange the following pivotal questions continue to receive much investigative attention:

1. **Do individuals with similar body weight, body composition and degrees of physical activity differ in energy expenditure?**

2. **In which components of energy expenditure do these differences, when they occur, become manifest?**

3. **To what extent do personal characteristics relate to individual differences in energy expenditure?**

4. **Does the genetic make-up of an individual affect the efficiency of energy utilization.**

5. **Does energy expenditure adapt in response to environmental stimuli, in particular, to chronic alterations in the plane of nutrition?**

6. **Did individuals become obese because they had lowered rates of energy expenditure? Alternatively, do relatively lowered rates of energy expenditure occur among the obese, and, if so, are these of pathogenetic significance?**

The present thesis does not address all these questions. It investigates the following questions that are relevant for answers to some of the aforementioned topics of human energy exchange:

1. **How large is the intra-individual variation in resting metabolic rate and diet-induced thermogenesis? Do sex, degree of body fatness and control of the antecedent diet affect the size of the intra-individual variation in resting energy expenditure?**

2. **What is the nature and magnitude of inter-individual differences in resting metabolic rate and diet-induced thermogenesis in lean and obese individuals? Does the type of body fat distribution in obesity affect these differences?**

3. **To what extent is resting metabolic rate affected by non-nutritional factors, ie, previous exercise, psychological stress and time of the day, and to what degree by nutritional factors, ie, ethanol ingestion and short-term carbohydrate overfeeding?**

4. **To what extent is diet-induced thermogenesis affected by non-nutritional factors, ie, previous exercise, psychological stress, time of the day and palatability of food, and to what degree by nutritional factors, ie, ethanol ingestion and short-term carbohydrate overfeeding?**

In this thesis intra-individual variation is defined as the variation of an individual about his or her true mean. Inter-individual variation is the variation among the true means of individuals within a population. To assess the relative importance of inter-individual variation in resting energy expenditure, knowledge of the magnitude of the intra-individual variation is important. If intra-individual variation in resting energy expenditure is large, it may prove difficult to characterize individuals from another, ie, to correctly assess the nature and magnitude of inter-individual variation.

A review of the literature (19,20) shows that it has been difficult to distinguish the lean from the obese with respect to diet-induced thermogenesis. There is no satisfactory explanation for the divergence in opinions concerning the importance of diet-induced thermogenesis for the pathogenesis of human obesity. The possibility that a relatively large

intra-individual variation in diet-induced thermogenesis has contributed to this controversy, has not been investigated. The problem of distinguishing lean subjects from obese subjects with respect to diet-induced thermogenesis could also be related to the occurrence among the lean and the obese of subgroups of individuals with relatively low and high rates of energy expenditure. This thesis addresses these topics in some depth. In addition, to the estimation of the size of intra- and inter-individual variation in resting energy expenditure, this thesis contains various studies on the impact of specific stimuli on resting metabolic rate and diet-induced thermogenesis. These stimuli are subdivided into nutritional factors and non-nutritional factors. The factors were chosen to study their impact on resting energy expenditure because of two reasons. Firstly, because it is generally assumed for some of these factors, ie, for psychological stress, previous exercise, time of the day, that they affect energy expenditure. In fact, however, their effect on energy expenditure is controversial or hardly documented. The second reason is that some of these stimuli were expected to affect the biochemistry of postprandial energy expenditure in a specific way as to cause a hypothetized change in the rate of resting energy expenditure. Studies were carried out to test these hypotheses in order to gain insight in the nature of diet-induced thermogenesis.

1.3. OUTLINE OF THESIS

The major part of this thesis contains articles which are in press or submitted for publication.

Chapter 1 contains a general introduction.

Chapter 2 gives a short review of the components, regulation, measurement and problems of energy exchange in humans. It provides a theoretical framework for the study of resting energy expenditure in humans and includes a formulation of the major hypotheses tested in this thesis.

Chapter 3 considers methodological aspects of the assessment of resting metabolic rate, diet-induced thermogenesis and in vivo fuel utilization by indirect calorimetry using a ventilated hood system. This chapter provides data on the nature and magnitude of intra-individual variation in resting

metabolic rate and diet-induced thermogenesis.

Chapter 4 describes a study on the impact of diurnal variation in resting metabolic rate and diet-induced thermogenesis. It is frequently assumed that resting metabolic rate shows considerable diurnal variation. This variation would seriously affect the assessment of diet-induced thermogenesis, if not properly corrected for.

In Chapter 5 the impact of previous exercise on resting metabolic rate is discussed. The duration and magnitude of the impact of previous exercise on resting metabolic rate is a controversial issue. A prolonged and significant after-effect of exercise on resting energy expenditure may represent a significant loss of energy for the individual, which could be useful in weight control or weight reduction therapies.

Chapter 6 contains a study on the effect of motion-picture induced psychological stress on resting metabolic rate and diet-induced thermogenesis. It is generally assumed that "stress" affects energy expenditure. However, little hard data is available to support this assumption.

The nature and magnitude of inter-individual differences in resting metabolic rate and diet-induced thermogenesis in lean and obese subjects are discussed in Chapter 7.

Chapter 8 describes the impact of obesity in women and of the type of body fat distribution in obesity on resting metabolic rate and diet-induced thermogenesis. The type of body fat distribution in obesity is an important predictor of metabolic complications of obesity. In recent years obesity research has investigated the relationships between body fat distribution in obesity and hormonal status, insulin and glucose metabolism, lipid metabolism, but not in assocation with energy metabolism.

The thermic effect of ethanol and the impact of ethanol on diet-induced thermogenesis are reported in Chapter 9. Ethanol provides on average up to 5 percent of daily energy intake in adults in Western countries. Alcohol is reported to be added on top of the diet. Surprisingly, however, its use is not associated with increased levels of adiposity. This has raised the suggestion that ethanol is less efficiently used as an energy source compared to fat or

carbohydrates. We studied the efficiency of ethanol energy utilization by assessing its thermic effect in fasting and fed subjects.
Chapter 10 addresses the question whether palatability affects diet-induced thermogenesis. Man eats to satisfy physiological and non-physiological needs. Ingestion of food, but possibly also the mere sight or smell of food, elicits a prompt response in the secretion of various hormones. These early (pre-absorptive) responses may modulate diet-induced thermogenesis. Food varying in sensory characteristics is known to differentially influence these early hormonal responses. In Chapter 10 it was tested whether food with different palatability would differentially affect diet-induced thermogenesis.

In Chapter 11 we discuss the interaction between exercise and short-term carbohydrate overfeeding on resting metabolic rate and diet-induced thermogenesis. Prolonged heavy exercise is known to deplete the body's glycogen stores. Carbohydrate overfeeding may have the opposite effect. Diet-induced thermogenesis would be increased when the body's glycogen levels are high and decreased with low levels of liver and skeletal-muscle glycogen. In this Chapter the effects of exercise and carbohydrate overfeeding on resting metabolic rate and diet-induced thermogenesis are discussed.

Chapter 12 contains a general discussion of the experiments reported in this thesis and includes the formulation of general conclusions.

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Chapter 2.
Energy exchange and energy balance in humans: a review

CONTENTS

2.1. INTRODUCTION

In this review I will briefly discuss the components of energy exchange and their relation to energy balance.

2.2. HISTORICAL PERSPECTIVE

The study of energy exchange in biological systems started more than two hundred years ago with the inference of Lavoisier that respiration in animals was based on consumption of 'oxygène', a hitherto unknown element. It took more than seventy years before another fundamental scientific advance was made in the study of energy exchange in biological systems. In 1842 the German Robert Meyer formulated the Principle of Energy Conservation. This Principle is now known as the first Law of Thermodynamics. It states that energy can not be destroyed or created, but only changed in form. More than half a century later Rubner showed that an animal's heat or energy balance was consistent with the first Law (1). At the turn of the century similar findings were reported by Atwater and Benedict for humans (2). In the decades following, biochemists unravelled the metabolic pathways involved in cellular heat production and energy transduction (3,4). During this period interest among physiologists and nutritionists for the study of human energy exchange was negligible. It was assumed that the fundamental problems of energy exchange were solved. Energy balance would be regulated by energy intake, which was under tight hypothalamic control (5).

Interest in the study of the 'fire of life' revived in the 1960s and the 1970s (6-8). Impetus for the revived interest in the study of human energy metabolism came from two directions. Firstly, animal experiments showed that genetic-based differences in body composition were related to differences in energy expenditure (9,10). Related experiments showed that rats induced to eat a highly palatable diet, increased their food intake substantially without gaining excess weight due to a marked stimulation of brown adipose tissue thermogenesis (11). Secondly, studies in humans indicated that the efficiency of energy utilization and, consequently, the level of energy requirements, varied in relation to degree of adiposity (12,13).

Thus, almost a century after it was shown that human energy exchange proceeds according to the first Law of Thermodynamics, the study of human energy exchange is at a point where it needs the second Law of Thermodynamics to

provide the key to understanding two remaining problems of human energy metabolism. These are the partitioning of energy transformations in the body into those leading directly to heat and those leading to internal work and subsequent heat ('efficiency of uncoupled metabolism'), and, secondly, the efficiency of the transformation of chemical energy in food stuffs to internal work ('efficiency of coupled metabolism'). Related to these problems are some important questions of human energy exchange, ie, whether individuals may show systematic differences in the efficiency of energy utilization, whether certain personal characteristics covary with such differences, in what way such differences relate to environmental stimuli, and, finally, whether the genetic make-up of an individual affects the efficiency of energy utilization.

2.3. THE COMPONENTS OF ENERGY EXCHANGE

The human body is in constant energy exchange with its surroundings. Chemical energy is provided to the body by food ingestion. The energy contained in macronutrients is liberated by oxidation involving a continuous uptake of oxygen and disposal of carbondioxide. During oxidation of nutrients heat is generated to maintain body temperature and for the provision of chemical work (biosynthesis), osmotic work (ion gradients) and mechanical work (muscular contractions). The transfer of energy from the body to its surroundings is designated energy expenditure. The uptake of energy from its surroundings by the body is known as energy intake.

2.3.1. ENERGY EXPENDITURE

The transfer of energy from to body to its surroundings comprises energy expenditure. Total energy expenditure can be subdivided into three components: resting metabolic rate, diet-induced thermogenesis and work-induced thermogenesis. These elements of energy expenditure are discussed in the following three paragraphs.

2.3.1.1. RESTING ('BASAL') METABOLIC RATE

Resting ('basal') metabolic rate (RMR or BMR) is the rate of energy

expenditure of an individual, awake and at complete physical rest some hours after the last meal and physical activity (14). Generally, it is similar to what is known as 'basal' metabolic rate. Basal metabolic rate is measured under highly standardized conditions and is defined as the rate of energy expenditure of an individual at complete physical rest, lying down, shortly after being awake, measured in a thermoneutral state, 12-14 hours after the last meal, with the individual at sexual repose and emotionally undisturbed, without disease or fever (14). The term 'basal' suggests that energy expenditure measured under these conditions would correspond to minimal energy expenditure. However, during sleep, drowsiness or meditation the rate of energy expenditure can be lower compared to 'basal' metabolic rate (14,15). Most recent authorative reviews on energy expenditure use the term resting metabolic rate (16,17). Throughout this thesis this term will be used.
Resting metabolic rate represents the sum of the energy liberated by the metabolic activities of all the cells of the body under the specific conditions of the measurement. It includes the costs of maintaining the integrated systems of the body and the homeothermic temperature at rest. Under thermoneutral conditions sufficient heat is generated to maintain body temperature during oxidation of nutrients to provide ATP for biosynthesis, active transport and muscular contraction. In the average adult individual resting metabolic rate comprises 60-75 percent of total daily energy output (16,17).
Figure 1 gives a schematic representation of various factors affecting resting metabolic rate in humans under thermoneutral conditions and the mechanisms involved.
Resting metabolic rate is depended on constitutional factors, ie, sex, age and genes, and environmental factors, ie, diet, temperature, stress and drugs. The constitution of a subject determines his or her mass of metabolically 'active' cells and the composition of the mass of active cells. In this respect composition refers to the proportion of tissues with different metabolic rates (18). Obviously, in the long term environmental factors, in particular diet, also affect a subject's mass of metabolically 'active' cells and, perhaps also, the proportion of tissues with different metabolic rates. Both factors determine primarily the ATP needs for biosynthesis, active transport and muscular contraction, and, consequently, resting metabolic rate.
Thus, resting metabolic rate differs among people of different sex, body weight and age (19-28). However, when differences in body weight and body composition are considered most of these differences tend to disappear

(23-28).

In metabolic studies the size of the 'active' mass is usually estimated by a measure of the amount of fat-free or lean mass in the body (23-28). Fat-free mass comprises all 'non-fat' tissues and organs in the body, including bone, skin and extracellular water. The importance of the size of the 'active' mass

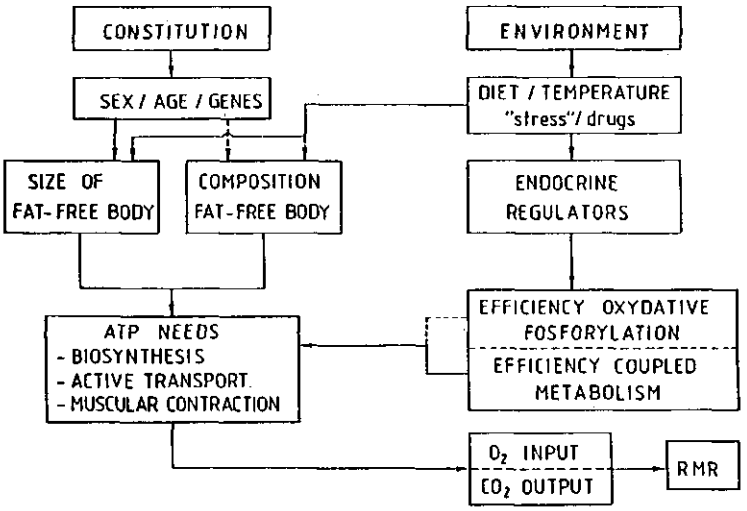

Figure 1. 'Model' of the impact of various factors on resting metabolic rate (RMR). Composition of the fat-free mass represents the proportion of tissues with different metabolic activities in the total fat-free mass. Thick lines indicate established contributions. Interrupted lines indicate contributions still in doubt.

for determining resting metabolic rate was shown in numerous studies reporting high correlations between resting metabolic rate and the size of the fat-free mass (23-28). Average resting metabolic rates per unit weight of fat-free mass are reported to vary between 110 and 130 kJ/d/kg fat-free mass (24-28). Since cells of different tissues and organs have widely different levels of metabolic activity (18), it follows that the average metabolic rate per unit weight of cell mass will change when the proportion of tissues with different metabolic rates changes. Currently it is very difficult, perhaps impossible, to quantify exactly in vivo the proportion of different tissues (composition) in the fat-free mass of the body (29). This means that differences in resting metabolic rate per weight unit of fat-free mass do not necessarily indicate a

difference in basic, energy-demanding biochemical processes at the cellular or molecular level. It is at this basic level that environmental factors, through endocrine regulators, may more directly affect the rate of energy expenditure and cause inter-individual differences in the rate of resting metabolic rate. Among these environmental factors diet and the environmental temperature are probably the most important, but stress and certain drugs may also affect resting metabolic rate (16,17).

Hormones could either affect the degree of coupling of oxidation to phosphorylation, ie, affect the efficiency of oxidative phosphorylation, or influence the use of ATP in metabolism, ie, affect the net efficiency of coupled metabolism (17). Thyroid hormones appear to be the major hormonal determinant of mammalian cellular thermogenesis (30). Thyroid hormones affect directly the rate of ionic pumping (31,32). A considerable part, perhaps a very large part, of our metabolic energy is, to cite Sims and Danforth (17), "...devoted to keeping the primordial brine out of our cells". However, whether the effect of thyroid hormones on sodium pumping contributes significantly to thyroid thermogenesis remains to be established (32). In addition to thyroid hormones, insulin (33) and norepinephrine (34) may also affect ionic pumping. Finally, norepinephrine and thyroid hormones are reported to increase the use of ATP in substrate ('futile') cycling (35,36). Whether hormones may affect in man the efficiency of mitochondrial coupling of oxidation to ATP production is not known. In small rodents it has been shown that norepinephrine reduces the efficiency of oxydative phosphorylation in brown adipose tissue (35).

The extent to which subtle differences in the hormonal control of cellular thermogenesis are associated with differences in the rate of energy expenditure per unit weight of fat-free mass remains to be investigated. Related to this question, is the impact of nutrition on endocrine regulators of thermogenesis. In this respect nutritionally-induced changes in sympathetic nervous system activity and the peripheral metabolism of thyroid hormones are interesting. It was shown that overfeeding increases, on the one hand, the activity of the sympathetic nervous system and thereby catecholamines secretion rates, and, on the other hand, the production of free triiodothyronine, whereas energy restriction induced opposite effects (37-41). The observed reduction in resting metabolic rate during energy restriction (42-59) has frequently been ascribed to hormonally-induced changes in the efficiency of energy utilization. Recently, Ravussin et al. (54) showed that the decrease in resting metabolic rate in subjects during weight loss could be

completely accounted for by the loss of fat-free mass. Bessard et al. (52) and Barrows and Snook (51), however, report a decrease after weight loss in resting metabolic rate per unit weight of the fat-free mass. Rozen et al. (55) showed in a short-term study that the decrease in metabolic rate in obese euthyroid women during weight loss could be prevented by a 'physiological' dosis of triiodothyronine. The latter study supports a role for hormonally-induced changes in the efficiency of energy utilization during weight loss. However, more research seems needed to settle this issue.
Two intrigueing observations on individual differences in resting metabolic rate should be mentioned. Recently, Bogardus et al. (27) showed a familial dependency of resting metabolic rate in American Pima Indians. The results of the study of Bogardus et al. do not offer an answer to the question whether this familial dependency was related to a genetic resembling of family members or to similarity among family members in nutritional habits or, to generalize, in lifestyle factors. It has, however, been reported that resting metabolic rate was more alike between monozygotic twins in comparison to dizygotic twins (56).

2.3.1.2. DIET(ARY)-INDUCED THERMOGENESIS

Diet(ary)-induced thermogenesis (DIT) is the increase in the rate of resting energy expenditure in response to feeding (14). In this thesis diet-induced thermogenesis is defined as the acute effect of food ingestion on resting metabolic rate, also referred to as the thermic (thermogenic) effect of food or the heat increment of feeding. In the average individual diet-induced thermogenesis would account for about 10 percent of total daily energy expenditure (16,17).
There are many synonyms in use to describe diet-induced thermogenesis, these terms are listed in Figure 2. In addition to the plethora of terms used to describe diet-induced thermogenesis, some investigators (57) consider diet-induced thermogenesis not as the acute impact of food ingestion on energy expenditure, but as the chronic effect of diet on the rate of energy expenditure. In that case diet is synonym to the plane of nutrition, which may affect energy expenditure. The impact of diet on energy expenditure would manifest itself in adaptive changes of resting metabolic rate, diet-induced thermogenesis or work-induced thermogenesis. To prevent semantical problems, I propose to refer to such adaptive responses as diet-induced facultative

changes in resting metabolic rate, diet-induced thermogenesis or work-induced thermogenesis, respectively.

The history of diet-induced thermogenesis starts with the work of Rubner (58). Rubner observed an increase in oxygen consumption of fasting dogs after ingestion of protein, fat or sugar. This increase in oxygen consumption was, in particular, manifest after protein ingestion. Rubner called the increase in oxygen consumption after food ingestion by fasting animals the specific dynamic effect (action) of food.

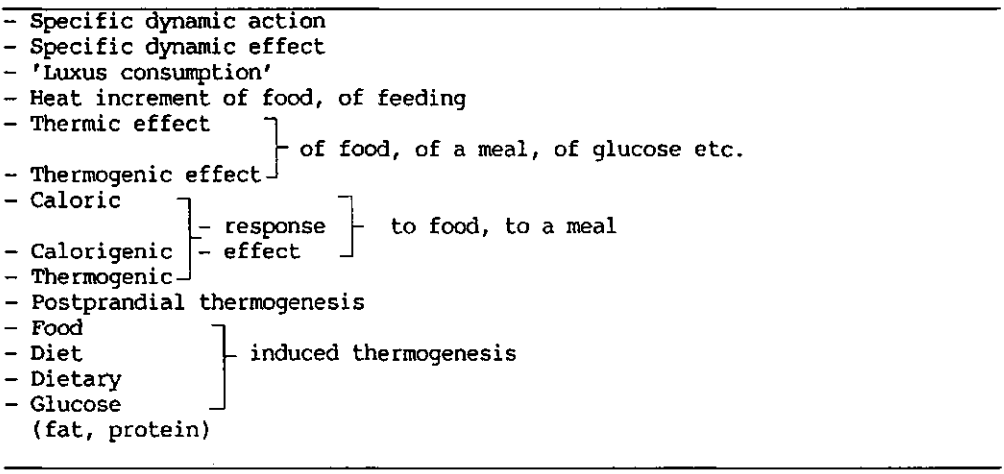

Figure 2. Terms encountered in the literature describing the increase in energy expenditure following the ingestion of food or a meal (14).

In the first 50-60 years of the 20th century diet-induced thermogenesis was not a matter of great concern to most nutritionists (59-61). In the last two decades, however, the number of publications on DIT has rapidly risen (12,13,52,53,62-85). The reason for the revived interest in diet-induced thermogenesis was the suggestion that the obese would have a lower diet-induced thermogenesis when compared to the non-obese (12,13). This difference would be of pathogenetic significance. It was suggested that diet-induced thermogenesis was an effector of differences in energy requirements between lean and obese subjects.

More recently, diet-induced thermogenesis has been proposed as an effector of an adaptation of energy expenditure to lower energy intakes or to sustained increased metabolic energy needs, ie, in pregnancy and lactation (86-88). Whether diet-induced thermogenesis may act as an effector of inter-individual

differences in energy requirements is unclear. Controversy in this area of the study of human energy exchange is disappointingly high. Seventeen recent studies (12,13,52,53,62-74) report a blunted diet-induced thermogenesis in the obese, whereas eleven studies (75-85) did not observe a systematic difference in diet-induced thermogenesis between the lean and the obese. According to D'Alessio et al. (85) the difference, in results is not simply related to a systematic difference in methodology between the studies supporting a role for diet-induced thermogenesis in the etiology of obesity and studies denying such a role.

Recently, it was reported that diet-induced thermogenesis would be reduced, in particular, among older diabetic obese subjects (64). Such subjects are insulin resistant and would have an impaired cellular transport of glucose, causing lower rates of glucose oxidation and storage. Restoring the insulin resistance by means of clamp studies showed that the thermogenic defect was not related to a defect in energy metabolism at the cellular level, but to impaired cellular glucose transport (89).

Controversy on the role of diet-induced thermogenesis is not only present with respect to the impact of the degree of body fat content on diet-induced thermogenesis, but for almost any other factor that has been studied for its effect on diet-induced thermogenesis (90). A recent example is the impact of the degree of physical activity on diet-induced thermogenesis (91-94). Whether adaptive changes occur in diet-induced thermogenesis during overfeeding is also unclear (5,41).

There is no disagreement on the primary determinants of diet-induced thermogenesis. These are the level of energy intake and the nutrient composition of food (80,83,85,93,95). With increasing energy intake, proportion of protein or carbohydrates, diet-induced thermogenesis increases. According to a recent study of D'Alessio et al. (85) energy intake is linearly related to diet-induced thermogenesis. The results of the study of D'Alessio et al. are confirmed by findings of Hill et al. (93).

Flatt (96) has provided a valuable account of the biochemistry of energy expenditure associated with protein, fat or glucose ingestion. Diet-induced thermogenesis would be highest for proteins, followed by carbohydrates and, finally, by fats. The balance between storage of nutrients and oxidation of nutrients would determine nutrient- or diet-induced thermogenesis. According to Flatt, the energy expended in digesting, absorbing and transporting nutrients is negligable in comparison with the metabolic processing of the ingested nutrients. Evidence for this notion comes from a study that observed

no significant difference in diet-induced thermogenesis between enteral and parenteral feeding (97).

Using Flatt's model of the biochemistry of energy expenditure associated with nutrient ingestion, it can be predicted that metabolic states favoring storage of nutrients prior to oxidation will enhance diet-induced thermogenesis. Flatt's model offers a possibility to calculate from substrate balances the amount of energy expended in postprandial metabolism of nutrients.

Various authors have shown that the amount of energy expended in the postprandial metabolism of the ingested nutrients can not completely account for the observed thermic response (98,99). Current theory on diet-induced thermogenesis states that diet-induced thermogenesis is composed of two distinct components, one, the 'obligatory' component, is involved in the postprandial metabolism of the nutrients (16,17,95). Using Flatt's model the size of the obligatory component can be calculated. In adddition to the obligatory component, there would be a 'facultative' component. This component is assumed to contribute about 30-40 percent to the total thermic response (17,95). The biochemical basis of this facultative component is not yet elucidated. Food ingestion, in particular carbohydrates, can stimulate the activity of the sympathetic nervous system (83,100). It is assumed that this activation leads to an increase in the turnover of catecholamines affecting, in its turn, the rate of energy expenditure and consequently diet-induced thermogenesis.

Empirical evidence for the two-component model of diet-induced thermogenesis is given by the observed reduction in diet-induced thermogenesis (41) and glucose-induced thermogenesis (99,101,102) in subjects infused by the beta-blocker propanolol. It has, however, been difficult to correlate diet-induced thermogenesis with plasma levels of norepinephrine (65,82,83). Recently, it was observed, however, that the increment after meal ingestion in plasma appearance rates of norepinephrine correlated significantly with diet-induced thermogenesis (103).

It is not clear which factors determine the facultative component of diet-induced thermogenesis. It has been suggested that personal characteristics, ie, the degree of adiposity may be important (104), in particular, the degree of insulin resistance that frequently accompanies the obese state. In addition, the plane of nutrition may affect facultative diet-induced thermogenesis. Overfeeding, in particular with carbohydrates, would enhance this component (17). A third factor that might be involved in the thermic response to food are the sensory characteristics of food (105).

The existence of a facultative component in diet-induced thermogenesis is inferred from a difference in the observed thermic response to food ingestion and the thermic response that should have been observed using Flatt's model to calculate the energy expended in postprandial nutrient oxidation and storage pathways. There are some problems here. The relevance of facultative thermogenesis for energy expenditure under normal circumstances after normal mixed meal ingestion has been questioned, since its presence has been inferred predominantly from unphysiological clamp studies (106). Secondly, nutrient utilization patterns as estimated from indirect calorimetry do not reflect dynamic states in nutrient oxidation and storage, but only net storage and oxidation (107). This means that turnover of a nutrient between its storage and mobilisation forms can not be assessed with indirect calorimetry. If such turnover would be high, this could increase thermogenesis. In this respect the quantitation of lipogenesis from glucose is interesting. The energy expended in transforming glucose to fat represents about 25 percent of the energy content of the glucose (96). This indicates that transformation of a large part of the ingested glucose to fat prior to oxidation will increase diet-induced thermogenesis when compared to a situation where most of the ingested glucose will be immediately oxydized. The indirect calorimetric method for assessing nutrient utilization gives estimates of net lipogenesis only. This means that concomitant fat synthesis and fat oxidation will not be perceived from measurements of gaseous exchange and urinary nitrogen excretion. Only the effects of fat synthesis in excess of fat oxidation, or of fat oxidation exceeding fat synthesis on gaseous exchange will be measured. This implies that estimating the amount of energy expended in the net transformation of glucose into fat may underestimate the total amount of energy expended in lipogenesis. Thus, estimation of the amount of energy expended in the so called obligatory component of diet-induced thermogenesis may be in error too. Consequently, the inference of the existence of a facultative component representing 30-40 percent of total diet-induced thermogenesis could also be wrong.

Figure 3 presents a 'model' for the current theory on the nature of diet-induced thermogenesis.

Ingestion and digestion of food or nutrients cause the obligatory expenditure of energy. After absorption nutrients are transported to storage and oxidation sites. Storage and oxidation of nutrients require the obligatory expenditure of energy. Absorbed food may directly stimulate the sympathetic nervous system (SNS) activity, or indirectly through insulin. Increased sympathetic nervous

system activity may lead to facultative thermogenesis. Insuline acts as gatekeeper of the amount of glucose directed to immediate oxidation or storage. Increased storage prior to oxidation will lead to increased obligatory thermogenesis. Insulin may also directly act to stimulate facultative thermogenesis.

Using this model we formulated hypotheses on the impact of various nutritional and non-nutritional factors on diet-induced thermogenesis. In this thesis we investigated the impact of ethanol, previous exercise, psychological stress, palatability of food and carbohydrate overfeeding on diet-induced

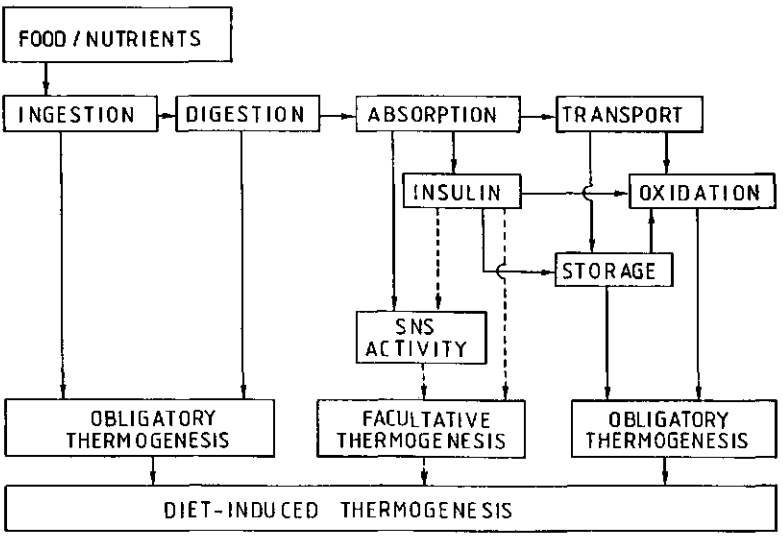

Figure 3. 'Model' of current theory on the nature of diet-induced thermogenesis (DIT). Thick lines indicate established contributions, interrupted lines indicate contributions still in doubt (SNS = sympathetic nervous system).

thermogenesis. Ethanol was assumed to increase nutrient storage and, hence, diet-induced thermogenesis. In resting conditions, ethanol elimination by

oxidation would cause ingested nutrients to be directed not to oxidation pathways, but to storage pathways. Prolonged exercise is known to deplete carbohydrate storage in the body (108) and may also affect the activity of the sympathetic nervous system (109). We expected that storage of carbohydrates from food after previous exercise would be increased in order to restore the tissues levels of glycogen, hence diet-induced thermogenesis would be increased. Psychological stress was expected to increase diet-induced thermogenesis by effects on the secretion of stress hormones, or by a stimulation of the activity of the sympathetic nervous system. Palatability was hypothetized to affect the early insulin rise, also known as the cephalic insulin response. Meals with high palatability would evoke a relatively high cephalic insulin response (110). The level of the cephalic insulin response is positively associated with the total insulin reponse, at least in rats (111). Loss of the early phase of insulin release has been shown in humans to impair glucose tolerance and to reduce the thermic effect of infused glucose (112). We expected that, due to increased insulin secretion, carbohydrate storage on palatable meals would be increased and, hence, diet-induced themogenesis. In addition, palatability could increase the activity of the sympathetic nervous system with stimulatory effects on thermogenesis. Carbohydrate overfeeding was expected to increase diet-induced thermogenesis in two ways. Firstly, by affecting the state of the body's glycogen stores. Secondly, through activation of the sympathetic nervous system. It was expected that the results of our studies would enable us to refine the model on the nature of diet-induced thermogenesis.

2.3.1.3. WORK-INDUCED THERMOGENESIS

Work-induced thermogenesis (WIT) is the energy expended above resting conditions due to the performance of physical work. It is determined by the type, duration and intensity of physical work. Physical activity is a much more potent stimulus of energy expenditure compared to diet. Due to physical activity the rate of resting energy expenditure can rise with a factor 10-20. For the average individual, work-induced thermogenesis would contribute 15-30 percent to total daily energy expenditure (16,17). It is, however, difficult to estimate accurately the energy expended in physical activity. This applies not only to persons leading their normal lives, but also to persons whose energy expenditure is studied in whole body calorimeters. In particular the

degree of spontaneous, relatively small, movements ('fidgeting') may be quantitatively important as an effector of inter-individual differences in energy requirements (28).

Dynamic muscular work, for example cycling, is generally performed with an efficiency of 20-25 percent (113). Theoretically, differences in energy requirements among individuals could be related to individual differences in the amount and intensity of physical activity and in the efficiency of the performance of physical work. An evaluation of differences in the amount and intensity of physical activity of free-living persons is currently very difficult. However, variation in work efficiency among individuals can be assessed with standard laboratory tests. There is little evidence for differences in work efficiency between the lean and the obese (114). The delta efficiency of muscular work seems similar between the lean and the obese (115). Delta efficiency is proposed as the best indicator of the efficiency of the performance of physical work (116). The delta work efficiency is the efficiency of work accomplished calculated by difference between two steady state levels of work, whereas the net work efficiency is the work done by an individual on an external system per unit of energy expended in the performance of that work (14). Weight loss was reported to affect the net efficiency of physical work, but not the delta efficiency (116).

It has been suggested that exercise would potentiate diet-induced thermogenesis and that the obese would be defective in this respect (5). This is another area of controversy on the impact of the degree of body fat content on diet-induced thermogenesis. More research under strictly standardized conditions seems necessary to settle this issue (114,115).

Recently, an interesting finding was reported. It was reported that African women expended less energy in carrying loads than did their Caucasian counterparts (117). The study of Maloiy et al. (117) does not give an answer to the question whether this difference was the result of a higher work efficiency of physical work among the African women compared to the Caucasian women, or of behavioral differences between the groups in the way the loads were carried.

2.3.1.4. MEASUREMENT OF ENERGY EXPENDITURE

Energy expenditure can be assessed by direct calorimetry, indirect calorimetry and by noncalorimetric methods. I will not discuss these methods in depth in

this thesis. Recently, a few excellent reviews (107,118,119) have been published on the measurement of human energy expenditure and of in vivo fuel utilization. A few comments will suffice in this respect.

Direct calorimetry

Direct calorimetry involves the measurement of heat losses. This is the oldest of the three techniques. It has been used since the time of Lavoisier at the end of the 18th century. Direct calorimetry measures the sum of radiant heat exchange and of convective -, conductive - and evaporative heat transfer. In a resting subject the sum of these heat losses equals, in the long-run, the heat released by metabolism in the body. Direct calorimetry has the disadvantage that it can not be used to assess short-term effects of thermogenic stimuli on heat exchange due to the relatively large heat storage capacity of the body. For example, during feeding or short-term exercise a large part of the heat that is generated is stored within the body. Only over a period of 24 hours or longer the body will be in heat balance with its surroundings. Over prolonged periods of time direct calorimetry provides the most accurate estimate of the rate of energy expenditure. However, it is a less suitable technique for assessing diet- or work-induced thermogenesis and it does not provide information on in vivo fuel utilization.

Indirect calorimetry

Indirect calorimetry is the method by which metabolic rate is estimated from measurements of oxygen consumption and carbondioxide production. When it is assumed that all the oxygen consumed is used to oxydize degradable fuels and all the carbondioxide thus produced is recovered, it is possible to calculate the total amount of energy 'produced'. If, in addition the rate of nitrogen excretion is known, indirect calorimetry can be used to quantitate nutrient, ie, fuel or substrate, utilization patterns. Energy 'production' means in this respect conversion of chemical energy of nutrients into chemical energy of 'energy-rich' substances, ie, ATP, plus loss of some energy as heat during the oxidation process. Eventually, however, in a resting adult subject all energy derived from the metabolism of food stuffs will be converted into heat. It is essential to realise that in the long run direct and indirect calorimety will give similar estimates of heat losses from the body. In the long run changes in body temperature will cancel out and the rate of formation and degradation

of energy-rich bonds will be equal.
Indirect calorimetry can be used to assess acute effects of thermogenic stimuli on metabolic heat production in the body. Since the body does not posses an oxygen storage capacity of quantitative importance, oxygen consumption will immediately rise in response to exposure of the organism to thermogenic stimuli.
There is a variety of equations used to calculate metabolic rate from oxygen consumption, carbondioxide production and urinary nitrogen excretion (118). Differences between these formulas are quantitatively unimportant (118). They are generally due to the use of slightly different constants for the amount of oxygen used and carbondioxide produced during combustion in the body of protein, fats and carbohydrates.
Two techniques based on indirect calorimetric principles are worthwhile to mention. These are measurements of energy expenditure with indirect whole body calorimeters, also known as respiration chambers, and with ventilated hood systems. Respiration chambers are, in particular, suitable for studying total daily energy expenditure of subjects or animals for one up to several days. The ventilated hood system is useful for measuring the individual components of total daily energy expenditure, ie, resting metabolic rate, diet- or work-induced thermogenesis. The ventilated hood technique can be traced back, at least, to Benedict in 1930 (120). However, the development of modern physical gas analyzers, eliminating the time-consuming volumetric analysis of gaseous exchange, and of modern physical flowmeters, ie, mass flowmeters, has stimulated the construction and use of ventilated hood systems in metabolic studies. Additional impetus for the use of ventilated hood systems came by the recognition that indirect calorimetry can provide information on the type and rate of substrate utilization in vivo. This type of information may be important for gaining insight in patterns of nutrient assimilation in various pathological states, for example, in diabetes and obesity (107).

Noncalorimetric methods

There is a variety of noncalorimetric methods (119) that can be used to estimate energy expenditure in man. These methods are based on, for example, the use of activity diaries, pulmonary ventilation or heart rate and mechanical activity meters. A more recent development is the use of isotopic dilution of doubly labelled water (121). This method was introduced in 1955 by Lifson et al. (122) for the study of energy expenditure in small animals.

Until recently the cost of the isotope was too high to permit its use in humans (123). However, in the 1980s the first publications appeared in which energy expenditure was assessed in humans by means of the doubly labelled water method (121,124,125). The principle of the method is to dilute the body water component of a person with a known amount of D_2O^{18}. Deuterium will label the water pool of the body, whereas the O^{18}, in addition, will also label the body's bicarbonate pool, due to the action of carbonic anhydrase. Deuterium will leave the body by water output only and O^{18} will leave the body by water plus carbondioxide output. As a consequence, the turnover in the body of O^{18} exceeds that of deuterium. The difference between the rates of deuterium and O^{18} turnover in the body water is used to calculate the carbondioxide production rate (126). To convert the carbondioxide production rate to an estimate of energy expenditure, the energy equivalent of carbondioxide has to be used. Depending on the respiratory quotient of the metabolic mixture oxydized in the body, the energy equivalent of carbondioxide may vary between 21.0 kJ and 27.7 kJ. If the dietary intake of the subject is estimated, and, if the subject is in energy equilibrium during the period of measurement, the respiratory quotient of the daily diet, also known as the food quotient, can be used to derive an estimate of the energy equivalent of carbondioxide (118). The major advantage of the doubly labelled water method for the assessment of energy expenditure is its applicability for use in persons living their normal lives (127). The use of the doubly labelled water method in free-living subjects requires the use of various assumptions, for example, with respect to steady state in water and energy balance of the subjects during the experimental period, the respiratory quotient of the diet and the proportion of water that is fractionated by evaporation at epithelial surfaces (118). Opinions on the applicability on the doubly labelled water method vary. Various authors regard the method as an exceptional technological advance that permits accurate estimates of the rate of energy expenditure of free-living adults (127,128). Other investigators are more cautious and argue that more research is needed to establish the validity of various assumptions of the method when applied to free-living individuals under various conditions of climate, food intake, and physical activity (118). It should also be realised that the doubly labelled water method gives an estimate of the average energy expenditure during the period of measurement, usually 2-3 weeks. It is not possible to measure 24-hours energy expenditure, day-to-day variability in energy expenditure or to obtain information on the quantitative importance of the various components of energy expenditure for total average energy

expenditure. In this respect a combination of the classical calorimetric methods and the doubly labelled water method may be promising.

2.3.2. ENERGY INTAKE

In contrast to energy expenditure, which is a constant process, energy is ingested only periodically at meal times and between meals during snacking. It is generally assumed that the body controls its food intake, ie, its energy intake, to meet current energy needs and to maintain an energy reserve for times when food is unavailable. The literature on factors affecting the regulation of food intake is enormous and will not be reviewed here in depth, see for reviews Kissileff and VanItallie (129), Nicholl et al. (130) or Forbes (131). In this review I will discuss briefly some theories on the physiological control of food intake and the control of food intake in humans.

2.3.2.1. THEORIES ON THE PHYSIOLOGICAL CONTROL OF FOOD INTAKE

That food intake is under some physiological control is inferred from observations on the relative consistency in daily or weekly food intake of animals maintained on a diet of homogenous composition under constant ambient temperature (132,133). These observations suggested the existence of a physiological control system for food intake (134). It was hypothetized that such a control system would correct deviations in daily energy balance in order to maintain long-term energy balance. Additional support for the existence of control of food intake came from the observations that food intake in animals varied reciprocally with caloric dilution of food (135,136). Evidence, that it was, in particular, the energy content of the food that was controlled, was provided by experiments that showed compensation in food intake when energy expenditure was varied (134,137). The existence of a physiological control of food, ie, energy, intake in humans was inferred from the observation that, although man may eat tons of food over the years, body weight stays relatively constant (138).
Is the evidence for a physiological control of food intake sufficient? Two comments can be made in this respect. Animals, ie, rats, can be easily overfed by offering them a highly palatable 'cafetaria' diet (139). Secondly, relative weight in man is not as constant as frequently is assumed. Gordon and Kannel

showed in a longitudinal study (the 'Framingham' study) that in adulthood, body weight increases on average 10 kg over a period of 18 years (140). These observations indicate that control of food intake in animals can be overruled by environmental stimuli, ie, palatability of food. In humans some form of physiological control of food intake is probably manifest, otherwise the increase in weight during adulthood might have been even larger than the 10 kg observed by Gordon and Kannel. The findings of Gordon and Kannel, however, clearly show that the control of food intake in humans is probably not as precise as previously thought (5).

When it is accepted that both humans and animals have some form of physiological control of food intake, various theories may be used to explain this control (134,141-144).

The homeostatic theories of the physiological control of food intake have received most attention. In homeostatic theories it is proposed that a variable is regulated and that certain effectors accomplish such regulations. Homeostatic theories have been subdivided into two types. There are those theories that propose that specific chemicals in the body are regulated ('chemostatted'), ie, fat ('lipostatted') (134), carbohydrates ('glucostatted') (141), or proteins ('aminostatted') (142), and those that propose regulation of energy, or some function of it such as body temperature ('thermostatted') (143), or energy expenditure by metabolizing cells ('energostatted') (144). Nutrient specific homeostatic theories are now known as the lipostatic, glucostatic and the aminostatic theories. The current opinion is that these theories present a too limited account of the control of such a complex phenomenon as food intake (145-148). However, a few decades ago much research was carried out to validate the various theories. Therefore, I will briefly mention the quintessence of each theory. The lipostatic theory stated that depot fat was the regulated variable and that rates of food intake and energy expenditure were its effectors (141). The glucostatic theory proposed that utilization of glucose by privileged cells was regulated by initiating feeding when utilization was low and inhibiting eating when utilization was high (134). The aminostatic theory proposed that excesses and deficiencies of plasma amino acids were responsible for the control of food intake (142). An integration of the lipostatic, glucostatic and aminostatic theories was the thermostatic theory which considered metabolic heat production as the control of food intake (143). The thermostatic theory has been further refined in the energostatic theory (144). The latter theory states that the energy 'produced' by the metabolism of absorbed nutrients is

monitored to regulate food intake. Specific temperature receptors in the brain would activate and terminate ingestive behavior.

The central problem in the validation of these theories has been to discover the location of detectors and effectors for the homeostatic response. In the past decades four anatomical sites have been suggested. About 30 years ago Stellar stated the hypothalamocentric theory of the control of food intake (149). It states that hunger is directly proportional to the output of a lateral hypothalamic center, the 'hunger' centre. This center would elicit feeding. Feeding would be inhibited by a ventromedial hypothalamic center, the 'satiety' center, as a result of specific postfeeding signals. Experimental evidence for the dual center theory of hypothalamic control of food intake came from observations that lesions of the lateral hypothalamus induced hypophagia (150) and stimulation induced eating (151), whereas lesions of the ventromedial hypothalamus induced hyperphagia (152) and stimulation inhibited eating (153). In addition, evidence was obtained that glucose and free fatty acids affected the neuronal activity in these areas, thus relating the dual center theory to the chemostatic, ie, glucostatic and lipostatic theories (134,141).

It is now generally accepted that the concept of the hypothalamus as having two distinct control centres is a gross oversimplification (145). The concept of discrete hunger and satiety centres has been replaced by one of diffuse excitatory and inhibitory systems (145). The 'satiety' centre has been associated with two major tracts, ie, the serotoninergic pathway, which originates in the raphe nuclei of the pontine-midbrain area and passes through the ventromedial hypothalamus, and the ventral adrenergic bundle which passes through the periformical area in the vicinity of the ventromedial hypothalamus. The hunger centre is associated with the dopaminergic nigrostriatal tract.

Another suggested major site for homeostatic control of food intake is the liver (154). According to this theory, hunger is the result of a decrease in the liver glycogen reserves, which is signaled to the brain by discharge from glucoreceptive hepatocytes. A role for the liver in the control of food intake is supported by the fact that intraportal injection of glucose inhibits feeding while equivalent doses elsewhere are less effective (155).

The gastro-intestinal system has also been proposed as a major site for regulation of food intake (156-158). According to this theory, neural and humoral signals arise in the gastro-intestinal tract that inhibit food intake. Experimental evidence for this model came from observations that sham-feeding

was inhibited by the infusion of food in the small intestine (156). In addition, evidence for a role of the gastro-intestinal tract in the control of food intake came from observations on the satiating effect of the gut hormone cholecystokinin (159), which is probably neuronally mediated by gastric vagal efferents (146). Other possible mediators of the satiating effect of food in the small intestine are somatostatin and pancreatic glucagon (146).
It seems that various factors are involved in the physiological control of food intake. The modern theory of the control of food intake consists of a feedback system in which various signals derived from the gastro-intestinal tract, liver, the circulating energy, ie, nutrient, pool, depot fat or metabolizing cells, transmit information to a central system, ie, the brain, which is responding to and integrating information from the various peripheral components (148). The brain also responds to and integrates external information. The control of food intake has been diagrammatically presented in figure 4. In this system peripheral signals act as satiety signals, some time after food intake these signals become attenuated and food intake is terminated. Food intake is initiated by putative hunger signals initiating in the brain. There has been an intensive search to identify the physiological correlates of putative satiety and hunger signals. Currently, a variety of signals is known that act satiating, ie, act to terminate feeding or act to suppress appetite (145,146). These are cholecystokinin, bombesin, calcitonin, corticotropin-releasing factor, thyrotropin-releasing hormone, neurotensin and serotonin. The recent search for signals that would initiate hunger has shown that, in particular, opioid peptides would act as hunger signals (145). An interesting hypothesis is that obesity, as effected by overeating, would be the result of auto-addiction to endogenous opioid peptides (145).
Concluding I can state that the physiological control of food intake is complex and that no factor in isolation seems to offer sufficient explanation for the complex behavior of food intake.

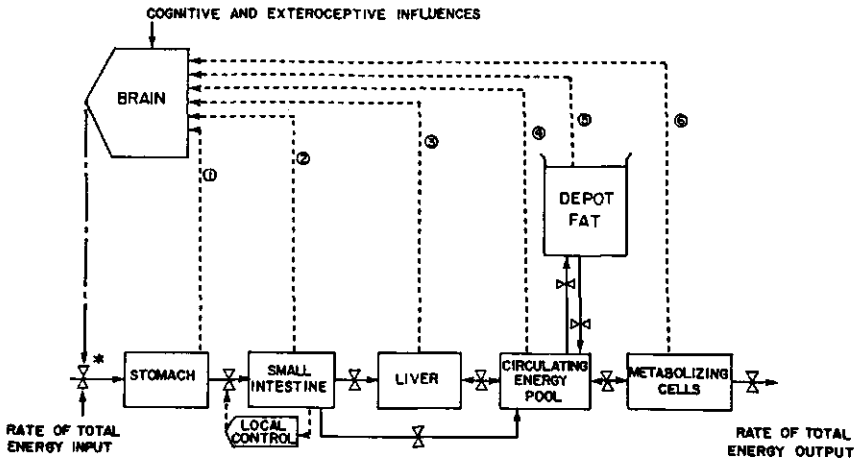

Figure 4. 'Model' of current homeostatic theory on the control of food intake in humans (148).
The components of the overall system are shown as blocks, which correspond to quantities of 'fuel' within anatomical or physiological entities: the stomach, the small intestine, the liver, the circulating energy pool, the adipose tissue depot, and the total mass of metabolically active cells. The blocks are connected to one another by solid lines, which represent the flow of substrate between components. Control of flow rates are represented by valve symbols. In this diagram, the rate of flow of energy into the body is shown as being under the control of the brain, which is responding to and integrating information (represented by the dashed lines) transmitted from various components of the system. The asterisk at the first valve symbol indicates that the rate of energy intake is not constant but occurs instead in discrete packets (meals) whose onset and termination are controlled by the brain in response to a variety of inputs (adapted from Van Itallie and Kissileff (18)).

2.3.2.2. CONTROL OF FOOD INTAKE IN HUMANS

In humans food intake is affected not only by physiological factors, in particular, energy needs, but also by non-physiological factors. A variety of

non-physiological signals may act on the brain to terminate or to initiate feeding (147). These non-physiological factors may differ in origin, ie, hedonic (palatability, taste, texture, odor), social (culture, religion), psychological (learned preferences and aversions), environmental (temperature), economic (cost, availability) or pharmacological (anorectants). Garrow concluded that 'In man control of food intake is complex, and the primitive hypothalamic reflexes are so buried under so many layers of conditioning, cognitive and social factors that they are barely discernible' (160). The question may rise whether food intake in humans is still under physiological control or whether it is largely determined by exogenous factors.

The importance of physiological control of food intake in newborns was demonstrated by Fomon et al. (161,162). In newborns the effect of non-physiological factors on the control of food intake may be assumed to be of negligble importance when compared to in adults. Fomon et al. (161,162) showed in studies on the food intake in newborns that babies fed less energy-dense milk adjusted their intake and ate more. The compensation was remarkably well, ie, between 80 percent and 100 percent. In an elegant study using normal foods, Porikos et al. (163) showed that three days after energy content of the diet had been covertly reduced by substituting the synthetic sweetener aspartame in some food items for sucrose, lean adults subjects, studied in a metabolic ward, started to compensate for the induced energy deficit. Compensation was not complete, but stabilized after some days at 85 percent of the energy intake observed in the control period. In a subsequent study of the same group with adult obese and lean subjects, no difference in caloric compensation between lean and obese subjects was observed (164). These results indicate some form of control of energy intake in adults. Similar results were obtained by Durrant et al. (166). Since in the studies of Porikos et al. the subjects were unaware of the caloric dilution, the response of food intake to the caloric dilution may be considered to be physiologically in origin. It can not be discerned from Porikos et al.'s studies whether the subjects were kept on a weight maintenance diet at the start of the study. When it is assumed that subjects were fed their energy requirements during the control period, the results suggest that a covert reduction in the energy density of the diet could be beneficial for weight reduction. However, in Porikos et al's studies no control group was investigated to estimate a spontaneous gradual decline in food intake due to adaptation to the experimental conditions.

Little is known about the relative contribution to the control of food intake of physiological and non-physiological factors in free-living subjects. This is a subject that should be studied more intensively.

2.3.3. ENERGY BALANCE

Two major components are involved in the regulation of energy balance: energy intake and energy output. According to the Law of Conservation of Energy a complete balance between these two components must exist. The components of energy balance, its control and its relation to obesity will be discussed.

2.3.3.1. ENERGY BALANCE EQUATION

The energy balance equation can be expressed in the following form (166):

gross energy of food = energy of faeces
+ energy of urine
+ energy of methane
+ metabolic heat produced
+ retrievable work
+ energy retained

Not all food ingested is entirely digested, resulting in a loss of energy in the faeces. Faeces also contains endogenous material from the alimentary tract. The difference between the energy content of the food and the energy content of the faeces is therefore referred to as the apparently digested energy of food. Some food constituents are metabolized by the bacterial flora in the colon. This may lead to the production of small amounts of combustible gasses, ie, methane, representing a loss of energy for the individual. The remaining part of digestible energy are nutrients that are absorbed into the blood stream to be metabolized. The metabolism of protein results in some waste products that are lost as energy in urine. The remaining energy is known as the metabolisable energy of food. This metabolisable energy (ME) is used after oxidation for, respectively, basal energy requirements, indicated by resting ('basal') metabolic rate, diet-induced thermogenesis, work-induced thermogenesis and, sometimes, for cold-induced thermogenesis. Under thermoneutral conditions, or in subjects well insulated by clothing against

the cold, the latter process is probably of negligable importance for daily energy expenditure. In very limited circumstances part of the energy expended during physical work is retrieved for use later (retrievable work), ie, in winding up a spring. Heat loss is then delayed until the spring is released. Finally, part of the metabolisable energy may be retained in the body for growth, production of milk, foetus, and also for an increase in the body's energy reserves, ie, fat stores.
The energy balance equation is usually presented in a slightly different form: metabolisable energy intake (ME) - energy expenditure (EE) = gain/loss in body's energy reserves ($\triangle$ER), ie,

$$ME - EE = \triangle ER, \text{ which equals}$$
$$ME - (RMR + DIT + WIT) = \triangle ER$$

In weight stable persons the metabolisable energy intake matches energy expenditure and no net change in the body's energy stores is observed. The question how the body preserves its energy balance without significant net storage or loss of energy from the body continues to be of interest to investigators.

2.3.3.2. CONTROL OF ENERGY BALANCE

It follows from the energy balance equation that an increase in energy intake relative to energy expenditure produces energy surfeits, increased fat storage and eventual gain in body weight. If, on the other hand energy expenditure exceeds energy intake, energy reserves are lost from the body, fat stores are mobilized and body weight decreases.
Which factors contribute to the development of a new balance between energy intake and energy output without any net change in fat stores?
Figure 5 and figure 6 show the changes by which the body will gain a new balance between energy intake and energy output in a situation of energy imbalance, either caused by a reduced food intake, ie, a negative energy balance (Figure 5) or by an increase in food intake, ie, a positive energy balance (Figure 6).
In negative energy balance, energy requirements are lowered. A decrease in body weight reduces the cost of weight bearing activities. The loss of fat-free mass which accompanies weight loss causes a lowering of resting

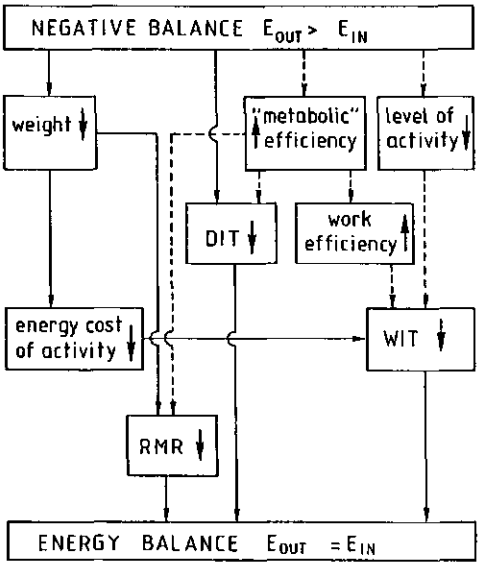

Figure 5. 'Model' of impact of a negative energy balance on energy expenditure. Thick lines indicate established contributions. Interrupted lines indicate contributions still in doubt (RMR = resting metabolic rate; DIT = diet-induced thermogenesis; WIT = work-induced thermogenesis).

In negative energy balance, energy requirements are lowered. A decrease in body weight reduces the cost of weight bearing activities. The loss of fat-free mass which accompanies weight loss causes a lowering of resting metabolic rate. The reduction in food intake decreases diet-induced thermogenesis. Individuals may show (facultative) behavioral changes that reduce the amount of energy expended in physical activities.
A further reduction in energy expenditure could be caused by an increase in the efficiency of energy utilization, ie, in 'metabolic' efficiency. The latter effect would become manifest in 'unexpected' (not accounted for by the processes, headed under 1, 2 and 3) decreases in resting metabolic rate, diet-induced thermogenesis and work-induced thermogenesis.

Recently, it was shown, however, that the reduction in energy expenditure during weight loss could be completely accounted for by the reduction in body weight and body composition, decreasing the cost of physical activity and of resting metabolic rate, and by the reduced energy intake, decreasing the amount of energy expended after food ingestion (54). There was no need to postulate the existence of an increase in 'metabolic' efficiency. However, Leibel and Hirsch presented evidence for an increase in 'metabolic' efficiency when they found that post-obese had significantly lower energy requirements in comparison to lean controls (167). However, in their study no measurements were performed of resting metabolic rate, diet-induced thermogenesis or work efficiency. Recently, Geissler et al. (168) showed that post-obese women had 15 percent lower daily energy expenditure rates when compared to lean controls, matched in body composition and degree of physical activity to the post-obese. These findings do suggest the existence of some change in the efficiency of energy utilization in the post-obese. Such a change would involve environmentally-induced alterations in the partitioning of energy transformations in the body into those leading directly to heat and those leading to work and subsequent heat and, secondly, in the efficiency of the transformation of chemical energy in food stuffs to internal work. It is clear that more research is needed, not only in post-obese, to investigate whether humans show 'unexpected' metabolic responses to a negative energy balance.
In developed countries with its calorie pollution a situation where energy intake exceeds energy expenditure is more frequently encountered. Figure 6 shows the way the body reacts to a positive energy balance. There is an increase in body weight, usually accompanied by an increase in the size of the metabolically 'active' mass, ie, in fat-free mass. The effects of energy overnutrition on body weight and body composition increase the amount of energy expended in weight bearing activities and at rest. Since more energy is ingested, diet-induced thermogenesis is increased. In addition, behavioral adaptation favoring expenditure of energy in physical activity may occur. Similar to the situation of an energy deficit, the question remains whether there are additional mechanisms favoring expenditure of energy. The existence of such mechanisms has been postulated for the first time by Neumann (169) when he introduced the 'Luxus consumption' phenomenon. Many years later the results of the Vermont overfeeding studies of the group of Sims and Horton supported the existence of such mechanisms (170). In these studies it was observed that weight gain was less than the weight loss expected to occur on the basis of the energy surplus in the diet (179). Evidence for the existence

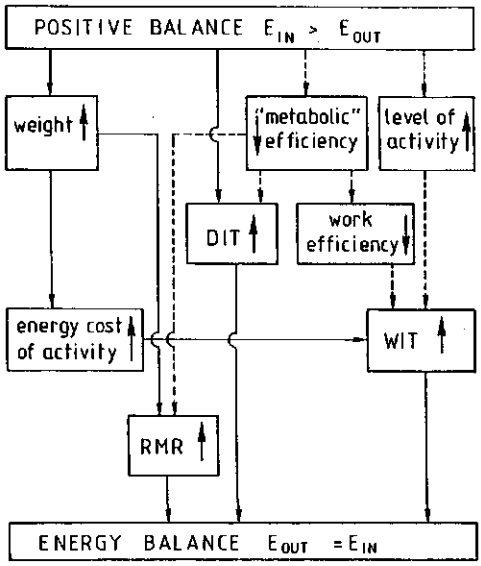

Figure 6. 'Model' of impact of a positive energy balance on energy expenditure. Thick lines indicate established contributions. Interrupted lines indicate contributions still in doubt. (RMR = resting metabolic rate; DIT = diet-induced thermogenesis; WIT = work-induced thermogenesis).

in humans of metabolic responses to energy overnutrition favoring energy expenditure is, however, far from conclusive (17,160).

Overfeeding-induced changes in thyroid status and the activity of the sympathetic nervous system could decrease the efficiency of uncoupled metabolism or of coupled metablism, ie, increase the use of ATP in so-called substrate or round-about cycles, or ion pumping. In small rodents it has been found that overfeeding decreases the efficiency of oxydative fosforylation in brown adipose tissue (171), although this has been disputed as well (172). When thermogenesis increases disproportionately during overfeeding, this could

act as a protective response to overeating.
The question whether the obese are defective in the capacity to show a protective response to energy overnutrition continues to be a topic of interest both to clinicians and to investigators (17). Another interesting question is whether the change in the composition of the diet over the last century has affected the capacity to show a protective response to energy overnutrition. The major change in composition of the diets of people living in developed countries has been the increase in the proportion of total energy intake delivered by fat (173). The proportion of energy delivered by fat in the diet has increased relative to calories derived from carbohydrates. This change in the composition of the diet may be important for overall energy balance for a variety of reasons. Firstly, carbohydrates generally evoke a higher thermic effect in comparison to fats (95). Converting ingested lipids to depot fat will incur a loss of energy from the body of about 4 percent of the ingested fats. In transforming carbohydrates to depot fat about 25 percent of the energy ingested, will be lost from the body as heat (96). It may be hypothetized that energy surfeits with a relatively high fat content will lead to higher fat storage in the body compared to similar energy surfeits with a higher carbohydrate content. In addition, carbohydrate intake may increase the activity of the sympathethic nervous system relative to fat intake (17,100). Empirical evidence for the importance of the fat/carbohydrate ratio in the diet in determining overall energy balance and body weight was given by Lissner et al. (174). Lissner et al. showed that subjects on an ad libitum diet with a fat content of 15-20 percent reduced calorie intake in comparison to a diet with 30-35 percent or a diet with 40-45 percent fat. Body weight was also reduced. It is not possible to partition the body weight loss observed in the study of Lissner et al. in a component due to decreased energy intake and a component due to increased energy expenditure. However, the results suggest that habitual unrestricted consumption of low-fat, ie, of calorically diluted, diets may be an effective approach to weight control.

2.3.3.3. ENERGY BALANCE AND OBESITY

Obesity is a situation with an increased storage of fats in the body. Obesity develops when energy intake exceeds energy expenditure for a prolonged period of time. A simple conclusion from this observation is, that the obese are

overindulgers who do not physiologically control food intake as precisely as the lean. Experimental evidence for this conclusion is, however, lacking (164,165). In absence of a clear defect in the obese of the (short-term) physiological control of food intake (164,165), it seems justified to conclude that the obese eat more than their lean counterparts, for example, due to some particular susceptibility to food intake inducing non-physiological factors. Surprisingly, however, evidence for increased food intakes among the obese compared to the lean is far from conclusive (175-179). This seemingly paradoxical finding could be related to methodological problems of accurately quantifying food intake in humans. It is well known that food intake may show considerable intra-individual variation, implying that repeated assessments of food intake may be important in comparisons of food intake between relatively small groups of lean and obese subjects (180,181). Another problem in assessing food consumption in free-living persons is that the simple act of monitoring food intake may affect food intake, possibly with a differential effect on the lean and the obese. It has been suggested that the obese, when monitored for their food intake, tend to underestimate their food intake to a greater extent when compared to lean subjects (127).

In the absence of clear indications that the obese would overeat, research has shifted its attention to the study of energy expenditure in obesity. If the obese would have an increased 'metabolic' efficiency, this could explain their obesity while ingesting similar amounts of energy as the lean.

The hypothesis of an increased efficiency of food energy use in the obese has, however, been difficult to prove. In addition, there are no indications for gross differences in the degree of physical activity between obese and lean subjects (104).

Since, according to the First Law of Thermodynamics, energy can not be created, there must be some explanation for these paradoxical results. If obesity is the result of an increased efficiency of food energy use, the absence of finding an increased efficiency in the obese state, indicates that obesity is an adaptation to a metabolic state favoring energy retention. Only prospective studies can provide a definite answer to this question. Alternatively, studying obese after weight loss could be used to solve this problem, but, only under the assumption that a change in metabolic state from obese to post-obese has similar effects on the body's energy economy compared to a change from a pre-obese to an obese state. Secondly, if among lean and among obese there are individuals with differences in 'metabolic' efficiency, comparing heterogenous groups of lean and obese individuals may not reveal any

significant differences in energy expenditure between both groups. A third explanation would be that the obese and the lean are equally efficient in use of food energy, but methodological problems in obtaining a representative and accurate estimate of habitual food intake, or of energy expenditure, in particular, due to physical activity, among the obese or the lean lead to unexpected results. Finally, a systematic difference in digestibility of food between the lean and the obese could be important for the etiology of obesity. If digestibility would be relatively low in the lean and relatively high in the obese this could favor energy retention in the obese. However, studies pertaining to this subject have shown that there are no differences in digestibility between the lean and the obese (182,183).
Whatever the cause of obesity is, it can be predicted that any investigator looking for the 'holy grail' of the etiology of obesity will encounter numerous problems in proving his or her hypothesis. Obesity is probably multifactorial in origin. It may develop due to energy retention caused by some form of energy overnutrition, aggravated in certain groups of the obese by an increased susceptibility to store fat as a result of relatively lowered rates of energy expenditure. Evidence for this hypothesis on the etiology of obesity comes from an elegant study by Ravussin and colleagues (184). Ravussin et al. recently showed in a prospective study that a reduced rate of energy expenditure is a risk factor for body weight gain. A reduced rate of energy exenditure could, however, in their study, account only for about 35 percent of the energy retained in body weight gain. The extent to which physiological and environmental factors contribute to the supposed hyperphagia or lowered rates of energy expenditure as well as how these factors interrelate with the genetic make-up of a pre-obese individual remain to be investigated. That the genetic make-up of a person may be important in the response to energy overnutrition of energy expenditure was shown by Poehlman et al. (185).

2.4. CONCLUSIONS

The study of human energy exchange has experienced a revival the last 2-3 decades. During this period substantial insight was gained in factors affecting energy output. Salient findings have been that individuals can differ in their efficiency of energy utilization and that a reduced rate of energy expenditure can contribute to body weight gain in free-living

individuals. However, the nature and magnitude of inter-individual differences in the efficiency of energy utilization as well as their relation to 'nature' and 'nurture' are largely unknown.

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Chapter 3.
The assessment of resting metabolic rate, diet-induced thermogenesis and in vivo fuel utilization using a ventilated hood system: methodological considerations

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ABSTRACT

Different aspects of the methodology of assessing postabsorptive resting metabolic rate (RMR), diet-induced thermogenesis (DIT) and in vivo fuel utilization rates (FUR's) were investigated in humans using an indirect calorimetric ventilated hood system. The reproducibility and between-person variability in RMR, DIT, postabsorptive and postprandial respiratory quotients (RQ's) and FUR's were assessed in a group of 37 non-obese men and in 22 non-obese women and 32 obese women. RMR reproducibility was assessed in a group of 49 men. The impact of strict control of the antecedent diet on the reproducibility of RMR, DIT, RQ's and FUR's was measured in a separate study. Diurnal variation in RMR, the effect of the phase of the menstrual cycle on RMR and DIT, the effect of the energy content of the test meal and of the duration of the postprandial period on the calculation of DIT were also investigated. The average method-free within-person coefficients of variation of RMR and RQ's were 5-6 percent. The within-person variation in DIT and FUR's were, respectively, on average 28 percent for DIT and 20 to 50 percent for FUR's, depending on the type of nutrient and the prandial state. RMR did not show a significant change from early morning to early afternoon. No systematic effect of the phase of the menstrual cycle on RMR and DIT was observed. In both sexes DIT was positively associated with the energy content of the test meal. With energy intakes below 2600 kJ in men and below 2100 kJ in women around 90 percent of the total DIT response, observed in four hours, could be assessed within three hours. DIT was not significantly associated with body weight, the size of the fat mass or the fat-free mass.

It is concluded that the low reproducibility of DIT measurements has important considerations for the design and evaluation of studies on DIT.

Keywords: indirect calorimetry, resting metabolic rate, diet-induced thermogenesis, respiratory quotient, fuel utilization, methodology, reproducibility, obese, non-obese, antecedent diet, diurnal variation, menstrual cycle, human

INTRODUCTION

At the turn of the century Rubner observed that the increase in heat production, ie, in energy expenditure, was greater with protein than with fat or sugar ingestion (1). Rubner called the increase in heat production after food ingestion the 'specific dynamic effect of food'. At the same time that Rubner developed his concept on the specific dynamic effect of food, Neumann described the 'Luxus consumption' phenomenon (2). Luxus consumption refers to a process of metabolic wastage of ingested excess energy. During Luxus consumption excess energy is not used for proportional increases in growth or in body energy reserves, but for increased thermogenesis.

Both Rubner and Neumann could not possibly have foreseen that more than 60 years later interest in the specific dynamic effect of food and in Luxus consumption would increase dramatically.

Until the 1960s the specific dynamic effect of food and Luxus consumption were matters of little concern to most nutritionists or physiologists (3,4). In the last three decades interest in both topics has, however, been reawakened (5,6). During this time a plethora of terms have been used to describe the specific dynamic effect of food and the Luxus consumption phenomenon. The terms most used nowadays are the thermic effect of food and diet-induced thermogenesis (DIT) (5,6). Both terms are considered here to refer to the immediate response of energy intake to food ingestion, irrespective of the nutritional status of the subject.

The reasons for the revived interest in DIT was the emerging view that thermogenesis would play an important role in the regulation of body weight and body energy stores (7,8). Previously, the central problem of weight or energy balance regulation was considered to be the regulation of energy intake (9,10). Animal experiments, however, showed that, in addition to this factor, the regulation of energy output was important for the control of body weight

(7,11,12). These findings suggested that in humans a defective regulation of energy output could also be responsible for the development or maintenance of obesity. This hypothesis led to a proliferation of studies comparing the obese with the non-obese or post-obese on total daily energy expenditure or on some component of daily energy output, usually the resting ('basal') metabolic rate (RMR) and DIT. With respect to DIT the most significant outcome of all these studies was, that DIT would be blunted in the obese, and in the post-obese, and therefore of significance in the pathogenesis or maintenance of obesity (13-29). The evidence for this view is, however, far from conclusive (30-42). Since 1924 at least 17 reports (13-29) have been published in which it was shown that the obese had a defect in DIT; in at least 13 articles (30-42) no such defect was reported. Conflicting results are reported for almost any other factor affecting DIT that has been investigated (43-58).

The reasons for these conflicting results are obscure (42). It is clear that, progress in gaining insight in the etiology of problems of energy balance is hampered considerably by these controversies.

The discrepancy in results in this field of study of postprandial metabolism may, in part, be related to some specific methodological problems of the measurement of DIT. For example, none of the studies reporting on DIT, present data on the within-person variability, ie, the reproducibility, of DIT measurements.

In the report presented here, the methodology for assessing DIT in humans is systematically reappraised. A description is given of a computerized open-circuit ventilated hood system, constructed for assessing RMR and DIT. Results are presented of repeated DIT and RMR measurements obtained in 91 subjects, males and females, the latter group with a wide range in body fat content. Special attention is given to the reproducibility of RMR and DIT measurements, the quantitation of the thermic response, the relation between DIT and the energy intake of the ingested meal and the duration of DIT measurements. In addition, the impact of the phase of the menstrual cycle on RMR and DIT is investigated. The results of this study provide important information on methodological aspects of DIT measurements.

SUBJECTS AND METHODS

The ventilated hood system

An open-circuit indirect calorimetric ventilated hood system was built to measure continuously the rate of energy expenditure for a prolonged period of time (up to 6 hours) with minimal discomfort for the subject. The principle of a ventilated flow-through system is, that a stream of air is forced to pass across the face of a subject and mixes with and collects any expired air as it does. The expired air is collected by a transparent perspex hood placed over the subject's head, which is made airtight around the neck with a cord. The rate of energy expenditure can be calculated by determining the amount of air flowing through the hood and by measuring the oxygen (O_2) and the carbondioxide (CO_2) concentrations in the incoming and outflowing air. Figure 1 gives a schematic representation of the system used in Wageningen.

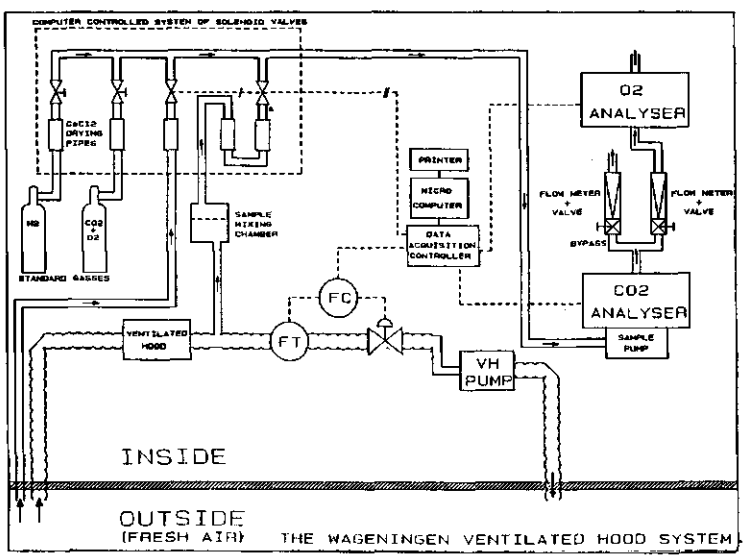

Figure 1. Schematic outline of the Wageningen Ventilated Hood System. VH indicates ventilated hood, FT is flowmeter (flowtransmitter) and FC is flowcontroller. Not shown in this outline are the multi-pen chart recorder and the load cells.

Through a perspex hood (volume 30 l) with an air inlet on top and an air outlet at the right side of the hood fresh filtered atmospheric air is drawn by negative pressure created by a pump downstream (Ocean SCL210, Dieren, the Netherlands). Airflow through the hood is measured in the outlet airstream by a thermal mass flowmeter (Brooks 5812N, Veenendaal, the Netherlands). This device measures the mass of gas and is unaffected, within certain limits, by temperature and pressure variations. It is, however, affected by humidity and at the point where liquefication starts, it is impossible to measure the mass flow of a gas accurately. In resting subjects condensation of water vapour in the outlet airstream of a ventilated flow-through system does not occur and the impact of small variations in humidity on the measurement of airflow is negligible. The mass flowmeter is calibrated at least once a year to within 0.2 percent of indicated flowvalues in the range between 20 and 50 l/min at the supplier's factory using a very precise gasvolume meter with built-in temperature and pressure sensors. Flow readings of the mass flowmeter are directly converted to STPD conditions. Prior to the flow measurement a small and constant quantity (0.4 l/min STPD) of air is continuously withdrawn by an airtight pump for gas analysis. Sample gas is thoroughly mixed in a small (0.4 l/min) perspex mixing chamber containing a small circulating fan (Micronel AG V244L, Zürich, Switzerland). Before gasanalysis the sample is passed through two serially placed perspex tubes, each 22 mm in diameter and 250 mm in length, containing the drying agent CaCl2 (Merck 2387, Darmstadt, West-Germany). Through a system of computer-driven solenoid valves (Bürkert 211A, Ingelfingen, Germany) sample gas is obtained from either the ventilated hood, standard gas mixtures or fresh filtered atmospheric air. The oxygen analyzer (Servomex 1100A, Zoetermeer, the Netherlands) is placed at the outlet of the carbondioxide analyzer (Analytical Development Company SS100, Hoddesdon, UK). A bypass system of two visual flowmeters (Brooks 1355, Veenendaal, the Netherlands) with needle valves measures the total sample flow and is used to regulate the flow through the oxygen analyzer (0.10-0.15 l/min). Gas analysis is performed with, respectively, an infrared carbondioxide analyzer with a full scale range of 0-1 percent, and a paramagnetic oxygen analyzer set to a full-scale range of 20-21 percent. Both analyzers have a resolution of 0.01 percent and are accurate to, respectively, 2 percent of full scale (carbondioxide) and 1 percent (oxygen). Both analyzers were found to be very stable in zero and sensitivity setting over the course of multiple (n = 10) 5-6 hours measurement sessions. Zero drift was -0.002 ± 0.002 percent (of full scale) for the CO_2 analyzer and 0.003 ± 0.002 percent

(of full scale) for the O_2 analyzer. Span, ie, sensitivity, drifts were, respectively -0.003 $\pm$ 0.003 percent (of full scale) for the CO_2 analyzer and 0.003 $\pm$ 0.005 percent (of full scale) for the O_2 analyzer. Part of the span drift in the O_2 analyzer is not caused by random drift of the analyzer, but by shifts in barometric pressure of the air used to calibrate the analyzer. Analyzers are calibrated using dried standard gas mixtures and dried filtered fresh atmospheric air. Zero settings are performed by passing 100 percent nitrogen through the analyzers (0.4 l/min, at a pressure of 0.4 bar). The span of the carbondioxide analyzer is set by passing a special gas mixture (0.60 percent carbondioxide, 20.50 percent oxygen, 78.90 percent nitrogen) through the analyzer (0.4 l/min, at a pressure of 0.4 bar). The span of the oxygen analyzer is set at 20.95 percent using fresh dried filtered atmospheric air. During the course of measurements of energy expenditure the span of the oxygen analyzer is controlled once every 30-60 minutes. The flow through the hood is adjusted to keep carbondioxide readings in the range of 0.50 to 0.80 percent and oxygen in the range of 20.10 to 20.40 percent. Flow adjustments are made by a flow measure and a flow control-unit (Brooks 5871, Veenendaal, the Netherlands) whose output is connected to a motor-driven valve (Brooks 5837, Veenendaal, the Netherlands) placed downstream the thermal mass flow meter. In adults, flow rates between 25 and 50 l/min, depending on body size and body composition, are used. Subject's movements are recorded by a load cell (Tokyo Sokki Kenkuyjo TKA-200A, Tokyo, Japan) placed under a leg of the hospital bed, that is used by the subject during the gas exchange measurement. The principle of a load cell is the registration of changes in a vertical load, ie, pressure. Pressure is exerted on strain gauges in the centre of the load cell. A change in pressure causes an electrical signal which is amplified and digitized by an analogue-digital converter for computer processing.
Output voltage signals of the carbondioxide and oxygen analyzer, the mass flow meter and load cell are measured and digitized by a data-acquisition system (Hewlett Packard 3497A, Palo Alto, USA) interfaced to a desktop computer (Hewlett Packard 86B or 85, Palo Alto, USA), which is connected to, respectively, a diskdrive (Hewlett Packard 9121, Palo Alto, USA), containing the program for the calculation of energy expenditure, and a printer. Flow and gas concentration measurements were taken every 10 seconds, the output of the load cell was measured with a frequency of 5 times/sec. The computer calculated and printed the values of oxygen consumed ($\dot{V}O_2$ at STPD), carbondioxide produced ($\dot{V}CO_2$ at STPD), the respiratory quotient ($\dot{V}CO_2/\dot{V}O_2$) and the rate of energy expenditure (in kJ/min) integrated over periods of either

1, 2 or 5 minute(s). Output signals of the gas analyzers, mass flowmeter and load cell are also visualized on a multi-pen chart recorder (Kipp&Zonen BD101, Delft, the Netherlands). Energy expenditure measurements are performed in a noise- and temperature controlled room (23-26 °C). Room temperature is measured by a ceramic-platina electrode (Heraeus PT100, Wijk bij Duurstede, the Netherlands), calibrated to 0.02 °C) and also printed at 1, 2 or 5 minute(s) intervals.
A first ventilated hood system was built in 1985, a second system was constructed in 1986 and in 1988 two more systems were built.

Calculation of energy expenditure and fuel utilization

The rate of oxygen consumption and carbondioxide production (at STPD) are calculated using equations published by Jéquier et al. (59) .

$$(1)\ \dot{V}O_2 = \frac{1}{1 - F_{in}O_2} * [\ \dot{V}_{out} * (\triangle FO_2 - \triangle FCO_2 * F_{in}O_2)].$$

$$(2)\ \dot{V}CO_2 = \dot{V}_{out} * FCO_2.$$

where:

$F_{in}O_2$ = fraction of oxygen at the inlet.
$\dot{V}_{out}$ = rate of air flow at outlet (l/min at STPD).
$\triangle FO_2 = F_{in}O_2 - F_{out}O_2$.
$\triangle FCO_2 = F_{out}CO_2 - F_{in}Co2$.
$F_{out}CO_2$ = fraction of CO_2 at the outlet.
$F_{in}Co2$ = fraction of CO_2 at the inlet.

When the respiratory quotient is different from 1.0, the inspired volume is not equal to the expired volume and therefore the inflow of air ($\dot{V}_{in}$) is different from the measured outflow ($\dot{V}_{out}$). Measurements of gas concentrations at the inlet are thus made in a different volume of gas when compared to measurements of O_2 and CO_2 at the outlet. The Haldane correction (59) is used to adjust measurements of inlet gas concentrations for a difference between the inlet and outlet gas volumes. The Haldane correction of inlet gas concentrations is particularly important for O_2, since O_2 has a relatively large concentration in the inspired air.

The rate of energy expenditure or metabolic rate is calculated using the formula (59):

(3) $MR = 4.184*[((4.686+1.096*(RQ_{np}-0.707))*\dot{V}O_{2np}+4.60*\dot{V}O_{2p})]$.

where:

MR = metabolic rate, ie, rate of energy expenditure, in kJ/min.
RQ_{np} = the nonprotein respiratory quotient, ie, $\dot{V}CO_{2np}/\dot{V}O_{2np}$.
$\dot{V}CO_{2np}$ = the volume of carbondioxide produced in nonprotein, ie, carbohydrate and fat, oxidation = $\dot{V}CO_2 - \dot{V}CO_{2p}$, in l/min (STPD).
$\dot{V}O_{2np}$ = the volume of oxygen used for nonprotein oxidation = $\dot{V}O_2 - \dot{V}O_{2p}$, in l/min (STPD).
$\dot{V}CO_{2p}$ = the volume of carbondioxide produced in protein oxidation = N * 6.25 * 0.7739, in l/min (STPD).
$\dot{V}O_{2p}$ = the volume of oxygen used in protein oxidation = N * 6.25 * 0.9936, in l/min (STPD).
N = total urinary nitrogen excretion, in g/min.
P = protein = 6.25 * N, in g/min.

At an RQ_{np} of 0.707, in addition to protein, fat is oxydized in the body and the energetic equivalent of 1 l of oxygen used for oxidation is equal to that of fat, ie, 19.61 kJ. At an RQ_{np} of 1.00, in addition to protein, carbohydrates are oxydized and the energetic equivalent of 1 l of oxygen used for oxidation is equal to that obtained at glucose oxidation, ie, 20.95 kJ. During relatively short energy expenditure measurements, for example, for assessing resting metabolic rate, total urinary nitrogen excretion can not be assessed. Under these circumstances metabolic rate is calculated with the following formula:

(4) $MR = 4.184*[(4.686+1.096*(RQ-0.707))*\dot{V}O_2]$

where:

RQ = "on line" measured respiratory quotient, ie, respiratory exchange ratio, = $\dot{V}CO_2/\dot{V}O_2$.
$\dot{V}O_2$ = "on line" measured oxygen consumption, in l/min (STPD).
$\dot{V}CO_2$ = "on line" measured carbondioxide production, in l/min (STPD).

We have established that with this formula energy expenditure is determined with a very small error (<1 %) compared to metabolic rate determined with formula no 3.

In vivo net fuel utilization rates are calculated using the Lusk's constants for fat, carbohydrate and protein oxidation (60):
6.25 g of protein are oxidized in the body to produce 1 g of urea nitrogen, requiring 966.3 ml of O_2 and producing 773.9 g of CO_2. The RQ for lipid oxidation is 0.707 and for carbohydrates 1.00. Oxygen consumed is 2019.3 ml/g of fat, 828.8 ml/g of glycogen and 745.8 g/ml of glucose. The constants for glycogen were used to calculate carbohydrate utilization rate in the fasting state.
Thus, the following metabolic mixture equations are derived:

in the fasting state:

(5) $\dot{V}O_2 = 0.8288*Gly + 2.019*F + 6.25*N*0.9663,$

(6) $\dot{V}CO_2 = 0.8288*Gly + 1.4276*F + 6.25*N*0.7739,$

in the postprandial state:

(7) $\dot{V}O_2 = 0.7456*Glu + 2.019*F + 6.25*N*0.9663,$

(8) $\dot{V}CO_2 = 0.7456*Glu + 1.4276*F + 6.25*N*0.7739,$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are expressed in l/min and Gly,Glu, F and N in g/min.

Gly = amount of glycogen oxidized
Glu = amount of glucose oxidized
F = amount of fat oxidized
N = amount of total- or urea-nitrogen excreted.

Since N and $\dot{V}O_2$ and $\dot{V}CO_2$ are measured, this system with metabolic mixtures equations can be solved and the amounts of fat, glucose, glycogen or protein that are oxydized, can be calculated using the following formula's:

carbohydrates:

in the fasting state:

(9) $Gly = 4.118*\dot{V}CO_2 - 2.911*\dot{V}O_2 - 2.325*N.$

in the postprandial state:

(10) $Glu = 4.5768*\dot{V}CO_2 - 4.5768*\dot{V}O_2 - 2.595*N.$

fat:

(11) $F = 1.69*\dot{V}O_2 - 1.69*\dot{V}CO_2 - 2.032*N.$

protein:
(12) $P = 6.25*N.$

A negative fat oxidation rate occurs when the RQ_{np} exceeds 1.00. This is indicative of net lipogenesis from glucose, ie, lipogenesis in excess of concomitant fat oxidation. The rate of net newly synthesized fat is equal to the negative fat oxidation rate. It is taken that 2.825 g of glucose are used to synthesize 1 g of a representative fat, ie, of palmitoyl-stearoyl-oleoyl-glycerol (61). The amount of glucose stored (non-oxidative storage) as lipid is measured in indirect calorimetry as glucose oxidation. If net lipogenesis occurs, observed glucose oxidation rates are corrected for the amount of glucose converted into lipid.

SUBJECTS

In total 103 subjects, 49 non-obese men, 22 non-obese women and 32 obese women, participated in the studies presented here. All studies were conducted between january 1986 and august 1988. In 91 of these subjects, 37 men and 54 women, repeated measurements of RMR and DIT were obtained. For 12 men repeated measurements of RMR only were available. Fifty-four of the subjects participated in studies carried out specifically for investigating methodological aspects of RMR and DIT measurements; in the other 49 persons, investigating aspects of RMR and DIT methodology was not the major objective

of study. Subject characteristics are shown in Table 1 for the men and in Table 2 for women. In addition to studies in these groups, a group of 6 men and 5 women participated in a study to assess diurnal variation in RMR. Subjects were recruited for participation by advertisement in regional newspapers, the University weekly periodical, by poster or through informal contacts. After applying for participation, subjects received a brochure with detailed information on the nature and purpose of the experiment(s). In addition, subjects received a questionnaire for obtaining information on past and present health status, nutritional habits, smoking and (alcoholic beverages) drinking behaviour, habitual activity pattern and sporting activities. Subjects eligible for participation were invited for a first visit to the laboratory to familiarize them with the ventilated hood system. After receiving their informed consent subjects were allowed to participate in the studies. The protocol of each study was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

Table 1. Characteristics of men participating in reproducibility studies of RMR, DIT and fuel utilization rates.

		Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Total	< Range >
Sample size	(n)	12	5	4	10	6	12	8.2±1.5	4-12
RMR replicates	(k)	4	4	4	6	6	4	4.7±0.4	4-6
DIT replicates	(k)	2	2	4	4	6	-	3.6±0.7 *	2-6
Age	(yr)	27.3±1.5	22.8±0.9	24.5±1.7	22.2±0.5	22.3±0.6	24.8±0.6	24.3±0.5	20-41
Weight	(kg)	78.3±3.0	71.6±3.9	69.2±4.0	75.5±2.2	71.2±1.2	70.2±1.2	73.5±1.1	58.4-101.8
Body fat	(%)	15.9±1.2	10.4±1.4	15.4±2.2	15.1±1.2	15.0±1.2	15.0±1.4	14.8±0.6	5.5-22.3
Fat-free mass	(kg)	65.9±2.9	64.1±3.3	58.4±3.7	64.0±1.7	60.6±1.9	59.5±0.7	62.5±1.0	3.4-19.2
Fat mass	(kg)	12.4±1.0	7.5±1.1	10.7±1.7	11.5±1.0	10.7±1.1	10.7±0.5	10.9±0.5	48.8-91.4

Values are given as mean ± sem
* Study 6 not included in calculation

Entry criteria could vary among the different studies, for example, with respect to body fat content. In general, however, a subject was eligible for participation when he or she was apparently healthy (no evidence of past or present thyroid disorders and diabetes mellitus), did not use drugs known to affect energy metabolism, had no proteinuria or glycosuria, was eating a normal balanced diet, smoked less than 10 cigarettes, consumed no more than 5 alcoholic drinks/d, did not exercise more than 7 hours/week and had had a

stable body weight (within 2.5 kg of body weight at entry) for a period of at least 6 months prior to the beginning of the study. Women had to be in the premenopausal state. Obesity was defined as a body fat content, assessed with the underwater weighing technique, exceeding 25 percent in men, or exceeding 35 percent in women. In the obese women fasting blood glucose levels were all within the normal range.

Table 2. Characteristics of women participating in reproducibility studies of RMR, DIT and substrate utilization rates.

		Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Total	< Range >
Sample size (n)	6	5	5	6	17	15	9.0±2.2	5–17	
RMR replicates	(k)	4	4	12	6	4	2	5.3±1.4	2–12
DIT replicates	(k)	2	4	6	3	4	2	3.5±0.6	2–6
Age	(yr)	21.2±0.8	22.8±0.5	22.8±0.9	21.8±0.5	39.1±1.5	37.9±1.1	31.8±1.2	20–45
Weight	(kg)	61.0±2.0	61.8±2.9	65.9±2.7	61.3±4.3	92.6±4.3	89.3±2.9	79.4±2.6	50.3–125.9
Body fat	(%)	28.8±1.2	27.7±2.3	28.4±1.1	28.1±2.0	47.8±1.3	44.8±0.9	39.0±1.4	19.9–55.9
Fat-free mass	(kg)	43.3±1.1	44.4±1.2	47.2±2.0	43.8±2.2	47.6±1.4	49.0±1.2	46.8±0.7	36.6–60.6
Fat mass	(kg)	17.6±1.2	17.4±2.1	18.8±1.1	17.5±2.3	45.0±3.2	40.3±2.0	32.6±2.1	10.0–70.4

Values are given as mean ± sem

METHODS

Variability and accuracy of the method

The precision of the gas analyzers and the calibration procedure was measured over a period of 2.5 years by 60 ethanol (Merck 983, Darmstadt, West-Germany) combustion tests of on average 2 hours duration (range 1–4 hours). Twenty of these tests were used to check the precision and validity of the entire ventilated hood system. To oxydize 1 g of ethanol 1.460 l of oxygen is needed and 0.972 l of carbondioxide is produced (RQ = 0.666). When a known quantity of ethanol is combusted, the volumes of oxygen consumed and carbondioxide produced, involved in the oxidation process can be calculated. These calculated values can be compared to the observed values. To calculate the variability of the method in a comprehensible form, we extrapolated the ethanol combustions to an ethanol combustion rate needed for the average male sedentary individual with a resting metabolic rate of 7000 kJ/d. This would

correspond to an ethanol combustion of 236 g assuming an energetic equivalent of ethanol oxidation of 29.7 kJ. For the oxidation of 236 g of ethanol 345 l O_2 are needed and 230 l of CO_2 are produced.

Within-person variability in RMR, DIT, RQ and fuel utilization rates

RMR variability was measured in 49 men participating in 6 different studies, DIT, RQ and fuel utilization rate variability was evaluated in 5 different studies in 37 men. In 54 women, 6 studies were used to estimate the within-person variability in variables of energy metabolism. All studies were conducted within a period of 6 weeks, except for study no 3 and 4 in women, which were carried out over a period of 3 months. A brief description of the different studies is given below:

- Men:

Study 1: n=12
These subjects participated in a study to measure the thermic effect of ethanol and the impact of ethanol on DIT. RMR was measured four times under the same conditions and DIT (4 hours) was measured two times under the same conditions. Between repeated measurements was a time interval of at least two days.

Study 2: n=5
These subjects participated in a study to assess the impact of palatability on DIT. The number of replicates and time-interval between replicates of RMR and DIT measurements were similar to those in study 1.

Study 3: n=4
These subjects participated in a study to measure the effect of a two-weeks controlled dietary trial on the within-person variability in RMR, DIT, RQ and fuel utilization rates. RMR and DIT were measured four times under the same conditions with a time-interval of 3-4 days between replicate measurements.

Study 4: n=10
This study was performed to measure the impact of diurnal variation (morning versus afternoon) on RMR and DIT (62). The time-interval between replicate RMR and DIT measurements was at least two days.

Study 5: n=6
This study was specifically designed to estimate the within-person variability in RMR, DIT, RQ and fuel utilization rates. RMR and DIT were measured on three consecutive mornings in two periods, separated by a five weeks period. This study was the first study we performed. The results of this study showed a significant time of measurement effect on RMR, but not on DIT. RMR measured on the morning of the first day of each period was slightly higher than on the mornings of the second and third day, respectively, 3 and 7 percent. This was probably due to a small carry-over effect of the first measurement day on the second day, and of the second day on the third day. On a day that energy exchange is measured food intake and activity pattern are generally quite different from food intake and activity as observed on a 'normal' day. As a result of this study a time-interval between replicates of RMR and DIT of at least two days was chosen.

Study 6: n=12
In this study the impact on RMR and DIT of type of motion picture was assessed. The type of motion picture did not significantly affect the RMR. Of each subject four replicates of RMR were available.

- Women:

Study 1: n=6
This study was designed to test the impact of palatability on DIT. In each women four replicates of the RMR and two of DIT were measured. In four women the effect on RMR and DIT of phase, ie, pre-ovular versus post-ovular, of the menstrual cycle could be assessed. Ovulation was estimated to occur two weeks before the onset of menses. Replicate measurements of RMR and DIT were performed with a time-interval of at least two days between replicates. The women were measured at least three days after the start of the menstruation.

Study 2: n=5
In this study the effect of a two-weeks controlled dietary trial on the within-person variability on RMR, DIT, RQ and fuel utilization rates was assessed. Four replicate measurements of RMR and DIT were available of each women. The time interval between replicate measurements was 3-4 days. Women were measured at least three days after the onset of menstruation. In two woman the impact of the phase of the menstrual cycle on RMR and DIT could be determined. The other women used oral contraceptives.

Study 3: n=5
This study was specifically performed to measure the effect of the stage of the menstrual cycle on RMR and DIT. The women were measured over a period of three cycli. RMR was measured 6 times in the pre-ovular and 6 times in the postovular phase of the cycle. DIT was measured, respectively, 3 times pre-ovularly and 3 times post-ovularly. Gas exchange measurements in the pre-ovular phase were always performed at least three days after the onset of menstruation. The time-interval between replicates in each stage of the cycle was in general three days. Ovulation was assessed with an LH ovulation test (Nouvpharma LH Color, Oss, the Netherlands).

Study 4: n=6
This study was carried out to determine the within-person variability in women using oral contraceptives. RMR and DIT were measured for 6 and 3 times, respectively, over a period of three months. RMR measurements were performed in general with a two-weeks interval between the replicate measurements, DIT was assessed once every month with on average a four weeks time-interval between the successive measurements.
Gas exchange measurements were always performed at least three days after the onset of menstrual bleeding.

Study 5: n=17
In this study only obese women participated. The purpose of the study was to measure the within-person variability of RMR, DIT, RQ and fuel utilization rates in obese women. Of each women four replicate RMR and DIT measurements were available. The time-interval between the replicate measurements was at least two days. Gas exchange measurements were performed at least three days after the start of the menstruation. In 12 women 2 RMR and DIT measurements were obtained in the pre-ovular and in the post-ovular phase of the cycle.

Study 6: n=15
Similar to study 5, in study 6 only obese woman participated. The purpose of this study was to assess the impact of body fat distribution and weight loss on RMR and DIT. In 15 of these women two initial RMR and DIT measurements were obtained. Measurements were performed at least three days after the start of the menstruation, but no attempt was made to measure the women in a specific phase of the cycle. Part of this study has been published (63).

Impact of control of the antecedent diet on the within-person variability in RMR, DIT, RQ's and fuel utilization rates

Nine subjects, 4 men and 5 women, ie, study 3 in men (Table 1) and study 2 in women (Table 2), participated in a two-weeks controlled dietary trial. Subjects received their total daily food intake, adjusted to individual energy needs, from the laboratory. They were instructed to eat all the food we provided to them and not to eat other food items during the course of the study and to keep to the same activity pattern. Energy needs were assessed using the mean of two RMR measurements, performed on the same day, ie, in the morning and in the afternoon. This value was extrapolated to 24 hours. Energy needs were calculated assuming that the RMR contributed, respectively, 70

percent to total daily energy output in sedentary subjects with on average 1-2 hours/week sporting activities and 67 percent in subjects having an average of 3-5 hours/week sporting activities. Each day one supplementary consumption of an alcoholic beverage or of orange juice was permitted. Food items were provided in a 4-days rotating menu with minor differences in energy content (coefficient of variation (CV) < 5 percent)) and nutrient composition (CV-protein,fat < 10 percent, CV-carbohydrates < 15 percent) between the four menus. Energy intake and nutrient intake were determined from chemical analysis of three duplicate portions of a total 4-days menu for a hypothetical individual with an estimated energy intake of 8-8.5 MJ/d, 8.5-9 MJ/d or 9-9.5 MJ/d. Protein (64), fat (65) and carbohydrates (66) were determined after assessing moisture and ash content (67). Mean energy intake was 9.3 $\pm$ 0.5 MJ/d (range: 7.3 - 12.4 MJ/d), with on average 15 percent of the energy derived from protein, 35 percent from fat and 50 percent from carbohydrates.

Constancy of the RMR

It is sometimes advocated to use a non-caloric sham feeding treatment in studies on DIT as a control on the constancy of the baseline, ie, the RMR (68). The constancy of the baseline is disputed, since the RMR would show

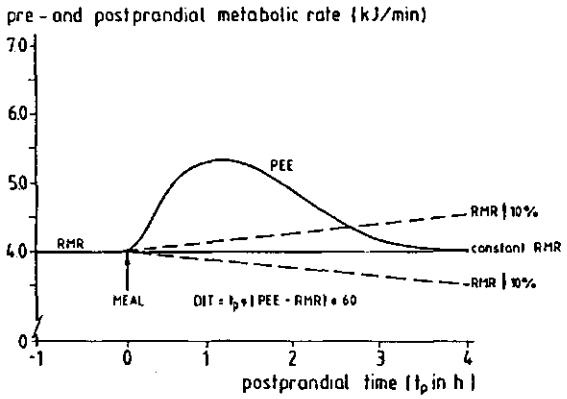

Figure 2. Postprandial energy expenditure (PEE) with an extrapolated changing or constant resting metabolic rate (RMR).

considerable diurnal variation (68). Various investigators report the use of

such non-caloric sham feeding tests (34-36,38). Others, however, do not report the use of such a control treatment, assuming no significant diurnal variation in RMR (15,17,20). When the RMR shows an increasing or decreasing trend during the day, this may seriously affect the calculation of the DIT response. This is illustrated in Figure 2.

With a constant RMR the total thermic effect would be:

240 (duration of postprandial time (Tp))* [PEE - RMR], eg, 240*[4.65 - 4.10] = 156 kJ, assuming an average postprandial energy expenditure (PEE) of 4.65 kJ/min and an RMR of 4.10 kJ/min.

When the RMR would increase 10 percent, DIT would be reduced by 38 percent to 96 KJ. On the other hand when the RMR would decrease 10 percent, DIT would increase 38 percent to 216 kJ. These are substantial effects and it is important to check the constancy of the baseline, ie, the suitability of extrapolating the RMR over the postprandial period for calculating the thermic response.

We assessed the constancy of the RMR in three different ways. In a first study in men (Table 1, study 4), the difference was determined between morning and afternoon RMR and DIT measurements, assessed on different days. We have reported previously on the results of this study (62). In a second study, we measured in 20 subjects, 11 men and 9 women, the difference between two 1-hour RMR measurements, performed on the same day. The first RMR was measured in the morning between 7.30 and 9.00 hours after an overnight fast and the second one was performed in the afternoon between 13.30 and 15.00 hours with the subjects in a 4.5 hours postabsorptive state. For this study, subjects received a small (2 MJ) standardized breakfast. Until the second RMR measurement subjects were not allowed to engage in heavy prolonged exercise, sleeping or to drink coffee or tea. Mineral water and sugar-free non-alcoholic drinks were allowed ad libitum. In a third study, we measured RMR in 12 subjects, all men, for a period of 2.5 hours during a morning.

Impact of energy content of a test meal on DIT and duration of measurement on calculation of DIT.

In 34 women DIT was measured for on average 3.9 hours (3 - 4 hours) to a meal of on average 1884 kJ (varying on average between 1810 and 2000 kJ). In 20 women, we measured DIT for on average 3.1 hours (3 - 3.5 hours) to a meal of on average 1321 kJ (varying on average between 1315 and 1340 kJ). In 12 men DIT was measured repeatedly for a period of 4 hours to a meal with an energy

content of on average 2550 kJ. In 21 men DIT was assessed for on average 3.9 hours (3 - 4 hours) to a meal of on average 1952 kJ (varying on average between 1900 and 2000 kJ) and in 4 men the thermic effect of food was assessed for 3.5 hours exactly, to a meal of on average 1340 kJ. The average hourly increase in metabolic rate after ingestion of these different test meals was compared among the various groups, subdivided according to sex. In addition, the distribution (in percent of total response) of the entire DIT response over the first, second, third, and, if possible, over the fourth hour of the postprandial period was calculated and compared among the various groups. The completeness of the DIT response was evaluated by calculating the difference (expressed in a percentage of the RMR) between the average rate of energy expenditure over the last hour of the postprandial period and the RMR.

Gas exchange measurements

All gas exchange measurements were performed with a ventilated hood system, as described in detail before.

Resting metabolic rate

Resting metabolic rate (RMR) was generally measured in the morning between 7.30 and 9.00 hours after an overnight fast. In some studies, ie, in studies 1, 3 and 4 in men and in study 2 in women, RMR was assessed in the afternoon between 12.30 and 14.00 hours or between 13.30 and 15.00 hours, at least 4.5 hours after a small ($\leq$ 2 MJ) standardized breakfast. Subjects were always instructed not to perform heavy physical activity the evening before RMR measurements. For each gas exchange measurement subjects were brought by car to the laboratory. After voiding they lay in a supine (semi-recumbent) position, but awake and motionless under the ventilated hood on a hospital bed for 30 minutes before the actual start of the RMR measurement. RMR was then continuously measured for one hour. Metabolic rate, oxygen consumption, carbondioxide production and respiratory quotient readings were integrated by the computer over periods of two or five minutes. These readings were averaged over a period of 1 hour to obtain the RMR. Occasionally subjects hyper- or hypoventilated for some period during the RMR measurements. These readings were not included in the calculation of the RMR. Metabolic rate was also corrected for the impact of spontaneous physical activity, as assessed by load cells, on oxygen consumption and carbondioxide production rates. In addition,

values were adjusted for any drift in the span reading of the oxygen analyzer, for example, due to changes in barometric pressure during the course of a one hour measurement session. Measurements were performed in a noise and temperature controlled room. During the RMR measurements subjects were kept awake by showing them motion pictures.

Diet-induced thermogenesis

Diet-induced thermogenesis (DIT) was assessed after an initial RMR measurement, which was used as a baseline in the calculation of the DIT response. The meals used to evoke a thermic response were, in general, liquid mixed meals, containing yoghurt, sucrose, orange juice, a protein supplement and vegetable oil. Meals varied in energy content among the various studies from 1300 to 2600 kJ. Nutrient composition was very similar among the various meals. Table 3 shows average energy content and nutrient composition of the various test meals. In one study, ie, study 6 in women, a testmeal composed of normal solid foods, ie, bread, margarine, apple syrup, cheese and orange juice, was used. No significant difference in thermic effect between this meal and a liquid test meal of similar energy content was observed.

Table 3. Energy content and nutrient composition of meals in reproducibility studies of DIT.

	MEN							WOMEN						
	Energy	Protein		Fat		CHO		Energy	Protein		Fat		CHO	
	kJ	g	%	g	%	g	%	kJ	g	%	g	%	g	%
Study 1	2550	19.8	13.0	18.6	27.0	90.8	60.0	2000	14.4	12.0	17.6	33.1	67.5	54.9
2	2000	14.4	12.0	17.6	33.1	67.5	54.9	1340	10.4	13.0	12.0	33.6	43.9	53.4
3	1340	10.4	13.0	12.0	33.6	43.9	53.4	1950	13.0	11.1	18.5	35.7	63.6	53.2
4	1900	11.6	10.2	16.7	33.1	66.1	56.7	1920	12.8	11.0	18.1	35.4	62.7	53.4
5	2000	14.4	12.0	18.6	35.0	65.0	53.0	1810	12.2	11.3	16.4	34.1	60.7	54.6
6	-							1315	9.8	12.4	11.6	33.2	43.9	54.4
Mean ±	1960	14.1	12.0	16.7	32.4	66.7	55.6	1723	12.1	11.8	15.7	34.2	57.1	54.0
sem	194	1.6	0.5	1.2	1.4	7.4	1.3	1218	0.7	0.3	1.3	0.5	4.3	0.3

Liquid test meals have various advantages over solid meals. Liquid test meals can be prepared in bulk, which will minimize differences in composition among test meals. Secondly, liquid meals can be ingested with minimal disturbance of

the energy exchange measurements, for example, by passing a straw through an opening in the ventilated hood and asking the subjects to drink the meal. To ingest a solid meal, it is necessary to remove the ventilated hood from the subjects head, which will cause a relatively greater disturbance of the energy exchange measurements. Thirdly, the composition of liquid meals can be manipulated more easily than the composition of a solid meal.
Subjects ingested the liquid meals always within 5 minutes, the solid meal was eaten in a period of 10-15 minutes. After ingestion of the test meal the rate of energy expenditure was continuously assessed for either 180, 210 or 240 minutes.
Once every hour the span of the oxygen analyzer was checked against fresh dried filtered atmospheric air during five minutes. During this period subjects were allowed to move briefly. Subjects were asked to remain awake and motionless during the remaining time of the measurements. During the measurements subjects watched motion pictures. Occasionally, subjects moved a leg, arm or another part of their body. When necessary, metabolic rate readings were adjusted for this type of disturbance.
Oxygen consumption, carbondioxide production, respiratory quotient and metabolic rate were integrated over periods of five minutes. These periods were averaged to obtain average hourly postprandial energy expenditure rates (PEE). The total DIT was defined as the integrated (over the total postprandial period (Tp)) difference between the preprandial (RMR) baseline energy expenditure rate and the average postprandial energy expenditure rate, ie, DIT= Tp * (PEE-RMR). Occasionally energy expenditure at the end of the postprandial period was lower when compared to the RMR. For these subjects RMR was redefined as the mean of the actual RMR measurement and the lower postprandial rate of energy expenditure. After the assessment of the thermic effect of food, subjects voided and urine was collected for determining the total amount of total urinary nitrogen. Total nitrogen excretion was assessed by the Kjeldahl method.
The energy content and macronutrient composition of the test meals were chemically measured in a random 5 percent sample of each batch of test meals. Protein (64), fat (65) and carbohydrates and lactic acid (66) were determined after assessing moisture and ash content (66).
Generally, subjects were familiarized with the equipment for assessing gas exchange by measuring resting energy expenditure on a seperate day for between 30 and 45 minutes before the start of the actual experiment.

Body composition

Body weight was measured to the nearest 0.05 kg (Berkel ED-60T, Rotterdam, the Netherlands) in subjects wearing underwear or swimming clothes only, after an overnight fast, or at least 4.5 hours after the last meal, and after voiding. The weight of the fat and the fat-free body mass was determined by hydrodensitometry. Subjects were weighed underwater with simultanuous assessment of the residual lung volume by a helium dilution technique. Total body density was calculated and body fat percentage, fat-free body mass and body fat mass were estimated using Siri's formula. Fat-free body mass was used as an estimate of the metabolically active mass of the body (69).

Statistics

Variability of the method and within-person variability

Reproducibility trials were analyzed using the repeated measures model as described by Winer (70). Time of measurement was treated as the within-subject factor. The within-subject factor was treated in the analysis of variance as a fixed effect factor, a factor for cases (subjects) was used as the only random effect factor. The subject factor was crossed with the within factor, ie, each case was observed at all combinations of the within factor. The mean square of the error term of the within-subject factor was taken as the best unbiased estimator of the within-subject variance of the variable under study. In study 5 in men a significant time of measurement, ie, sequence, effect on RMR was observed. The estimator of the within-subject variance in RMR was corrected, according to Winer (70) for this effect. Assumptions required for the validity of F tests in the fixed-effects analysis of variance model are that the observations are mutually independent, normally distributed, and have equal variances. In the repeated-measures model observations obtained in the same subjects are generally correlated. As a consequence F test were made in a way that allows some relaxation of the assumptions of complete independency, as described by Winer (70) and in Dixon (71).
For each subject we also calculated the coefficient of variation (CV) of repeated RMR and DIT measurements.
Reproducibility of body composition measurements has been reported before (72).

Between-person variability

For each person RMR, DIT, RQ and fuel utilization rates were averaged over the repeated measures. Analyses of the between-person variance of RMR, DIT, RQ and fuel utilization rates were based on these average values. Since part of the total between-person variance in RMR, DIT, RQ and fuel utilization rates could be related to differences among individuals in body weight, body composition, age (RMR) or macronutrient and energy intake (RQ, DIT, fuel utilization rates), multiple regression analyses were used to estimate the variance among individuals in these variables accounted for differences in body weight, body composition, age or macronutrient and energy intake. The variance not accounted for by the linear effects of the various predictor variables (covariates) equals the residual mean square of the error term of the analysis of variance of the regression models. This residual variance is referred to as the 'adjusted' (for the linear effects of covariates) between-person variance. RMR, fasting RQ and fuel utiliation rates were adjusted for fat-free mass, fat mass and age. DIT and postprandial RQ and fuel utilization rates were adjusted for the same variables including the energy or macronutrient intake with the test meal. Normal probability plots of the residuals were used to evaluate differences from the normality assumptions of the various regression models. If no deviation from normality occurred the adjusted between-person variance was assumed to be distributed as a Chi-square distribution.

- Between-group differences in variables of energy metabolism were tested using normal analysis of variance procedures. In addition, analysis of variance statistics not assuming equality of group variances in each group, ie, the Welch and Brown-Forsythe statistic (70,71), were used to evaluate the significance of group differences.

- Differences in variables between two treatments, ie, pre-ovular versus post-ovular and morning versus afternoon energy expenditure rates, were evaluated using the paired (within-person) comparison t-test with a two-sided rejection region and a confidence level of 95 percent.

- Correlation coefficients were computed as Pearson's product-moment correlation coefficients. Adjusting for linear effects of a variable on a correlation was performed by the partial correlation procedure.

- All descriptive statistics are computed as mean ± sem.

RESULTS

Accuracy of the method

The respiratory quotient of 60 ethanol combustions test was found to be 0.666 ± 0.001 (range: 0.635 - 0.695) with a coefficient of variation of 1.5 percent. In 20 whole system checks the observed $\dot{V}O_2$ and $\dot{V}CO_2$ were 340 ± 10 l/d and 227 ± 8 l/d, respectively, with a RQ of 0.668. This would result in a calculated energy expenditure of 6929 ± 192 kJ/d, with a coefficient of variation of the method of 2.8 percent. Oxygen was underestimated with 1.5 percent and carbondioxide with 1.3 percent.

Variability of RMR, DIT, RQ and fuel utilization rates in persons

Table 4 shows the within-person variability of RMR and DIT in men. The within-person variances were, respectively, in RMR 0.095 ± 0.012 $(kJ/min)^2$, in DIT 2172 ± 526 $(kJ)^2$ or 5.78 ± 1.28 $(percent)^2$ of the energy intake. The

Table 4. Results of reproducibility studies of RMR and DIT in men.

		Study 1 (n = 12)	Study 2 (n = 5)	Study 3 (n = 4)	Study 4 (n = 10)	Study 5 (n = 6)	Study 6 (n = 12)	Total (n = 49)	< Range >
RMR	(kJ/min)	5.34±0.27	5.46±0.25	5.24±0.29	4.76±0.14	4.92±0.16	4.69±0.10	5.02±0.09	4.16-7.29
DIT	(kJ)	185±20	188±11	123±11	134±13	178±15	-	164±9*	76-329
DIT-% ME	(%)	7.17±0.76	9.32±0.53	9.08±0.83	7.13±0.71	8.91±0.76	-	7.94±0.37*	4.02-12.76
Within-person variance:									
RMR	$(kJ/min)^2$	0.112	0.117	0.108	0.115	0.044	0.072	0.095±0.012	0.044-0.115
DIT	$(kJ)^2$	3146	1311	1166	3722	1513	-	2172±526*	1166-3722
DIT-% ME	$(\%)^2$	4.90	3.28	6.58	10.38	3.77	-	5.78±1.28*	3.28-10.38
Within-person CV's:									
RMR		6.0±1.5	5.4±2.1	5.9±1.2	6.9±0.9	5.2±0.2	5.4±0.5	6.0±0.6	0.1-16.5
DIT		27.7±6.7	14.1±4.5	22.6±3.2	39.4±5.4	22.0±5.4	-	27.5±3.0*	0.9-68.7
DIT-% ME		27.9±6.7	14.2±4.7	22.8±3.1	39.4±5.4	21.7±3.3	-	27.6±3.0*	1.0-69.1

Values are given as mean ± sem

* Study 6 not included in calculation

RMR = resting metabolic rate; DIT = diet-induced thermogenesis; %-ME = percentage of energy content of test meal; CV = coefficient of variation

calculated coefficients of within-person variation were, respectively, on average 6.0 ± 0.6 percent for the RMR and 27.5 ± 3.0 percent for the DIT. The within-subject variability in RMR was, expressed as the root mean square of the pooled within subject variances (SD_w^2), 0.308 kJ/min. If it is assumed that the intra-individual variability in RMR of a subject is normally distributed and uncorrelated with the random measurement error (SD_m^2), then the contribution of the subject's variance (SD_s^2) to the total observed SD_w^2 is given by:

$$SD_s = \sqrt{(SD_w^2 - SD_m^2)}.$$

This results for men in an SD_s of 0.277 kJ/min corresponding to a method-free coefficient of within-subject variation of 5.5 percent.

The correlation between the calculated coefficients of variation for the RMR and for the DIT was not significant (r = 0.01). The average intercorrelations in men among the repeated RMR and DIT measurements were 0.72 and 0.22, respectively.

Table 5 shows the within-person variability of RMR and DIT in women. The within-person variances were, respectively, in RMR 0.070 ± 0.009 $(kJ/min)^2$, and in DIT 1247 ± 143 $(kJ)^2$ or 4.66 ± 0.70 $(percent)^2$ of energy intake. The calculated coefficients for the within-person variation were 6.0 ± 0.4 for the RMR and 28.5 ± 2.6 percent for the DIT. The SD_w of the RMR in women was 0.265 kJ/min, the method-free SD_s was 0.242 kJ/min corresponding to a method-free coefficient of within-subject variation of 6.1 percent.

Similar to men, in women the calculated coefficients of variation for the RMR showed a non-significant correlation with those of the DIT (r=0.06). The average intercorrelations in women among the replicate measurements of the RMR and DIT were, respectively, 0.80 and 0.41.

Except for study 5 in men, none of the studies showed a significant time of measurement effect on RMR and DIT.

Table 6 and 7 show within-person variances in fasting and postprandial RQ's and fuel utilization rates in men and in women, respectively. Both fasting and postprandial RQ's and nonprotein RQ's showed relatively low within-person variance. Average CV's for the intra-individual variation in these variables were between 4 and 6 percent. Intra-individual variation in fuel utilization rates was generally higher when compared to variation in RQ's. The lowest CV's were observed for protein oxidation rates, ie, on average 20 percent in men

Table 5. Results of reproducibility studies of RMR and DIT in women.

	Study 1 (n = 6)	Study 2 (n = 5)	Study 3 (n = 5)	Study 4 (n = 6)	Study 5 (n = 17)	Study 6 (n = 15)	Total (n = 54)	< Range >
RMR (kJ/min)	3.88±0.10	3.98±0.14	3.91±0.21	3.59±0.18	4.18±0.16	3.97±0.13	3.98±0.07	3.10–5.70
DIT (kJ)	127±17	66±10	114±17	128±14	128±7	89±8	110±5	25–205
DIT-% ME (%)	6.30±0.84	4.89±0.77	5.78±0.86	6.60±0.7	7.03±0.3	6.69±0.57	6.48±0.26	1.84–11.23
Within-person variance:								
RMR $(kJ/min)^2$	0.088	0.081	0.058	0.051	0.096	0.043	0.070±0.009	0.043–0.096
DIT $(kj)^2$	868	1008	1627	1089	1736	1155	1247±143	868–1736
DIT-% ME $(\%)^2$	2.72	5.80	4.24	2.68	5.82	6.69	4.66±0.70	2.72–6.69
Within-person CV's:								
RMR	6.8±1.5	6.2±1.6	6.2±0.8	5.7±0.8	7.0±0.4	4.8±0.6	6.0±0.4	0.5–13.2
DIT	24.0±13.8	41.2±9.1	32.5±6.5	22.7±3.1	28.9±2.6	35.2±6.4	28.5±2.6	0.1–72.0
DIT-% ME	24.1±13.8	41.4±9.1	32.7±6.5	21.5±3.1	29.3±2.7	35.6±6.4	28.6±2.6	0.1–72.2

Values are given as mean ± sem

RMR = resting metabolic rate; DIT = diet-induced thermogenesis; %-ME = percentage of energy content of test meal; CV = coefficient of variation

Table 6. Results of reproducibility studies of fuel utilization rates in men.

Variables studied		Study 1 (n = 12)	Study 2 (n = 5)	Study 3 (n = 4)	Study 4 (n = 10)	Study 5 (n = 6)	Total (n = 37)	< Range >
– Postabsorptive:								
1.	RQ	0.81±0.01	0.83±0.01	0.80±0.01	0.84±0.01	0.85±0.01	0.83±0.01	0.75–0.87
2.	npRQ	0.81±0.01	0.84±0.02	0.80±0.01	0.85±0.01	0.86±0.01	0.84±0.01	0.73–0.90
3. Fat oxidation	(mg/min)	62±6	61±9	68±5	48±5	44±5	56±3	26–92
4. CHO oxidation	(mg/min)	85±11	111±9	82±6	115±8	120±4	102±5	17–139
5. Protein oxidation	(mg/min)	78±5	57±4	58±12	56±3	57±3	64±3	40–112
– Postprandial:								
6.	RQ	0.88±0.01	0.88±0.01	0.83±0.01	0.88±0.01	0.89±0.01	0.88±0.01	0.79–0.94
7.	npRQ	0.90±0.02	0.90±0.02	0.83±0.01	0.90±0.02	0.92±0.01	0.90±0.01	0.79–0.99
8. Fat oxidation	(mg/min)	39±6	44±9	64±5	37±8	30±3	41±3	4–96
9. CHO oxidation	(mg/min)	198±13	215±9	134±10	178±11	215±11	190±7	112–268
Within-person variance:								
1.	$RQ.10^{-3}$	1.63	2.16	1.07	1.37	0.89	1.42±0.22	0.89–1.63
2.	$npRQ.10^{-3}$	2.84	3.81	1.78	1.51	1.61	2.31±0.44	1.51–3.81
3. Fat oxidation	$(mg/min)^2$	235	403	263	360	200	292±131	200–403
4. CHO oxidation	$(mg/min)^2$	2068	2592	989	1370	791	1562±337	791–2592
5. Protein oxid	$(mg/min)^2$	137	195	322	52	83	158±48	52–322
6.	$RQ.10^{-3}$	3.70	1.33	0.70	1.00	0.94	1.53±0.55	0.70–3.70
7.	$npRQ.10^{-3}$	6.06	1.86	1.66	1.82	1.37	2.55±0.88	1.37–6.06
8. Fat oxidation	$(mg/min)^2$	1127	174	214	241	204	392±184	174–1127
9. CHO oxidation	$(mg/min)^2$	7028	2961	1095	1252	1406	2749±1121	1095–7028

Values are given as mean ± sem

RQ = respiratory quotient; np =non protein; CHO = carbohydrate (glycogen or glucose); oxid = oxidation

and 19 percent in women. Fasting fat oxidation rates showed CV's of, respectively, 31 percent in men and 30 percent in women. Fasting carbohydrate, ie, glycogen, oxidation rates showed higher CV's, ie, of 40 percent in men and 30 percent in women. Within-person CV's for postprandial fat oxidation were 49 percent in men and 50 percent in women, whereas the CV's for postprandial glucose oxidation were 27 and 20 percent, respectively. Average fasting RQ's were 0.83 ± 0.01 with a range of 0.75 to 0.90. Postprandial RQ's were on average 0.88 ± 0.01 with a range of 0.79 - 0.94. In none of the subjects we observed average nonprotein postprandial RQ's exceeding 1.00.

Table 7. Results of reproducibility studies of fuel utilization rates in women.

Variables studied		Study 1 (n = 6)	Study 2 (n = 5)	Study 3 (n = 5)	Study 4 (n = 6)	Study 5 (n = 17)	Study 6 (n = 15)	Total (n = 54)	< Range >
- Postabsorptive:									
1.	RQ	0.83±0.01	0.82±0.01	0.86±0.01	0.87±0.01	0.83±0.01	0.82±0.01	0.83±0.00	0.75-0.90
2.	npRQ	0.84±0.01	0.82±0.01	0.88±0.02	0.89±0.01	0.83±0.0	0.83±0.01	0.84±0.01	0.73-0.93
3. Fat oxid	(mg/min)	43±3	49±3	30±6	25±3	44±3	40±4	40±2	13-76
4. CHO oxid	(mg/min)	83±7	73±3	103±5	103±4	81±4	69±7	81±3	13-123
5. Protein oxid	(mg/min)	38±4	42±2	47±2	42±4	54±2	62±4	51±2	21-88
- Postprandial:									
6.	RQ	0.89±0.01	0.87±0.01	0.88±0.01	0.90±0.01	0.88±0.01	0.88±0.01	0.88±0.00	0.80-0.93
7.	npRQ	0.90±0.01	0.89±0.01	0.91±0.02	0.92±0.01	0.90±0.01	0.90±0.01	0.90±0.01	0.80-0.99
8. Fat oxid	(mg/min)	30±2	32±2	27±7	21±2	30±2	27±4	28±2	2-52
9. CHO oxid	(mg/min)	162±6	140±10	153±7	159±8	160±9	141±9	152±4	71-220
Within-person variance:									
1.	$RQ.10^{-3}$	0.97	0.48	1.33	1.11	1.35	0.38	0.94±0.17	0.38-1.33
2.	$npRQ.10^{-3}$	1.50	0.76	1.33	2.21	2.51	0.71	1.50±0.30	0.71-2.51
3. Fat oxid	$(mg.min)^2$	137	82	193	150	235	57	142±27	57-235
4. CHO oxid	$(mg/min)^2$	640	420	808	329	959	381	590±104	329-808
5. Protein oxid	$(mg/min)^2$	15	102	85	58	118	174	92±22	15-174
6.	$RQ.10^{-3}$	0.99	1.10	1.10	1.71	1.53	1.23	1.28±0.12	0.99-1.71
7.	$npRQ.10^{-3}$	1.52	1.56	1.07	3.08	2.68	2.56	2.08±0.33	1.07-3.08
8. Fat oxid	$(mg/min)^2$	174	181	180	255	260	155	201±18	155-260
9. CHO oxid	$(mg/min)^2$	585	1296	998	406	1184	1126	933±145	406-1296

Values are given as mean ± sem

RQ = respiratory quotient; np = nonprotein; CHO = carbohydrate (glycogen/glucose); oxid = oxidation

Impact of control of the antecedent diet on within-person variability of RMR, DIT, RQ, and fuel utilization rates

The results, presented for men in Tables 4 and 6 (study 3) and for women in Tables 4 and 7 (study 2), indicate no clear impact of control of the antecedent diet on the within-person variability of RMR, DIT, RQ and fuel utilization rates, when comparing the various estimators of within-person variances in variables of energy metabolism among the different studies.

Variability of RMR, DIT, RQ and fuel utilization rates between persons

Figure 3 and Table 8 show variability of the method, within-person variability and between-person variability in RMR and DIT.

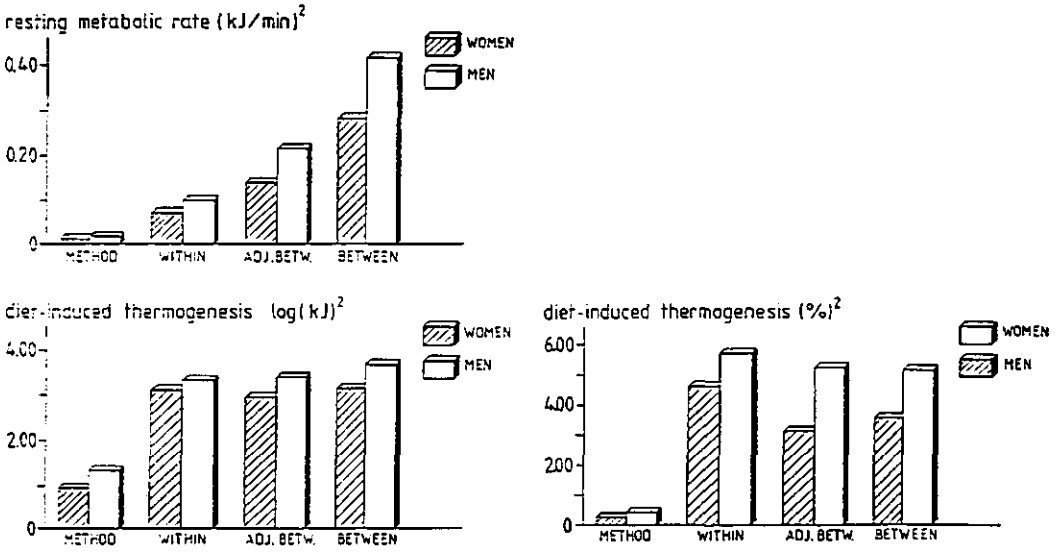

Figure 3. Within- and between-person variances in men and women of resting metabolic rate and diet-induced thermogenesis.

The between-person variability in RMR was significantly higher, even after adjusting for covariates, compared to the within-person variability. The ratio of (adjusted) between-person to within-person variance was 2.2 in men and 2.0

in women. In contrast, no significant difference between (adjusted) inter-individual and intra-individual variation in DIT was found in men, with ratios of between and within-person variability around 1. In women the adjusted beteen-person variance in DIT was even slightly lower than the within-person variation in DIT. The ratio's of (adjusted) between-person and within-person variation in RQ's and fuel utilization rates were all within the range of 0.5 to 1.7. Except for postprandial RQ's and fat oxidation rates, no significant differences between the (adjusted) inter-individual and intra-individual variations were found.

Table 8. Between-person variance of RMR, DIT, RQ and fuel utilization rates in men and women.

		Men		Women	
		Between	Adj. between*	Between	Adj. Between*
RMR	$(kJ/min)^2$	0.415	0.212	0.278	0.142
DIT	$(kJ)^2$	4643	2626	1385	892
DIT-% ME	$(\%)^2$	5.16	5.31	3.65	3.27
Postabsorptive:					
	$RQ \cdot 10^{-3}$	1.02	1.80	0.961	0.80
	$nRQ \cdot 10^{-3}$	1.76	1.00	0.73	1.50
Fat oxidation	$(mg/min)^2$	361	379	208	161
CHO oxidation	$(mg/min)^2$	872	1164	474	405
Protein oxidation	$(mg/min)^2$	243	160	183	86
Postprandial:					
	$RQ \cdot 10^{-3}$	1.23	1.20	0.73	0.70
	$nRQ \cdot 10^{-3}$	2.21	2.20	1.37	1.30
Fat oxidation	$(mg/min)^2$	444	424	134	119
CHO oxidation	$(mg/min)^2$	1716	1541	868	817

* = variance adjusted for fat-free mass, fat mass, age (postabsorptive) and energy or macronutrient intake (postprandial)

RMR = resting metabolic rate; DIT = diet-induced thermogenesis; % ME = percentage of energy content of test meal; RQ = respiratory quotient; np = nonprotein; CHO = carbohydrate (glycogen or glucose)

Constancy of the RMR

The results of the three studies on the constancy of the RMR are presented in Table 9. None of the three studies showed a significant diurnal variation in the RMR. In the first study the average difference between morning RMR and afternoon RMR was 0.14 $\pm$ 0.09 kJ/min, ie, 2.9 $\pm$ 1.9 percent of morning RMR.

In the second study the difference between morning and afternoon RMR was -0.03 ± 0.06 kJ/min, ie, -0.7 ± 1.4 percent of morning RMR. In the third study no significant change in RMR over a 2.5 hours measurement period was found. The difference between the RMR assessed over the first 30 minutes of this period and the RMR measured over the last 30 minutes was 0.09 ± 0.17, ie, 1.9 ± 3.6 percent of the initial RMR value.

Table 9. Results of studies on the constancy of the RMR.

Variables		Study 1 (n = 10)	Study 2 (n = 20)*	Study 3 (n = 12)
Age	yrs	22.2±0.5	23.1±0.5	24.8±0.6
Body weight	kg	75.5±2.2	65.0±2.1	70.2±1.2
Body fat	%	15.1±1.2	19.4±1.5	15.0±1.4
Morning RMR	kJ/min	4.81±0.18	4.58±0.14	-
Afternoon RMR	kJ/min	4.67±0.11	4.61±0.14	-
Morning RMR				
0 - 30 min	kJ/min	-	-	4.67±0.11
30 - 60	kJ/min	-	-	4.78±0.11
60 - 90	kJ/min	-	-	4.71±0.11
90 -120	kJ/min	-	-	4.68±0.10
120 -150	kJ/min	-	-	4.76±0.10

Values are given as mean ± sem

* M/F ratio 11/9, age in men: 32.5 ± 0.9 yrs, weight: 71.4 ± 2.3 kg, body fat content: 14.9 ± 1.2 percent; age in women: 22.6 ± 0.4 yrs, weight: 65.0 ± 2.0 kg, body fat content: 23.8 ± 1.4 percent

RMR = resting metabolic rate

Impact of phase of the menstrual cycle on RMR and DIT

Table 10 shows the results of the impact of the phase (pre-ovular versus post-ovular) of the menstrual cycle on RMR and DIT. No significant difference in RMR and DIT between the two phases of the cycle was found. The average number of replicates in each phase of the cycle was 2.9 for the RMR and 2.0 for the DIT. The within-person coefficients of variation in RMR and DIT of these women were compared with those of the women using oral contraceptives. CV's of RMR were, respectively, 5.7 ± 0.7 percent for the women with a normal menstrual cycle and 7.5 ± 0.7 for the women using oral contraceptives. CV's for DIT were, respectively, 31.5 ± 3.4 for women with normal menstrual cycles and 26.1 ± 4.0 for women using oral contraceptives. A significant difference ($p<0.05$) in CV's for RMR between both groups was found; CV's for DIT did not differ significantly between both groups.

Table 10. RMR and DIT of women in the pre-ovular and post-ovular phase of the menstrual cycle.

Subject no	Age (yrs)	Weight (kg)	FFM (kg)	RMR pre (kJ/min)	RMR post (kj/min)	DIT pre (kJ)	DIT post (kJ)	DIT pre (%)*	DIT post (%)
1.	20	74.6	54.5	3.78	4.00	100	62	5.09	3.18
2.	23	59.3	43.0	3.32	3.41	127	123	6.44	6.22
3.	22	62.1	43.9	3.87	3.76	104	111	5.29	5.62
4.	25	68.7	46.5	3.76	3.84	114	81	5.82	4.12
5.	24	65.0	47.9	4.70	4.65	164	126	8.39	6.41
6.	21	64.3	45.8	4.08	4.30	147	155	7.35	7.75
7.	21	61.1	43.5	3.90	4.21	169	152	8.45	7.60
8.	20	65.5	45.2	3.86	3.82	151	127	7.55	6.35
9.	25	51.8	39.1	3.62	3.83	157	171	7.85	8.55
10.	22	67.1	44.3	4.13	3.67	72	95	5.44	7.12
11.	22	50.0	40.3	3.93	3.97	49	45	3.71	3.35
12.	42	81.2	43.9	3.72	4.30	116	100	6.41	5.52
13.	32	101.7	50.4	3.72	3.67	79	158	4.47	8.73
14.	43	74.2	50.1	3.69	3.47	94	112	5.20	6.27
15.	41	74.2	42.6	3.54	3.25	98	112	5.43	6.29
16.	44	65.5	40.3	3.84	3.43	152	198	8.41	10.95
17.	39	72.2	43.1	3.75	3.81	158	94	8.75	5.26
18.	44	92.7	48.4	5.54	5.87	147	65	8.12	3.60
19.	40	103.4	47.5	5.62	5.54	123	148	6.99	8.18
20.	45	91.2	50.7	4.00	4.13	72	76	3.97	4.36
21.	40	101.3	46.6	3.94	4.03	138	112	7.63	6.21
22.	36	95.9	50.7	3.86	4.04	122	196	6.81	10.91
23.	40	83.6	41.5	3.90	4.15	93	111	5.32	6.37
Mean ± sem	31.7±2.0	75.1±3.3	45.6±0.8	4.00±0.12	4.05±0.13	119±7	119±8	6.47±0.32	6.47±0.44

* Expressed in percentage of energy content of test meal

For cases 1 – 5 number of replicate measurements for each phase of cycle are 6 for RMR and 3 for DIT; for cases 6 – 9 2 replicates for RMR and none for DIT and for cases 10-23 2 replicates for RMR and 2 for DIT

FFM = fat-free mass; RMR = resting metabolic rate; DIT = diet-induced thermogenesis; Pre = pre-ovular; Post= post-ovular

Impact of energy intake and duration of postprandial measurement on the calculation of DIT

Figure 4 shows postprandial energy expenditure rates in three groups of men, who ingested different amounts of energy in the meals used to evoke the thermic response. No significant difference among the three groups in the postprandial rise of energy expenditure during the first two hours of the postprandial period was found. In the third hour of the postprandial period, the postprandial rate of energy expenditure was significantly lower in the group with the low energy intake compared to the groups with energy intakes of

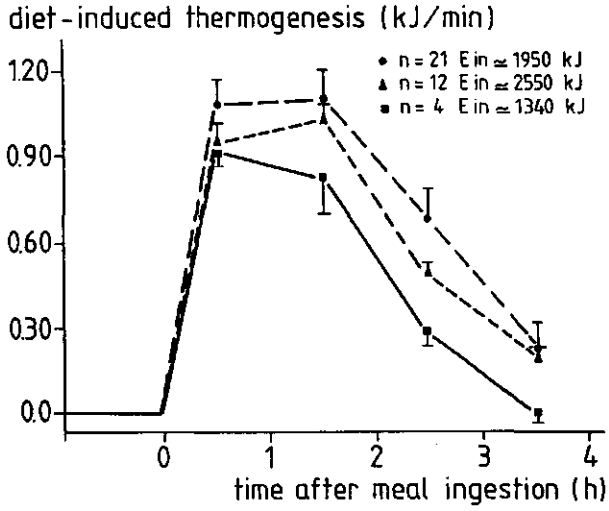

Figure 4. Diet-induced thermogenesis (mean ± sem) in men after ingestion of meals with different energy content.

on average 1952 kJ and 2550 kJ. In subjects with the low energy intake 86 percent of the total observed thermic effect (over 3.5 hours) was observed in the first two hours of the postprandial period, compared to 75 percent in subjects with higher energy intakes. At the end of the postprandial measurement period energy expenditure was elevated 4 percent above the RMR in subjects of the groups with higher energy intakes and 0 percent in subjects with low energy intake. In seven subjects energy expenditure at the end of the postprandial period was lower compared to RMR, with a minimum of 98 percent of the RMR. Total DIT values increased significantly on the meals with higher energy content. The correlation coefficient (r) between total DIT and energy intake in the testmeal was 0.39 ($p=0.05$).

No significant correlation was found in men between DIT and, respectively, body weight ($r = 0.11$), the fat-free mass ($r = 0.07$), fat mass ($r = 0.16$) or age ($r = -0.02$), adjusted for linear effects on the correlations of differences in energy intake in the test meal. No difference in DIT among the three groups was observed in DIT expressed as a percentage of the energy content of the test meal, ie, 8.16 ± 0.46 percent (1952 kJ), 7.17 ± 0.76

percent (2550 kJ) and 9.08 ± 0.83 percent (1340 kJ).
Figure 5 shows increases in postprandial energy expenditure for female subjects receiving either meals of on average 1884 kJ, or of on average 1321 kJ.

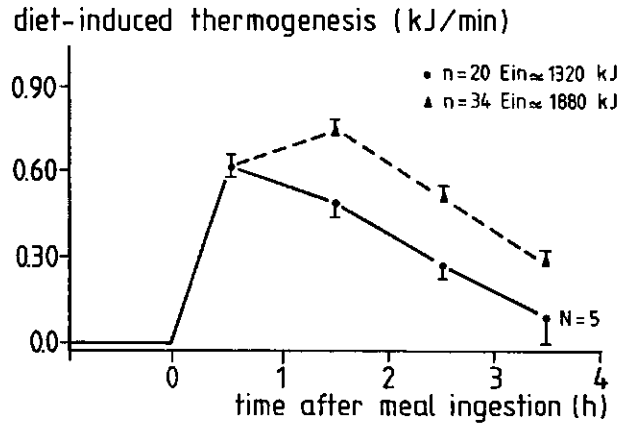

Figure 5. Diet-induced thermogenesis (mean ± sem) in women after ingestion of meals with different energy content.

There was a significant difference in the rise of postprandial energy expenditure during the second and third hour of the postprandial period between both groups. In subjects receiving the low energy meals, on average 83 percent of the total thermic response was measured in the first two hours of the postprandial period. In subjects receiving the higher energy meals, on average 66 percent of the total response was observed in the first 120 minutes. At the end of the postprandial measurement period, energy expenditure was still elevated by 7.2 ± 0.8 percent in the group receiving the higher energy meals and by 6.5 ± 1.3 percent in the group receiving the lower energy meals. In four women lower rates of energy expenditure were observed at the end of the postprandial measurement period when compared to the RMR, with a minimum of 98 percent of the RMR. Total DIT values were significantly higher ($p<0.05$) in women with higher energy intakes in the test meals. The correlation coefficient between DIT and the energy content of the test meal

was 0.53 ($p<0.001$) in women.
No significant correlations were found in these women between DIT and the size of the fat-free mass ($r = 0.02$). DIT was significantly correlated with body weight ($r = 0.28$, $p<0.05$)), body fat mass
($r = 0.33$, $p<0.05$) and age ($r = 0.35$, $p<0.05$), adjusted for linear effects on the correlations of differences in energy content of the test meal. No significant differences were found between both groups in DIT expressed in a percentage of the energy content of the test meal, respectively, 6.64 ± 0.29 percent in the group with high energy intake and 6.24 ± 0.50 in group with low energy intake.

DISCUSSION

This study was performed to evaluate various methodological aspects of the assessment of resting metabolic rate (RMR), diet-induced thermogenesis (DIT) and in vivo fuel utilization rates. It is the first study that reports data on the reproducibility of measurements of DIT in men and women using a ventilated hood system. At the start of the study a ventilated hood system was built in order to be able to measure the rate of energy expenditure continuously for prolonged periods of time with minimal discomfort for the subject. The accuracy of this method in assessing energy expenditure was tested and studies were performed to assess the within-subject variability in RMR and DIT. The impact of the energy intake of the ingested meal, of the duration of the postprandial measurement on DIT, of the phase of the menstrual cycle on RMR and DIT, of control of the antecedent diet on the within-person variability of RMR and DIT and of diurnal variation on RMR were also investigated. All studies, with the exception of two studies in women (Table 2, study no 3 and 4), were carried out in a period of at most 6 weeks duration to prevent seasonal effects on energy expenditure.
The results of this study have important consequences for the study of postprandial human energy metabolism. A finding of great importance is the relatively large within-subject variation in DIT, which could not be contributed to variation in the method and which seemed not subject to reduction when the antecedent diet was controlled.

Accuracy of the method and within-subject variation in RMR, DIT, RQ and fuel utilization

The random and systematic error of assessing energy expenditure with indirect calorimetry using a ventilated hood system was in accordance with the data on the accuracy of the indirect calorimetry method used in respiration chambers (73,74). The random error was 2.7 percent and the systematic error was 1.5 percent. The variability of the method was much smaller when compared to the within-subject variability in the RMR and in DIT. The variability in the method contributed less than 10 percent to the total observed within-person variability in RMR. The total CV for repeated within-person measurements of RMR were also calculated as the average of the individual CV's. Our results agree well with other reports on the within-person variability in RMR, which report average within-subject CV's for the RMR ranging between 2 and 7 percent (75-78). It is not possible to partition the method-free within-subject variation into specific sources. RMR measurements were performed under strictly standardized conditions, but there was some variation in conditions under which the RMR was assessed. At each RMR measurement subjects watched different motion pictures, which may have caused variation in RMR measurements. In addition, there may have been variation among the repeated measurements within a subject in subject's behavior, ie, one day a subject lies calmly, awake, motionless in the same semi-recumbent posture under the hood, breathing regularly, on another day a subject dozes off frequently, is restless, has many spontaneous small movements and changes of posture and is breathing irregularly with alternating periods of hyper-and hypoventilation. Finally, there may be biological within-person variation in RMR from day to day. This variation could be related to variations in feeding, drinking or activity pattern in the days before the RMR measurements. In most of the studies here reported, subjects were measured after an overnight fast. Subjects were eating normal balanced diets, but their diets were not under strict control.

There is considerable within-person variation in energy and nutrient intake from day to day (78). This variation may be a source of additional variation in the RMR, irrespective of the fact that the acute effects of food intake on RMR were minimized by measuring in the postabsorptive state. We tested this hypothesis by measuring the within-subject variation in RMR in subjects who were put on a controlled dietary intake regime for two weeks and whose activity pattern remained as constant as possible throughout the experimental

period. Control of the antecedent diet did not seem to have marked effects on the within-person variability, suggesting that variation in subject's behavior and in measurement circumstances may be greater sources of within-subject variation than the absence of strict control of the antecedent diet.

In contrast to the relatively small within-person variation in RMR, the intra-individual variation in DIT was much larger. In this study DIT reproducibility was found to be of similar magnitude in obese when compared to non-obese women. The within-subject variation seemed not subject to systematic reduction when the antecedent diet was controlled, but it should be noted that for these subjects no estimates were available of the within-person variability of DIT under normal measurement conditions.

Comparison of the within-person variability of DIT measurements as observed in this study with data of other studies is difficult. To the best of our knowledge no other groups have published data on the reproducibility of DIT measurements obtained by a ventilated hood system, have been published. Ravussin et al. (74) report a high within-person variability in DIT calculated from the difference between 24-hours energy expenditure, corrected for work-induced thermogenesis, and the basal metabolic rate extrapolated to a 24 hours basis. The lack of data on the reproducibility of DIT measurements is surprising. Differences in DIT have become a subject of paramount interest for many groups involved in research on, for example, the etiology of obesity (13-40).

The sources of the within-person variation in DIT may be similar to those of the RMR, ie, variation among trials in measurement circumstances (motion pictures), and in subject's behavior. Since DIT is a response of relatively small magnitude, assessed over a prolonged period, these factors may have a relatively greater impact on the reproducibility of DIT measurements compared to their impact on the reproducibility of the RMR measurements. Subjects with a relatively low reproducibility of RMR measurements had not necessarily the highest within-person variation in DIT. This was illustrated by the non-significant correlation between within-person variation in the RMR and in DIT. An additional source of intra-individual variation in DIT may be biological day-to-day variation in the postprandial processing of nutrients. Acheson et al. (79) showed that the state of the body's glycogen stores influenced DIT. The amount of glycogen stored in the body is affected by diet and physical activity (80). Therefore in this study only subjects eating normal mixed meals were allowed to participate. Subjects were also instructed not to perform heavy exercise on the day preceding the DIT assessment to

prevent serious depletion of the body's glycogen stores.
We found that most subjects considered the DIT measurements as tedious measurements, because of their long duration and the requirement to perform the measurement under RMR conditions. Diverting their attention by showing motion pictures was essential to prevent the subjects from falling asleep or becoming restless and bored.

Within-subject variation in RQ and fuel utilization rates

Postabsorptive and postprandial respiratory quotients showed good reproducibility. Within-person CV's for repeated measurements were, in general, below 6 percent. Fuel utilization rates, both postabsorptively as postprandially, showed greater within-subject variances. This indicates that the type of metabolic mixture utilised in the postabsorptive or postprandial phase is relatively constant in an individual. The amount of nutrients oxydized to obtain a given type of metabolic mixture may show much larger within-subject variation. Control of the antecedent diet did not seem to reduce the within-subject variation in RQ's and fuel utilization rates.

Between-subject variation in RMR, DIT, RQ and fuel utilization rates

In this study it was found that the between-person variation in RMR, with or without adjusting for the covariates age, fat-free mass and fat mass was significantly greater when compared to the within-subject variation. This finding confirms results obtained in a previous study (74). The reasons why individuals of a given age, fat mass and fat-free mass may differ in RMR are now subject of much research (6). Part of the (adjusted) inter-individual differences in RMR may be related to differences in hormonal, ie, thyroid, status or activity of the sympathetic nervous system (6).
The inter-individual variations in DIT, RQ and fuel utilization rates were, after adjusting for covariates, generally of the same magnitude or smaller in comparison to the intra-individual variation.

Importance of within- and between-person-variation in RMR, DIT, for study design and evaluation

Knowledge of the within-person and between-person variation in variables of energy metabolism is of paramount importance for the design and evaluation of studies assessing changes in RMR or DIT within the same individual during a treatment, or in the comparison of RMR or DIT between independent samples of individuals classified as high or low with respect to, for example, body fat content. In the latter case, average RMR and DIT for individuals classified into these two levels are generally compared to see if they are significantly different. If intra-individual variability is large and the number of measurements for each subject is small, the power of the within-subject or between-group comparisons may be low. This means that the probability of rejecting the null hypothesis of no effect of treatment (paired comparison) or of no difference in variables of energy metabolism (between group comparison) may be small, even if the null hypotheses are false and the true treatment effects or true between-group differences are quite large. A well-designed study aiming at the assessment of treatment-induced within-person changes in a variable under study or at the comparison between interesting variables of independent sample means should, on the one hand, have a great chance, ie, power, of detecting a true effect of treatment or difference and, on the other hand, a small chance, ie, confidence, on falsely detecting an effect or difference. In other words the type 1 error (false positive) and the type 2 error (false negative) should be sufficiently small. The probability of the type 1 error, generally called α, is specified by the investigator. However, the probability that a significant result will be obtained if a real effect or difference exists, depends largely on the sample size. As the total sample size increases, the type 2 error, generally called ß, decreases. This means that if a statistical test fails to reach significance, the power of the test becomes a critical factor in reaching an inference. The failure to achieve statistical significance may be related more to the low power of an experiment than to the actual lack of effect of a treatment or difference between groups. This means that an experiment with inadequate sample size is doomed to failure before it begins. This may serve only to confuse the issue of determining the effect of a treatment or of assessing differences between groups. Therefore each investigator should take steps to ensure that the power of an experiement is sufficient to justify the effort involved. If an experiment has great power, say 0.95, the failure to achieve significance may be properly interpreted as probably indicating a negligible treatment effect or negligible

relevant between-group differences. Many statisticians, but not all, argue that the proper interpretation of a negative result should be based upon a consideration of the power of an experiment (81-83). In appendices 1 and 2 sample size calculations are given at specified α- and β-levels and specified true treatment effects or between-group differences in DIT.
From these calculations it appears that studies with sample sizes smaller than 10 persons and performing one measurement per subject may have power levels below 80 percent of assessing true, relatively large, ie, 25-50 percent, treatment effects or between-group differences in DIT.
The question rises whether these findings have consequences for the interpretation of studies on differences in DIT between the obese- and non-obese. A literature survey shows that eight (30,31,34,36,37,39,40,42) of the thirteen studies reporting no difference in DIT between obese and non-obese, probably had a power lower than 80 percent of finding a true between-group difference in DIT of 25 percent.

Constancy of the RMR

The results of Table 9 show that RMR did not show systematic diurnal variation from morning untill early afternoon. This indicates that the RMR remains constant during the assessment of postprandial energy expenditure, at least during measurements of energy expenditure over a morning. This implicates that in the assessment of the thermic effect of food no sham feeding control experiment is needed to correct for changes in the basal energy expenditure. Our findings are in accordance with most other studies assessing changes during the day in RMR (34-36), with the exception of some older studies (84,85). It is well known that RMR shows diurnal variation due to the thermic after-effects of exercise and food ingestion or due to sleep (20). This study shows that in the absence of thermogenic stimuli the postabsorptive RMR shows very little change in the morning over the course of a 2.5 hours measurement period or between morning and afternoon assessments, comparing single RMR measurements performed on the same day, or comparing RMR's assessed repeatedly on mornings or afternoons.

Impact of the phase of the menstrual cycle on RMR and DIT

Table 10 shows that the phase of the menstrual cycle did not significantly influence RMR or DIT. RMR was slightly, ie, 1.2 percent, higher during the post-ovular phase of the cycle compared to the pre-ovular phase. On average pre-ovular and post-ovular RMR and DIT were, respectively, assessed for about three and two times in 23 subjects, although the variation in number of replicates was quite large. This means that with this sample size, true differences between the two phases of the cycle of at least 2 percent in the RMR or at least 20 percent in DIT could have been detected with a power of 90 percent and a confidence level of 95 percent (two-sided), assuming a correlation between the two phases of the cycle of 0.80 in RMR and of 0.40 in DIT. Smaller differences in RMR or DIT between the pre- and post-ovular stage of the cycle may be physiologically irrelevant. With respect to the RMR the results of this study support the findings of several authors (86-90), but are in contrast with results of other studies (91-94). The reasons for these differences are not quite clear, but may be related to differences in experimental protocol, techniques used for measuring energy expenditure, the number of subjects studied, the frequence of measurements during the menstrual cycle and the method used to assess ovulation.

In the present study measurements were always performed at least three days after the onset of menses. The rationale behind this protocol demand is the fact that many women have health complaints shortly before or during menstruation. This may affect a subject's behavior during energy expenditure measurements, which may cause unwanted effects on measurements. For example, already in the 1920s it has been reported that basal metabolic rate is lowered during menstruation in comparison with other phases of the menstrual cycle (95).

In this study most of the pre-ovular energy expenditure measurements were made in a period between 4 and 10 days after the onset of menstruation. For most subjects ovulation was assumed to occur 14 days before the start of menstrual flow. In subjects 1 to 5 ovulation was conclusively identified using an ovulation test. Also, in these subjects no significant effect of the stage of the ovular cycle on RMR or DIT was observed.

No studies have reported on the impact of the stage of the menstrual cycle on DIT. Frequently it is assumed that the phase of the menstrual cycle affects the RMR (96). In fact, the number of studies not showing any effect of the stage of the cycle on the RMR are in balance with the number of studies that

do report an effect. In general, the post-ovular rise in RMR is attributed to the increased progesterone secretion in that phase of the cycle (91-94). The thermogenic effects of progesterone are, however, not clear (97). In a recent study, progesterone administration was found to decrease RMR in women instead of increasing RMR (97).

Variability in RMR and DIT was also compared between women using oral contraceptives and women having normal menstrual cycles. Variation in RMR was significantly higher in women using oral contraceptives in comparison to women having normal cycles. This was due to the fact that 5 women using oral contraceptives had particularly high CV's for their RMR, exceeding 10 percent. We expected that the within-person variability of RMR in women using oral contraceptives would be smaller when compared to the variability observed in women having normal menstrual cycles. In women using oral contraceptives hormonal profiles will be relatively more constant over the course of a cycle. As a consequence the biological within-person variation in RMR was expected to be smaller in women using oral contraceptives when compared to women with normal ovular cycles. This hypothesis was not supported by our findings. No systematic difference between both groups in variation in DIT was observed.

Impact of energy intake and duration of postprandial measurement on the calculation of DIT

There is a great variety among the various studies on DIT in the energy content of the meal given and in duration of the postprandial measurement period (13-42). Thus, meals or nutrient solutions are being given varying in energy content between 800 kJ (14) and 8000 (42) kJ, whereas the postprandial measurement period varies between 1.5 (27) and 8 hours (42). It has been pointed out by various authors (20,42) that the duration of the postprandial measurement period should be of sufficient length in order to measure the largest part of the thermic response to food. However, it has not been reported which proportion of the thermic effect of food, using different energy loads, is measured within fixed time intervals of the postingestive period.

In this study, it was found that DIT increases with greater energy intakes, confirming previous findings (42). In men, DIT was positively associated with energy intake, but no significant correlation between DIT and body weight, the size of the fat mass or fat-free mass was found. In women, DIT was also significantly positively correlated to the energy content of the test meals,

but not to the size of the fat-free mass. Low, but significant correlations were found between DIT and body weight, fat mass and age. This indicates that some of the older obese subjects had relatively higher DIT's when compared to the younger lean subjects. The postprandial response patterns indicate that in men and women a DIT response to ingestion of a mixed liquid meal with an energy content below 1500 kJ can be nearly completely assessed within three hours. The DIT response to meals with larger energy content, ie, between 1500 kJ and 2600 kJ in men and between 1500 and 2000 kJ in women, can also be assessed with sufficient accuracy within three hours. The data show that adding a fourth hour to the postprandial period increased the observed thermic effect by 10-15 percent only. We have experienced that after three hours many subjects become restless and bored by the measurement. Reducing the postprandial measurement period to three hours does not have substantial effects on the calculation of the DIT response to meals of an energy content of 2000 kJ, but will prevent some of the behavioural problems occurring when subjects are measured for a more prolonged period of time.
Sometimes the energy content of the meal or nutrient solution is given as a dosis per kg (ideal) weight (13,34,41) or fat-free body mass (20,42). When such a procedure is used, it is implicitly assumed that the thermic response is correlated to (ideal) body weight or to the size of the fat-free mass, and that by adjusting for inter-individual differences in these variables inter-individual comparison of DIT responses will be more valid. However, data to support this view have not been reported. The present study showed a low, and for the most, except in women, part non-significant correlation between DIT and body weight and the size of the fat-free mass. This is in accordance with the findings of a recent report (42) and supports a notion put forward by Garrow, quoted in (96), ie, that " a log of a given size gives of the same amount of heat in a small or large oven". Our findings suggest that comparing DIT responses among individuals to equal caloric loads should be advocated. This supports the view of Sjöström (98).

In conclusion, this study shows that RMR and postabsorptive and postprandial RQ's can be measured with sufficiently high reproducibility in humans with a ventilated hood system. DIT and fuel utilization rates show considerably larger within-person variation, comparable in magnitude to the between-person variation. The within-person variation in DIT seemed not subject to reduction when the antecedent diet was controlled. Postabsorptive RMR did not show substantial variation over a morning, eliminating the need for sham-feeding

control experiments in the assesssment of DIT. The phase of the menstrual cycle did not markedly affect RMR or DIT, suggesting that in measurements of RMR and DIT in women the stage of the cycle is a factor of negligible relevance in the study design. The thermic effect to meals with an energy content between about 1.5-2.5 MJ can be nearly completely assessed within three hours. There is no need to standardize the energy content of the test meal to body weight or to the size of the fat-free mass.

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Appendix 1. Number of subjects, according to sex, required to test a change within subjects of 10, 25 or 50 percent in diet-induced thermogenesis with a significance level of $\alpha = 0.05$ (two-sided) and ß-levels of 0.20 and 0.10, when one or two measurements are performed per subject with and without pre-post test correlation.

			Expected change (%)* in DIT					
			10		25		50	
			ß - level		ß - level		ß - level	
			0.2	0.1	0.2	0.1	0.2	0.1
No of measurements	Sex	Correlation+						
1	M	0	129	172	22	29	8	9
		0.2	103	138	18	24	4	6
	F	0	164	219	28	37	9	11
		0.4	99	132	18	23	6	8
2	M	0	66	87	13	16	5	6
		0.2	48	63	10	12	4	5
	F	0	83	111	15	20	6	7
		0.4	38	49	8	10	4	4

$\alpha = 0.05$, two-sided

Number of subjects calculated according to Lachin (83), using the average within-subject variation, according to sex, found in this study

* Expected change expressed as percentage of mean DIT values (in kJ) observed in this study, in men and women

+ Correlation coefficient as determined from repeated-measures modal analysis of variance according to Winer (70)

DIT = diet-induced thermogenesis.

Appendix 2. Total number of subjects, according to sex, required to test a difference of 10, 25 and 50 percent in diet-induced thermogenesis between two groups of equal size with a significance level $\alpha = 0.05$ (two-sided) and ß-levels of 0.20 and 0.10, when one or two measurements are performed per subject.

		Expected difference (%)* in DIT					
		10		25		50	
		ß - level		ß - level		ß - level	
		0.2	0.1	0.2	0.1	0.2	0.1
No of measurements	Sex						
1	M	308	412	50	67	14	18
	F	233	311	38	51	11	14
2	M	154	206	25	34	7	9
	F	117	156	19	26	6	7

$\alpha = 0.05$, two-sided

Number of subjects calculated according to Lachin (83), using the adjusted between-person variation, according to sex, found in this study

* Expected change expressed as percentage of mean DIT values (in kJ) observed, in men and women

DIT = diet-induced thermogenesis

Chapter 4.
Diurnal variation in postabsorptive resting metabolic rate and diet-induced thermogenesis

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ABSTRACT

Postabsorptive resting metabolic rate (RMR) and diet-induced thermogenesis (DIT) were repeatedly assessed on different days in ten young normal weight men on mornings and afternoons. No significant diurnal variation was found in RMR, DIT, total postprandial substrate oxidation and overall postprandial nutrient balances. The pattern of postprandial substrate oxidation indicated an increased glucose oxidation in the first hour after ingestion of a meal on morning tests compared to afternoon tests. This was probably related to differences in the degree of the postabsorptive state, ie, 12-14 hours on morning tests and 6-7 hours on afternoon tests.

Keywords: indirect calorimetry, resting metabolic rate, diet-induced thermogenesis, diurnal variation, substrate oxidation, human

INTRODUCTION

Rhythmic variations in energy metabolism are well documented for animals (1). Cyclical changes in energy metabolism have also been postulated for humans (1). Among them, changes in energy expenditure during the menstrual cycle have been studied relatively frequent (2-5). Less information is available for humans on variation in energy metabolism during the day, ie, on diurnal variation in energy expenditure (6-8). It is well known that energy expenditure varies during the day due to the thermic effects of food and physical activity (9). Very few investigators, however, have studied changes

during the day in energy expenditure in the absence of thermogenic stimuli (6-8). In addition, no studies report on diurnal variation of energy metabolism in response to thermogenic stimuli.

In previous studies on humans, evidence was obtained for a gradual increase during the day in postabsorptive resting energy expenditure, ie, in postabsorptive resting metabolic rate (6-8). More recent studies do not support these findings (10-13). In the latter studies, however, diurnal variation in postabsorptive resting metabolic rate was not the main objective of study, and energy expenditure was usually measured during the morning only. It is important to clarify the issue of diurnal variation in energy expenditure, since this may affect the interpretation of studies on diet-induced thermogenesis. Diet-induced thermogenesis can be defined as the increase in energy expenditure due to ingestion of food. Frequently, in studies on diet-induced thermogenesis a non-caloric control or sham feeding test is used to adjust for possible diurnal variation in postabsorptive resting energy expenditure (10-13). However, other investigators assume no significant changes in postabsorptive resting metabolic rate during the day and do not report such sham feeding tests (14-17).

Besides diurnal variation in resting energy expenditure in the absence of food, changes during the day in the response of energy metabolism to food may be important. Diurnal variation in the response of energy metabolism to food may affect daily nutrient and energy balance.

The present study was designed to investigate whether repeated measurements of resting metabolic rate and diet-induced thermogenesis varied systematically with time of the day, ie, between mornings and afternoons. No significant diurnal variation in resting metabolic rate and in diet-induced thermogenesis was found, but postprandial substrate oxidation showed a different pattern in the morning in comparison to the afternoon.

SUBJECTS AND METHODS

SUBJECTS

Ten young men, all students, participated in the study. Volunteers were invited for participation in the study by advertisement in the University weekly periodical. Data concerning some physical characteristics and age are presented in Table 1. Each individual was fully informed on the nature and

purpose of the experiment, before giving his written consent to participate. Each subject received a questionnaire to assess past and present health status and nutritional habits. None of the subjects was on a special diet, all subjects were eating normal balanced diets and none of the subjects was using any medication at the time of the study or had experienced greater body weight fluctuation than 2.5 kg in the half year preceding the study. All subjects were non-smokers and apparently healthy as assessed by medical questionnaire. The protocol was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

Resting metabolic rate (RMR) was measured six times on different days in each subject, three times in the morning and, similarly, three times in the afternoon. Between each session there was a time span of at least two days. All subjects completed the experiment in four weeks. Morning RMR's were measured with the subject in a 12-14 hours postabsorptive state. Afternoon RMR's were measured with the subject in a 6-7 hours postabsorptive state. At the day of the afternoon sessions the subjects were allowed to have a small normal Dutch breakfast of about 2000 kJ (10 percent protein, 35 percent fat, 55 percent carbohydrates) between 7.45 and 8.00 hours. The subjects were instructed to refrain from sleeping, coffee drinking and moderate to heavy physical activity on the mornings before the afternoon tests. They were also instructed not to perform heavy physical exercise on the evening before the morning tests. The order of RMR measurements was randomized between the morning and afternoon tests.

On two mornings and on two afternoons after the RMR measurement, energy expenditure in response to a meal was measured in each subject to assess diet-induced thermogenesis. The increase in resting metabolic rate due to the ingestion of the meal was defined as diet-induced thermogenesis (DIT). The meal was a yoghurt-based liquid formula containing on average 1900 kJ (10 percent protein, 33 percent fat, 57 percent carbohydrates). The order of the DIT measurements was randomized between the morning and afternoon tests.

To familiarize the subjects with the equipment for measuring RMR and DIT,

resting energy expenditure was measured in each subject on a separate day before the start of the experiment for about 30-45 minutes.

Gas exchange measurements

Resting metabolic rate

On the day of each RMR measurement, the subjects were taken to the laboratory by car. After voiding they lay supine but awake on a hospital bed. After a 30 minutes rest period, RMR was continuously measured between 08.00 and 09.00 hours in the morning or 14.00 and 15.00 hours in the afternoon at an environmental temperature of 23-25°C. During the RMR measurements the subjects watched video films.

Postprandial energy expenditure

After the RMR measurement the subjects were given the test meal, five minutes after they had finished eating the test meal, energy expenditure was measured continuously for four hours. During the postprandial energy expenditure measurements the subjects watched video films. Room temperature was always kept at 23-25°C.
The subjects were instructed to be as relaxed as possible and to minimize movements. After the gas exchange measurements, urine was collected for determining urea-nitrogen and total urinary nitrogen.
All gas exchange measurements were performed using a ventilated hood system. A transparent perspex ventilated hood was placed over the subjects head and made airtight around the neck. A pump (Ocean SCL210, Dieren, The Netherlands) drawed fresh outside air through the hood. The flow rate through the hood was measured by a thermal mass flowmeter (Brooks, 5812N, Veenendaal, The Netherlands). The flow rate was kept between 30 and 40 l/min by an electrically operated control valve (Brooks, model 5837, Veenendaal, The Netherlands). A small sample of about 0.4 l/min of the air leaving the hood was analysed for oxygen (O_2) and carbon dioxide (CO_2) content by, respectively, a paramagnetic O_2-analyzer, adjusted to a full scale range of 20-21 percent (Servomex, 1100A, Zoetermeer, The Netherlands) and an infrared CO_2-analyzer, full scale range 0-1 percent (Analytical Development Company, SS100, Hoddesdon, UK). The analyzers were calibrated each morning or afternoon session using dried gas mixtures with known O_2 and CO_2 content. O_2 and CO_2

concentrations in dried fresh outside air were measured for five minutes every 30 minutes during the RMR measurement and every hour during the postprandial energy expenditure measurements. Air flow rate, O_2 and CO_2 concentration of the inflowing and outflowing air were computed on line by an automatic data acquisition unit (Hewlett Packard 3497A, Palo Alto, USA) interfaced to a microcomputer (Hewlett Packard 85, Palo Alto, USA). The outputs of the gasanalyzers and the flowmeter were also continuously recorded by a multi-pen recorder (Kipp & Zonen BD101, Delft, The Netherlands). O_2 and CO_2 concentration of the outflowing air and air flow rate through the hood were measured every 30 seconds and integrated over five minutes interval periods to obtain O_2 consumption, CO_2 production, respiratory quotient (RQ) and metabolic rate according to equations described by Jéquier (18).

Calculation of diet-induced thermogenesis and substrate oxidation

The O_2 consumption and CO_2 production were corrected for movements of the subjects when necessary. To obtain the mean resting energy expenditure for each hour after ingestion of the test meal, the O_2 consumption and CO_2 production were averaged over each hour using the five minutes interval data. These values were used to calculate the average DIT for the individual hours after the test meal and for the total mean DIT over four hours. DIT was calculated by subtracting the RMR value from the respective average hourly or total mean postprandial energy expenditure. The DIT is expressed as a percentage of the energy content of the test meal.
Protein oxidation was estimated by determination of urinary urea-nitrogen excretion, with a testkit (Boehringer Mannheim BV kit 396346, Almere, the Netherlands) according to Gutman and Bergmeyer (19). Carbohydrate and fat utilization during RMR and during the four hours after ingestion of the test meal were calculated using the non-protein respiratory quotient (18). Oxygen and carbon dioxide equivalents for substrate oxidation before and after the ingestion of the test meal were obtained from Lillioja et al. (20). Carbohydrate oxidation was corrected for net de novo lipogenesis from glucose as previously described (17). A non-protein respiratory quotient exceeding 1.00 indicated net de novo lipogenesis, ie, lipogenesis exceeding concomitant fat oxidation.

Body composition

Fat-free mass was estimated by densitometry. Total body density of each subject was measured once during the experiment in duplicate by the under water weighing method with simultaneous measurement of the residual lung volume by a helium dilution technique. Fat-free mass was calculated from total body density using Siri's equation (21).

Statistics

The results are expressed as mean values with standard errors of the mean (sem). Analysis of variance using the model for a single factor experiment with repeated measurements was used to test whether RMR and DIT varied systematically between mornings and afternoons. Paired two-tailed Student's t-tests were performed to evaluate differences in mean RMR, DIT and substrate oxidation rates between morning and afternoon sessions. Confidence intervals are used to express mean differences in RMR, DIT and substrate oxidation rates between morning and afternoon sessions. Pearson's product-moment correlation coefficients were used in correlation analyses.

RESULTS

Table 1 shows that all subjects had body weights and body fat percentages within the normal range.

Table 2 gives resting metabolic rates in subjects, separately for the morning and afternoon sessions. Analysis of variance showed no significant day and time of day effect on RMR. The 95 percent confidence interval for the average difference between the mean morning and afternoon values in RMR is -0.07 to +0.35 kJ/min. Therefore, for each person mean morning and afternoon RMR values were used in subsequent analyses. Morning RMR's were significantly correlated with afternoon RMR's, ($r = 0.95$, $p < 0.01$).

Table 1. Age and some physical characteristics of the subjects.

subjects	age yrs	weight kg	body mass index kg/m^2	fat-free mass kg	body fat percentage %
1	22	65.5	21.7	53.9	17.6
2	20	86.1	25.9	71.3	17.1
3	23	74.3	21.8	59.9	19.3
4	20	78.9	21.3	68.0	13.7
5	21	75.2	21.4	61.7	17.9
6	24	74.9	22.8	61.9	17.3
7	25	79.4	22.0	69.5	12.5
8	22	65.2	19.5	59.9	7.8
9	23	71.9	22.2	64.5	10.3
10	22	83.5	24.4	69.1	17.2
mean ± sem	22 ± 0.5	75.4 ± 2.2	22.3 ± 0.6	64.0 ± 1.7	15.1 ± 1.2

Table 2. Resting metabolic rate in subjects on different times of the day.

subjects	Resting metabolic rate (kJ/min)		
	morning mean ± sem*	afternoon mean ± sem*	total mean ± sem†
1	4.45 ± 0.11	4.54 ± 0.07	4.50 ± 0.06
2	5.87 ± 0.24	5.45 ± 0.07	5.66 ± 0.15
3	4.61 ± 0.27	4.74 ± 0.35	4.68 ± 0.20
4	5.43 ± 0.35	4.82 ± 0.33	5.12 ± 0.62
5	4.56 ± 0.06	4.31 ± 0.20	4.43 ± 0.11
6	4.52 ± 0.24	4.66 ± 0.26	4.58 ± 0.16
7	5.24 ± 0.11	4.69 ± 0.11	4.96 ± 0.14
8	4.37 ± 0.28	4.47 ± 0.04	4.42 ± 0.13
9	4.09 ± 0.21	4.19 ± 0.05	4.14 ± 0.10
10	5.02 ± 0.04	4.87 ± 0.04	4.94 ± 0.04
mean ± sem	4.81 ± 0.18	4.67 ± 0.11	4.74 ± 0.15

* mean of three measurements
† mean of six measurements

Data on diet-induced thermogenesis in subjects over a four hours period after ingestion of a meal on different times of the day are presented in Table 3, separately for mornings and afternoons. Analysis of variance showed no significant day and time of day effect on total DIT. Therefore, for each person the mean morning and afternoon DIT values were used in subsequent

analyses. The 95 percent confidence interval for the mean difference between the morning and afternoon DIT values was -1.8 to + 2.6 percent of the energy content of the test meal (-34 to + 50 kJ). Average morning DIT values were significantly correlated with average afternoon DIT values ($r = 0.89$, $p < 0.01$).

Table 3. Diet-induced thermogenesis in subjects after ingestion of a meal on different times of the day.

subjects	Diet-induced thermogenesis (% of energy content of test meal)*		
	morning mean ± sem†	afternoon mean ± sem†	total mean ± sem‡
1	7.5 ± 0.6	5.2 ± 1.5	6.3 ± 1.0
2	6.9 ± 1.3	13.6 ± 6.3	10.2 ± 1.6
3	7.8 ± 0.7	4.8 ± 1.3	6.3 ± 1.0
4	9.3 ± 4.5	9.8 ± 1.2	9.5 ± 1.9
5	6.1 ± 0.5	6.4 ± 2.8	6.2 ± 1.2
6	6.3 ± 1.4	4.7 ± 1.0	5.5 ± 0.9
7	5.2 ± 0.9	7.6 ± 3.4	6.4 ± 1.6
8	6.3 ± 0.3	5.4 ± 0.5	5.8 ± 0.4
9	7.0 ± 0.8	4.6 ± 1.2	5.8 ± 0.9
10	10.2 ± 0.0	6.8 ± 1.6	8.5 ± 1.2
mean ± sem	7.4 ± 0.5	6.9 ± 0.9	7.1 ± 0.5

* test meal contained on average 1906 kJ. The coefficient of variation for energy intake was 0.5 percent.
† mean of two measurements
‡ mean of four measurements

Figure 1 shows diet-induced thermogenesis separately for each hour after ingestion of the meal in subjects on morning or afternoon tests. No significant time of day effect on the mean hourly DIT values was observed. On average DIT was slightly higher on morning sessions in the first hour after ingestion of the meal in comparison with afternoon sessions, whereas for the third and fourth hour the opposite case was true. The average DIT in the fourth hour after the ingestion of the test meal was about 0.1 kJ/min (2-3 per cent) above the corresponding postabsorptive resting metabolic rate on morning sessions and about 0.2 kJ/min (4-5 per cent) above the corresponding postabsorptive resting metabolic rate on afternoon sessions.
Figure 2 represents respiratory quotients in subjects before and after ingestion of a test meal on different times of the day. RQ's were

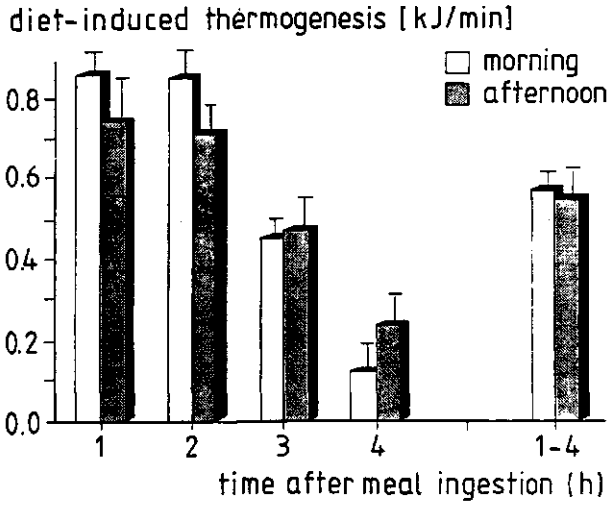

Figure 1: Diet-induced thermogenesis (mean ± sem) in subjects after ingestion of a meal on different times of the day.

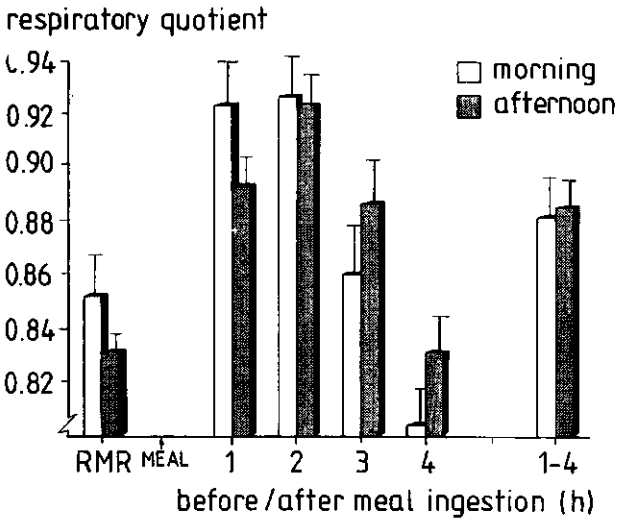

Figure 2: Respiratory quotients (mean ± sem) in subjects before and after ingestion of a meal on different times of the day.

significantly higher on mornings in the first hour after ingestion of the meal in comparison with afternoon RQ's. The reverse was true for the third and fourth hour after ingestion of the test meal.

Table 4 gives carbohydrate and fat oxidation in subjects before and after ingestion of a meal, separately for the morning and afternoon sessions. The results are expressed in absolute amounts and as a percentage of total energy expenditure. Before ingestion of the test meal, during the RMR measurement, no significant differences were obeserved in relative or absolute amount of oxidation of these nutrients between morning and afternoon sessions.

Table 4. Mean carbohydrate and fat oxidation in absolute amounts and as a percentage of total energy expenditure in subjects before and after ingestion of a meal on different times of the day.

oxidation during:		carbohydrates* morning mean	carbohydrates* afternoon mean	fats morning mean	fats afternoon mean
RMR†	%kJ/min	39	39	40	40
	mg/min	117	115	50	47§
PEE1‡	%kJ/min	64	57§	16	24§
	mg/min	234	196§	24	33
PEE2	%kJ/min	66	66	14	14
	mg/min	241	226	21	20
PEE3	%kJ/min	46	54§	33	25§
	mg/min	152	177	46	34§
PEE4	%kJ/min	27	37§	51	42§
	mg/min	84	133§	65	53
PEEt	%kJ/min	52	54	28	26
	mg/min	178	178	39	35

* expressed on a glucose basis
† RMR: postabsorptive resting metabolic rate
‡ PEE: postprandial energy expenditure; PEE1, during first hour; PEE2, during second hour; PEE3, during third hour; PEE4, during fourth hour; PEEt, during total postprandial period
§ significant difference between morning and afternoon values at $p < 0.05$

Carbohydrate oxidation was significantly higher and fat oxidation significantly lower on mornings in the first hour after ingestion of the test meal than on afternoons. During the third and fourth hour of the postprandial period, carbohydrate oxidation was lower in the morning in comparison with the afternoon, in contrast to fat oxidation. Protein oxidation was estimated for each session by the average (5.5 hours) urinary urea-nitrogen excretion. Protein oxidation was not significantly different between morning tests and

afternoon tests, 57 ± 3 mg/min versus 53 ± 3 mg/min.
Table 5 shows energy and nutrient balances in subjects after ingestion of meals on different times of the day. No significant differences between morning and afternoon tests in total oxidation and storage or balance of the various nutrients were observed. On average, energy balance, ie, energy retention, was slightly higher on afternoon than on morning sessions. The 95 percent confidence interval for the mean difference in energy retention between morning and afternoon tests is -1 to +133 kJ. Net "de novo lipogenesis" from carbohydrates did not differ between mornings and afternoons. On average less than 3 percent of the ingested carbohydrates was converted to fat.

Table 5. Nutrient and energy balance in subjects after ingestion of a meal on different times of the day.

	energy		carbohydrates		fat		protein	
	morning	afternoon	morning	afternoon	morning	afternoon	morning	afternoon
	kJ	kJ	g	g	g	g	g	g
intake	1902 ± 3	1911 ± 4*	69.2 ± 0.1	69.5 ± 0.2	16.6 ± 0.0	16.7 ± 0.0	11.7 ± 0.0	11.7 ± 0.0
oxidation			41.7 ± 3.1	41.8 ± 2.2	9.0 ± 1.9	8.0 ± 1.6	13.8 ± 0.8	12.8 ± 0.7
expenditure	1306 ±53	1248 ±43						
net lipogenesis					0.7 ± 0.3	0.4 ± 0.2		
balance	597 ±54	664 ±42†	27.5 ± 2.3	27.7 ± 2.3	8.4 ± 2.1	9.2 ± 1.7	-2.1 ± 0.8	-1.1 ± 0.7

* results expressed as mean ± sem

† significant difference between morning and afternoon values at p = 0.05 (borderline significance)

DISCUSSION

This study was designed to determine the effect of time of the day, morning versus afternoon, on resting metabolic rate and diet-induced thermogenesis. The results show no significant diurnal variation in resting metabolic rate and diet-induced thermogenesis. Postprandial substrate oxidation was affected by time of the day, but this effect was transient and overall postprandial nutrient balances did not show significant diurnal variation. Morning and afternoon measurements of energy expenditure were performed on different days. In this way, it was excluded that morning measurements would have a residual effect on afternoon assessments of energy expenditure. The design of the

study, however, allowed for normal intra-individual between-day variation in morning and afternoon RMR and DIT. This may have obscured the finding of diurnal variation in RMR and DIT of a relatively small magnitude. To minimize this possibility RMR and DIT were assessed repeatedly both on mornings and on afternoons.

Diurnal variation in RMR

Several investigators have studied in man temporal order in a number of physiological variables. For many variables, ie, hormone blood levels and urinary hormone excretions, diurnal rhythms have been well established (22). Information on cyclical changes during the day in human energy metabolism is scarce. The first part of this study is about diurnal variation in post-absorptive resting metabolic rate. Early reports (6-8) show that in man postabsorptive resting metabolic rate increases during the day. However, the results of these studies may have been influenced by effects of variation during the measurement period in the duration of the postabsorptive state. Thus, metabolic rate measurements later on the day were usually performed in subjects under greater postabsorptive stress. This may in itself have caused the reported rise during the day in postabsorptive resting metabolic rate. In spite of the absence of clear data on diurnal variation in postabsorptive resting metabolic rate in man, it is generally believed that such variation exists (10-13,23). Obviously, resting energy expenditure varies during the day due to the thermic effects of feeding and physical activity (9). In addition, nocturnal resting metabolic rate may be slightly different from diurnal resting metabolic rate (24). The question is, whether there is a diurnal rhythm in man in resting metabolic rate at a given fasting state. When such a rhythm exists, its physiological and biochemical basis could be related to hormonally-mediated changes during the day in energy-demanding processes, ie, in sodium pumping or protein synthesis. At present, however, no diurnal variation in turnover rates of thermogenic hormones has been reported (25) and a mechanism for possible diurnal variation in resting energy expenditure is unclear.

To study diurnal variation in energy expenditure, one should ideally measure resting metabolic rate in subjects under the same conditions at different times of the day. Usually in previous studies on this subject, variation was introduced in the duration of the fasting state between different times of the day (6-13). In the present study morning and afternoon tests also varied in

duration of the postabsorptive state. But in contrast to previous studies, the subjects had a shorter duration of the fast on the afternoon tests than on the morning tests. This may have confounded the afternoon measurements of resting metabolic rate with residual diet(breakfast)-induced thermogenesis. However, the results show that residual diet-induced thermogenesis did not significantly increase afternoon RMR's in comparison with morning postabsorptive RMR's. In contrast, average resting metabolic rate was slightly, but not significantly lower on afternoon than on morning tests. The absence of significant diurnal variation in postabsorptive resting metabolic rate was not due to large inter-individual differences in response to time of the day of energy metabolism, since morning RMR values were highly correlated with afternoon RMR values. In addition, the result of this study agrees with other reports in which, for several hours during the morning, no significant effect of sham feeding was observed on resting metabolic rate (10-13).

Diurnal variation in DIT

The second part of the study deals with the question of systematic variation with time of the day in the response of energy metabolism to food. This question has not been addressed before. The results show that time of the day did not significantly influence diet-induced thermogenesis (DIT). Similar to the findings for the RMR, this was not due to great inter-individual variability in difference in DIT between morning and afternoon tests. In addition, it is unlikely that the absence of diurnal variation in DIT was caused by a too short measurement period, since mean DIT had reduced almost to zero in the fourth hour after ingestion of the meal both in the morning as in the afternoon. DIT was assessed without using a noncaloric control test. It has been argued (23) that such a test should be used to assess diurnal variation in postabsorptive resting metabolic rate. However, several investigators calculate DIT in the way as has been done in this study (14-17). Furthermore, when metabolic rate measurements on such tests are confounded by variation in the duration of the fasting state during the test, measured changes in resting metabolic rate may be induced by an adaptation to an increasingly prolonged fasting state and may not reflect diurnal variation in postabsorptive resting energy expenditure. In addition, it is a matter of conjecture to infer that changes in resting energy expenditure in the absence of food will also occur after ingestion of food.

Total postprandial substrate oxidation and nutrient balances were not sig-

nificantly different between morning and afternoon tests. There was, however a transient effect of time of the day on substrate oxidation in the first, third and fourth hour after ingestion of the meal. The effect of time of the day on postprandial substrate oxidation is most likely related to differences between morning and afternoon sessions in the duration of the postabsorptive state. It can be expected that after a 12-14 hours fast total body glycogen stores are more depleted than after a 6-7 hours fast. Previous studies in man have shown that glucose given orally or intravenously is taken up predominantly by muscle for glycogen synthesis (26,27). In a more glycogen depleted state, the activity of glycogen synthetase seems increased (28), which may be associated with an enhanced insulin response or insulin action at the cellular level (29). This may, in turn, be related to an increased glucose oxidation, since in the resting state, glucose oxidation up to levels of about 4 mg/min.kg is associated with increased glucose storage, ie, glycogen synthesis (30). Such high rates of glucose oxidation did not occur in this study. Due to the relatively increased glucose oxidation in the first hour after ingestion of the meal, less glucose may be available in the circulation for oxidation in the third and fourth hour of the postprandial period. This may enhance fat oxidation to meet the tissue demands for energy. This mechanism is supported by the findings of this study.

In conclusion we can state that time of the day had no significant effect on resting metabolic rate and diet-induced thermogenesis. In addition, no systematic diurnal variation was observed in total pre and postprandial substrate oxidation. However, hourly postprandial substrate oxidation indicated an increased glucose oxidation in subjects in a more prolonged fasting state.

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Chapter 5.
Lack of a systematic sustained effect of prolonged exercise bouts on resting metabolic rate in fasting subjects

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ABSTRACT

Resting metabolic rate (RMR) and respiratory quotient (RQ) were measured in nine young normal weight men after an overnight fast on three occasions, twice with and once without exercise on the previous evening. Exercise was cycling for 90 min at 100 W or 175 W. A Latin square experimental design was used to balance the order of treatment with the type of treatment. Energy expenditure was measured by indirect calorimetry using a ventilated hood system.
No significant 10-hours after-effect of exercise on RMR was found. The differences in RMR between the control and the 100 W or 175 W treatments were, respectively, 0.1 $\pm$ 1.3 (mean $\pm$ sem) percent and 2.4 $\pm$ 1.6 percent of the control value (4.68 $\pm$ 0.13 kJ/min). On the 175 W exercise treatment RQ's decreased significantly from 0.85 $\pm$ 0.01 (control) to 0.82 $\pm$ 0.01 ($p<0.05$). This indicates that single bouts of prolonged exercise can have a persisting effect on the type of substrate oxydized in the fasting state.

Key words: indirect calorimetry, exercise, fasting, resting metabolic rate, respiratory quotients, human

INTRODUCTION

The question whether exercise has a sustained effect on resting metabolic rate remains controversial (1-10). There is no disagreement on the immediate effect of exercise on energy expenditure or oxygen consumption. It is also generally accepted that during the postexercise recovery period, oxygen consumption

declines gradually to its pre-exercise level (11). This increased oxygen consumption after exercise has been known as the lactic oxygen debt, indicating that it would correspond to the amount of oxygen needed to eliminate the lactate produced during exercise (11). More recently, this concept has been heavily criticized and it was proposed to rename the lactic oxygen debt to excess postexercise oxygen consumption (EPOC) (12). The major part of EPOC would be due to an elevation in tissue temperature and increased levels of circulating catecholamines (12). However, the biochemical and physiological basis of EPOC are not yet completely elucidated.

Controversy arises particularly in estimating the magnitude and duration of EPOC. Estimates of the duration of EPOC vary from forty minutes to more than twenty-four hours (1-10). It is important to clarify this issue, since a persisting EPOC may represent a significant loss of energy for the individual. This may be important for weight-conscious individuals. The reported differences in estimates of the duration of EPOC may in part be due to variation among the studies in methodology used to assess EPOC. Studies reporting on EPOC differ considerably in, for example, sample size, technique for measuring oxygen consumption, intensity, duration and type of exercise used, and, in control of food intake during the postexercise period (1-10). In most studies the number of subjects was less than six persons (1-3,6,9). Such studies may not have had enough power to detect a small persisting EPOC. However, studies that had relatively larger sample sizes (5,7,8,10) also vary considerably in methodology of the assessment of EPOC. Consequently, estimates in these studies of the duration and magnitude of EPOC are very dissimilar.

The present study was undertaken to determine whether prolonged exercise bouts have a sustained effect on oxygen consumption, ie, on resting metabolic rate, measured 10 hours after the exercise. Two levels of exercise intensity were used to determine whether there is a threshold level of exercise intensity before EPOC would be significantly increased. Since exercise may potentiate the thermic effect of food (13), subjects were fasted during and after exercise. The results of this study show that moderate degrees of exercise, performed the evening before assessment of resting metabolic rate, do not systematically affect resting metabolic rate.

SUBJECTS AND METHODS

SUBJECTS

Nine young men participated in the study. Volunteers were invited to participate in the study by advertisement in the University weekly periodical. Each individual was fully informed on the nature and purpose of the experiment, before giving his consent to participate. None of the subjects was on a special diet and none was using any medication. All subjects were non-smokers and were practicing sports for between 1 and 4 hours weekly. A questionnaire was used to obtain information on the past and present health status and on the nutritional habits of the participants. All subjects were considered by a physician (JGAJH) to be in good health. In addition, only subjects were selected who had not experienced greater weight changes than 2.5 kg in the half year preceding the start of the study. All subjects were eating normal balanced diets and were instructed to keep to the same eating and drinking pattern during the course of the study. The protocol of the study was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

Resting metabolic rate (RMR) was measured three times in each subject. RMR measurements were always performed between 08.00 and 09.00 hours with the subject in a 12-14 hours postabsorptive state. Between each session there was a time interval of at least 3 days. Each subject completed the experiment within 2 weeks. RMR was measured on two mornings, after the subjects had exercised on a cycle ergometer the evening before the test. A RMR measurement without exercise on the previous evening served as a control measure. Cycling was always performed between 20.00 and 22.00 hours, after the subjects had had their evening meal. The evening meal was composed of ordinary foods and provided an estimated energy intake of 3-5 MJ. During and after the exercise period, the subjects were allowed to drink water only. The exercise period consisted of cycling for 90 minutes at respectively 100 W, ie, at about 25-35 percent of maximal aerobic power (6,8,10,11), and at 175 W, ie, at about 60-70 percent of maximal aerobic capacity (6,8,10,11). The exercise was performed in

the laboratory and always supervised by one of the investigators. Since a systematic additive effect on RMR associated with the order of treatment could not be excluded, a Latin-square experimental design was used to balance the order of treatment with the type of treatment. To familiarize the subjects with the equipment for measuring RMR, resting energy expenditure was measured on a separate day before starting the study in the afternoon for about 30-45 minutes.

Gas exchange measurements

Resting metabolic rate

On the morning of each RMR measurement, the subject was taken to the laboratory by car at about 07.30 hours. After a 30 minutes rest period, RMR was measured continuously for one hour between 08.00 and 09.00 hours at an environmental temperature of 24°C with the subject lying in a supine position on a hospital bed. During the RMR measurements, the subjects watched video tapes. A ventilated hood system was used to assess energy expenditure. A transparent plastic ventilated hood was placed over the subjects head and made airtight around the neck. A pump (Ocean SCL210, Dieren, The Netherlands) drawed fresh outside air through the hood. The flow rate through the hood was measured by a thermal mass flowmeter (Brooks, 5812N, Veenendaal, The Netherlands). The flow rate was kept between 30 and 40 l/min by an electrically operated control valve (Brooks, 5837, Veenendaal, The Netherlands). A small sample (0.4 l/min) of the air leaving the hood was analysed for oxygen (O_2) and carbondioxide (CO_2) content by, respectively, a paramagnetic O_2-analyzer, adjusted to a full scale range of 20-21 percent (Servomex 1100A, Zoetermeer, The Netherlands) and an infrared CO_2-analyzer, full scale range 0-1 percent (Analytical Development Company, SS100, Hoddesdon, UK). The analyzers were calibrated each morning using dried gas mixtures with known O_2 and CO_2 content. O_2 and CO_2 concentrations in dried fresh outside air were measured thrice during the RMR measurement. Air flow rate, O_2 and CO_2 concentration of the inflowing and outflowing air were computed on line through an automatic data acquisition system (Hewlett Packard 3497A, Palo Alto, USA) interfaced to a microcomputer (Hewlett Packard 85, Palo Alto, USA). O_2 and CO_2 concentration of the outflowing air and air flow rate through the hood were measured every 30 seconds and integrated over 5 minutes interval periods to obtain O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), respiratory

quotient (RQ) and RMR according to a modification of an equation described by Jéquier (14), ie:

$$\text{RMR (kJ/min)} = 4.184 \cdot [(4.686 + 1.096 \cdot (\frac{\dot{V}CO_2}{\dot{V}O_2} 0.707)) \cdot \dot{V}O_2] \text{ and}$$

$$RQ = \frac{\dot{V}CO_2}{\dot{V}O_2} .$$

Body composition

Fat-free mass and fat mass were estimated by densitometry. Total body density of each subject was measured once during the experiment in duplicate by the under water weighing method with simultaneous measurement of residual lung volume by a helium dilution technique. Fat-free mass and fat mass were calculated from total body density using Siri's equation (15).

Statistical analysis

The results are expressed as mean values with standard errors of the mean (sem). Analysis of variance was used to evaluate the data from the Latin square design (16). Differences in oxygen consumption, resting metabolic rate and respiratory quotients between pairs of different treatments were evaluated by paired t-tests using a two-sided significance level of 5 percent.

RESULTS

Table 1 shows age and some physical characteristics of the subjects participating in this study. The subjects formed a homogeneous group with respect to age, weight, body mass index, fat-free mass and body fat percentage. None of the subjects had a body fat content lower than 12 or higher than 20 percent body weight.

Table 2 gives data on postabsorptive VO_2, VCO_2, RQ and RMR, separately for the control and exercise treatments. No significant differences in VO_2, VCO_2 and RMR were found among the different treatments. RMR's on the 100 W exercise treatment differed on average 0.04 $\pm$ 0.06 kJ/min from control RMR's. The mean difference between RMR's on the 175 W exercise treatment and the control RMR's was -0.11 $\pm$ 0.08 kJ/min. Generally, the RQ's were lower on the exercise treatment when compared to the control RQ's. However, only the difference, ie,

0.034 ± 0.013, in RQ's between the 175 W exercise treatment and the control treatment reached statistical significance (p = 0.03).

Table 1. Age and some physical characteristics of the subjects.

subjects	age	weight	body mass index	fat-free mass*	body fat percentage
	yr	kg	kg/m^2	kg	%
1	18	81.2	24.4	68.5	15.6
2	24	68.3	21.6	58.4	14.5
3	22	66.6	22.2	54.8	17.6
4	20	83.1	25.4	68.9	17.1
5	21	76.4	21.6	66.4	13.0
6	24	74.4	22.6	61.5	17.3
7	23	74.0	21.7	59.7	19.3
8	28	77.8	21.4	68.2	12.4
9	23	71.3	22.0	57.6	19.2
mean ± sem	22.6 ± 0.9	74.8 ± 1.8	22.5 ± 0.5	62.7 ± 1.8	16.2 ± 0.8

* assessed from total body density.

Table 2: Postabsorptive oxygen consumption, carbondioxide production, respiratory quotient and energy expenditure in subjects with or without previous exercise.

subjects	control				exercise 100 W				exercise 175 W			
	$\dot{V}O_2$ ml/min	$\dot{V}CO_2$ ml/min	RQ	RMR* kJ/min	$\dot{V}O_2$ ml/min	$\dot{V}CO_2$ ml/min	RQ	RMR kJ/min	$\dot{V}O_2$ ml/min	$\dot{V}CO_2$ ml/min	RQ	RMR kJ/min
1	231	208	0.90	4.73	240	204	0.85	4.86	244	206	0.84	4.94
2	216	173	0.80	4.33	214	181	0.85	4.33	224	178	0.80	4.48
3	202	170	0.84	4.09	209	179	0.86	4.24	222	180	0.81	4.46
4	262	240	0.92	5.39	274	226	0.83	5.52	255	205	0.80	5.11
5	251	212	0.85	5.08	239	195	0.82	4.81	265	211	0.80	5.30
6	229	189	0.83	4.61	218	175	0.80	4.37	221	186	0.84	4.47
7	233	197	0.85	4.72	225	176	0.78	4.49	254	208	0.82	5.11
8	234	195	0.83	4.72	227	190	0.84	4.59	231	195	0.84	4.67
9	218	185	0.85	4.41	225	190	0.84	4.55	227	181	0.80	4.55
mean ±	231	197	0.85	4.68	230	191	0.83	4.64	238	194	0.82	4.79
sem	6.0	7.2	0.01	0.13	6.5	5.4	0.00	0.13	5.6	4.5	0.01	0.11

$\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbondioxide production; RQ = respiratory quotient; RMR = resting metabolic rate

* RMR calculated as RMR = 4.184.((4.686 + 1.096.(RQ-0.707)).$\dot{V}O_2$), according to a modification of an equation published by Jéquier (14)

to their control value. In two subjects, no 3 and 7, the difference between control RMR and RMR on the 175 W exercise treatment was relatively large, ie, between 5 and 10 percent.

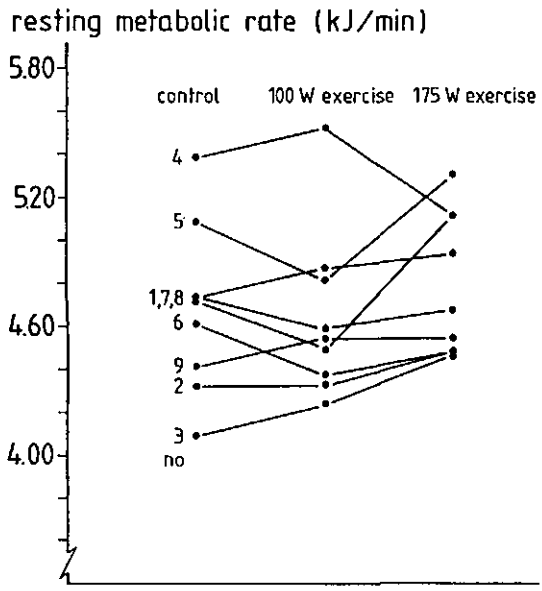

Figure 1: Postabsorptive resting metabolic rate in subjects with or without previous exercise.

DISCUSSION

The present study was designed to determine whether exercise causes an excess postexercise oxygen consumption (EPOC) under fasting conditions after 10 hours. The results show that postabsorptive resting metabolic rates were not significantly increased 10 hours after exercise in comparison with control values. However, the postabsorptive respiratory quotients on the morning after the 175 W exercise were lower when compared to the control morning. Thus, exercise, depending on its intensity, can have an after-effect on substrate oxidation.

Most recent reports on the duration and magnitude of EPOC agree with our findings (4-6,9), with the exception of two studies (7,8). One of these stu-

dies showed a variable effect of previous exercise on resting metabolic rate after 18 hours. In addition, no significant effect of exercise on sleeping metabolic rate was found (7). Maehlum et al. (8) found that oxygen consumption was elevated with about 15 per cent for about 12 hours after exercise in comparison with control values. However, a close inspection of the data of this study reveals that oxygen consumption was significantly elevated for about 5 hours after exercise and not for about 12 hours after exercise. This means that the results of our study do not contrast with the data of the latter study. Recently, Bahr et al. (10) reported that 12 hours EPOC was proportional to exercise duration at a work intensity of 70 percent of maximal oxygen consumption. Besides differences in the intensity of the applied exercise between Bahr et al.'s study and the present study, there are differences between both studies in the control of food intake. In Bahr et al.'s study subjects received meals during the assessment of EPOC, whereas in this study subjects were fasted during and after the exercise. Part of the EPOC observed by Bahr et al. may be related to a synergistic effect of previous exercise on diet-induced thermogenesis (13).

This study did not show a significant persisting 10 hours EPOC under fasting conditions. Using data from table 2 we calculated that the probability to detect an increase in RMR in response to previous exercise of about 0.2 kJ/min (4-5 percent) was 95 percent for the 100 W and 90 percent for the 175 W exercise treatments ($\alpha = 0.05$, one-sided). This means that with the present design, increases in postabsorptive RMR's less than 4 percent could not have been detected with sufficient power. Whether smaller increases in RMR are physiologically important is a matter of conjecture.

Only two persons showed consistently higher RMR's after exercise compared to control values, and only two persons showed relatively large increases in RMR after exercise, ie, between 5 and 10 percent of control values. In these persons the increase in RMR after exercise could be related to a persistent EPOC. On the other hand, even in these individuals differences in RMR's among the three treatments may be simply a reflection of ordinary intra-individual variation in RMR.

Using data from the literature (6,8,10,11) we estimated that in our subjects the 100 W exercise treatment represented an average exercise intensity of 25-35 percent of the maximal aerobic power, whereas the 175 W exercise treatment was conducted at 60-70 percent of the aerobic capacity. The latter treatment seems comparable in intensity with the treatments used by Bielinski et al. (7), Maehlum et al. (8) and Bahr et al (10). The 100 W exercise

treatment is comparable or slightly lower in intensity compared to the exercise level used by Pacy et al. (6). It has been suggested that variation in estimate of the magnitude and duration of EPOC is related to differences among the various studies in exercise intensity (17). Brehm and Gutin (17) suggested the existence of a threshold value of about 50 percent of the maximal oxygen consumption before EPOC would be significantly increased. The 175 W exercise treatment employed in this study is certainly above this threshold value, since our subjects were all normal males not engaged in athletics.

We calculated that the exercise treatments used in this study corresponded to an average energy expenditure of about 2.5 MJ (100 W, 90 min) and of about 4 MJ (175 W, 90 min). It cannot be excluded that more prolonged heavier exercise can cause a sustained increase in energy expenditure. Extreme prolonged intense exercise may cause long-lasting metabolic disturbances and may also result in damage of the tissues. Restoring the metabolic disturbances and tissue damage may, in this case, elevate energy expenditure during recovery for longer periods of time than after less extreme types of exercise. On the other hand, if EPOC is only significantly increased after extreme prolonged heavy exercise, this finding may be of no practical importance for the average individual engaged in recreational physical activity.

The effect of exercise on substrate oxidation has also been reported by other investigators (7-10). Sustained moderate to heavy exercise decreases the body glycogen stores (18) and may reduce postabsorptive hepatic glucose production. As a corollary, glucose oxidation by peripheral tissues will be reduced. Instead of glucose, free fatty acids are used as substrates for energy metabolism.

In generalizing the results of this study to other situations in which exercise-training is employed, for example, as an adjuvant in weight reduction programmes, a caveat is warranted. The results of this study can not be used to evaluate the possible use of exercise-training programmes on long-term energy expenditure (19-21). Exercise-training programmes may induce an increase in the fat-free body mass, which in itself can elevate energy expenditure (22). Furthermore, exercise programmes may have different biochemical and physiological consequences compared to single sustained bouts of exercise.

In conclusion we can state that single sustained bouts of moderate to heavy exercise did not have a systematic prolonged effect on postabsorptive resting metabolic rate. They may, however, increase the amount of fat oxydized in the postabsorptive state.

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Chapter 6.
Surprisingly large impact of psychological stress on diet-induced thermogenesis but not on resting metabolic rate

JAN A WESTSTRATE MSC, KARIN VAN DER KOOY MSC, PAUL DEURENBERG PHD, JOSEPH GAJ HAUTVAST MD

ABSTRACT

The effect of psychological stress on resting metabolic rate and diet-induced thermogenesis was assessed in 12 healthy young non-obese men, body weight 70.2 ± 1.2 kg (mean ± sem), age 25 ± 0.6 years. Two types of motion pictures (video films) were shown to the subjects during the measurements, ie, stress-inducing horror films and romantic family films (control).
Resting metabolic rate and respiratory quotients were not significantly influenced by the type of film shown to the subjects. Diet-induced thermogenesis was signicantly increased on the stress-inducing treatment, 0.95 ± 0.05 kJ/min (mean ± sem) versus 0.76 ± 0.06 kJ/min (control).
No significant effect of psychological stress on postprandial substrate oxidation rates, nutrient balances and urinary catecholamines excretion was observed.

Keywords: indirect calorimetry, resting metabolic rate, diet-induced thermogenesis, stress, anxiety, human.

INTRODUCTION

Daily human energy expenditure consists of three major components (1,2). The largest among these is the basal (BMR) or resting metabolic rate (RMR). The second component is the energy expenditure due to the performance of external work. The third major component is the increase in resting metabolic rate due

to the thermic effect of food, also known as diet-induced thermogenesis (DIT). RMR and DIT contribute about 70-85 percent to daily energy expenditure in the average individual.

In recent years there has been considerable interest in inter-individual differences in RMR and DIT (3-9). Inter-individual variation in RMR primarily reflects differences between individuals in body weight and body composition (5,9). Controversy exists on the nature and determinants of inter-individual variation in DIT (3,6,8).

Conflicting results in this area of research might be related to the methodology used for measuring the thermogenic response to meal ingestion (10). Various investigators (5,7,11) report that subjects were entertained by music or motion pictures during the assessment of energy expenditure. This may have had an impact on the mental state of the subject and likewise on energy expenditure. Unfortunately there are very little data on the impact of disturbance of the mental state on RMR and DIT.

The purpose of this study was to determine whether emotionally upsetting and frightening motion pictures affected RMR and DIT in comparison to romantic family films. We hypothetized that an emotionally upsetting film would lead to an increase in energy expenditure mediated by a stimulation of the sympathetic nervous system. Urinary catecholamines excretion was used as an index of sympathetic activity (12).

SUBJECTS AND METHODS

SUBJECTS

Twelve young healthy normal weight men participated in this study. Subjects were either graduates or undergraduates of the University. All subjects were aged between 22 and 29 years (25 years $\pm$ 0.6 mean $\pm$ sem), had a body mass (Quetelet) index between 18.9 and 22.9 kg/m^2 (21.0 $\pm$ 0.3 kg/m^2) and a body fat percentage between 7 and 22 percent (15.0 $\pm$ 1.4 percent). Body fat percentage was assessed by densitometry as previously described (11). All subjects were apparently healthy as evaluated by a medical questionnaire. None of the subjects was on a diet at the time of the study, used any medication or had experienced a body weight reduction of more than 2.5 kg in the half year preceding the study or had glycosuria. All subjects were eating normal balanced meals. The protocol was fully explained to the subjects, but none of

the subjects knew the purpose of the study. Subjects were informed that the study was intended to investigate the effect of meal ingestion and body temperature on total body impedance, a measure of body composition (13). The protocol of the study was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

In each subject energy expenditure was measured on four mornings between 08.00 and 13.00 hours within a period of six weeks. Between each test was a time-interval of at least two days.
In the first experiment (A), the effect of psychological stress on resting metabolic rate (RMR) was measured in duplicate in each subject after a 12-14 hours overnight fast. Half of the subjects, randomly selected, started the first RMR measurement with the stress-inducing treatment (horror films) and switched to the control treatment (romantic-family type films) after 1.5 hours. The other half of the subjects received the treatments in the reverse order. At the second RMR measurement the same procedure was followed, but subjects who had started the first RMR measurement with the stress-inducing treatment now started with the control treatment and vice versa.
In the second experiment (B), diet-induced thermogenesis (DIT) was measured twice in each subject after ingestion of a standardized mixed liquid test meal with an energy content of on average 1900 kJ (10.8 percent protein, 32.9 percent fat, 56.3 percent carbohydrates). Subjects were randomly allocated to the stress-inducing or control treatment after ingestion of the meal.
Energy expenditure was assessed with a ventilated hood system as previously described (11). For a DIT assessment, RMR, ie, premeal "baseline" resting energy expenditure, was based on an one hour continuous registration of oxygen consumption and carbondioxide production, while the subjects watched 'normal', ie, control, motion pictures. After meal ingestion postprandial energy expenditure (PEE) was measured continuously for four hours. The difference between the mean PEE and the RMR was used to calculate DIT in kJ/min and, integrated over the total measurement period, in kJ.
After the DIT assessment, urine was collected for determination of catecholamines excretion rates. Catecholamines excretion rates were also

assessed in urine collected over the night preceding the DIT measurements. These values were used as baseline excretion rates. Catecholamines were analyzed according to Smedes et al. (14) and Westerink et al. (15). Substrate oxidation rates and nutrient balances were calculated using the nonprotein respiratory quotients and urinary urea-nitrogen excretion rates for estimating protein oxidation as described by den Besten et al. (11).

Statistical analysis

Significance of differences in several variables between the experimental conditions was evaluated with two-sided paired t-tests. All values are given as mean ± sem.

RESULTS

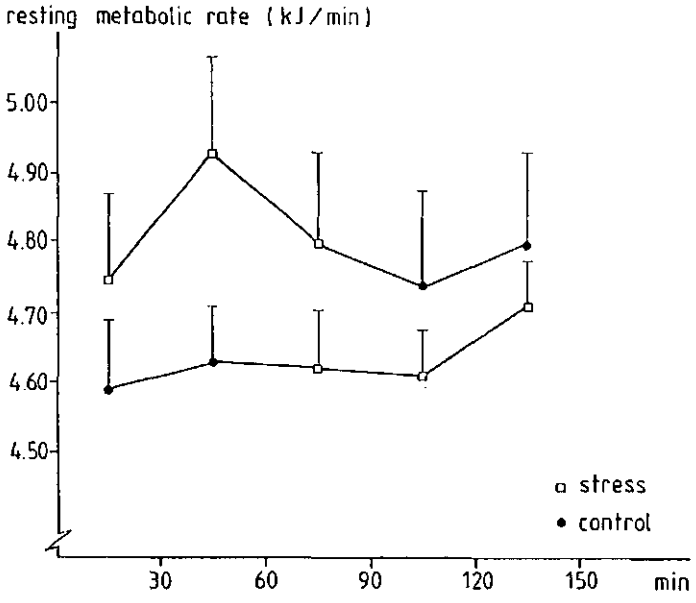

Figure 1. Resting metabolic rate (mean ± sem) measured for 2.5 hours in the same subjects on two separate mornings with different types of motion pictures (● = control, □ = stress-inducing) shown to the subjects during each measurement.

Figure 1 shows RMR's measured in experiment A. There was no significant difference in RMR between the two treatments. On average RMR increased 0.04 kJ/min, less than 1 percent, when films were changed from stress-inducing to control type.

Figure 2 shows pre- and postprandial energy expenditure measured in experiment B. RMR's were not significantly different between the two treatments, ie, 4.69 ± 0.10 kJ/min (control) versus 4.67 ± 0.08 kJ/min (stress). Diet-induced thermogenesis, was significantly higher on the stress-inducing treatment, ie, 0.95 ± 0.05 kJ/min compared to 0.76 ± 0.06 kJ/min for the control treatment (p=0.021). The difference in total integrated DIT between the treatments was about 45 ± 17 kJ or 2.5 ± 0.9 percent of the energy content of the test meal. Average postprandial respiratory quotients did not differ significantly between both treatments. They were, respectively, 0.91 ± 0.006 and 0.91 ± 0.009.

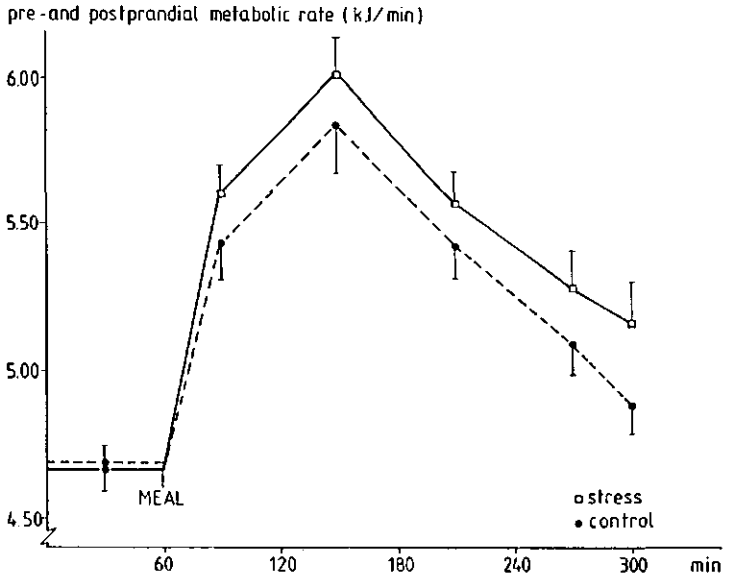

Figure 2. Postprandial energy expenditure (mean ± sem) measured for 4 hours in the same subjects with (□) or without (●) stress-inducing motion pictures.

Table 1 gives urinary catecholamines excretion for the two treatments. Baseline levels for the excretion of catecholamines were not significantly

different between the treatments, neither were the excretion rates measured over the treatment period or ratios of baseline and treatment period excretions. Total or individual catecholamines excretion rates or baseline treatment period ratios were not significantly correlated with DIT.

Table 1. Urinary catecholamines excretion in men before and during energy expenditure measurements under different attention diverting conditions.

Catecholamines	Control (n=12)			Stress (n=12)		
	before* μg/h	during† μg/h	during/before	before μg/h	during μg/h	during/before
adrenaline	0.43±0.09	0.81±0.10	2.72±0.46	0.34±0.08	0.81±0.09	3.18±0.50
noradrenaline	1.53±0.14	2.31±0.15	1.66±0.19	1.35±0.08	2.09±0.18	1.61±0.20
dopamine	17.3±1.7	22.9±2.5	1.41±0.14	16.9±0.9	21.0±1.7	1.23±0.06

All values are given as mean ± sem
*** measured over an 11 hours period immediately before the start of the energy expenditure measurements**
† measured over a 5.5 hours period during energy expenditure measurements

Postprandial substrate oxidation rates and nutrient balances did not differ either between both treatments (data not shown).

DISCUSSION

This study shows a surprisingly large impact of psychological stress on diet-induced thermogenesis, but not on resting metabolic rate.
Most reviews on factors affecting energy output in humans mention that anxiety or stress may influence energy expenditure (16,17,18). Relatively few studies, however, give quantitative data on the impact of some sort of emotional upset on human energy expenditure (19-22). The results of the latter studies do not permit an accurate estimation of the impact of stress on energy expenditure. Only one recent study reports on the impact of a more usual type of stress, ie, of arduous clerical work, on daily energy expenditure (23).
In the study here reported, stress was induced by motion pictures. The use of a motion picture to induce stress under laboratory conditions is common practice in the field of experimental psychology (24). It was expected that an emotionally upsetting and frightening film would lead to activation of the sympathetic nervous system, causing a rise in energy expenditure. To our surprise no significant effect of stress on RMR was found, whereas DIT was

significantly increased by about 0.20 kJ/min. On average only 0.04 kJ/min of this increase may have been due to the impact of stress on RMR. The absence of an effect of stress on RMR indicates that under fasting conditions an emotionally upsetting film is not a sufficient stimulus of the sympathetic nervous system. The combination of exposure to psychological stress and ingestion of food resulted in increased rates of energy expenditure, suggesting a synergistic action of stress and food ingestion on the activity of the sympathetic nervous system.

Ingestion of food, especially of carbohydrates, affects the activity of the sympathetic nervous system (25). A relatively large part, 30-40 percent, of the total DIT response is thought to be mediated by an increase in the activity of the sympathetic nervous system (2). An increased activity of the sympathetic nervous system is usually reflected in increased urinary catecholamines excretion rates (12). In this study, however, no significant correlation was found between total DIT and urinary catecholamines excretion rates, suggesting that urinary catecholamines excretion rates are not a sensitive indicator of stimulation of the sympathetic nervous system.

Postprandial substrate oxidation rates and nutrient balances indicated no significant differences between the treatments in the metabolism of the ingested nutrients. This indicates that the effect of stress on DIT was due to other factors increasing thermogenesis, for example by a stimulation of ionic pumping (26) or substrate cycling in skeletal muscles (27). In addition, it is possible that skeletal muscle tone was higher in subjects exposed to the stress treatment in comparison with the control treatment. The energy expended by such isometric muscle contraction may have elevated total energy expenditure. Finally, total postprandial energy expenditure may have been elevated by minor movements. However, an increase in muscle tone and in minor movements due to stress would also have elevated RMR, which was not found.

Whatever the cause of the increased DIT, this study clearly shows that stress-inducing motion pictures can significantly influence diet-induced thermogenesis. The findings of this study implicate that investigators of RMR and DIT should explicitly state under what conditions energy expenditure was assessed.

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Chapter 7.
Nature and magnitude of inter-individuel differences in resting metabolic rate and diet-induced thermogenesis in lean and obese individuals

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ABSTRACT

Resting metabolic rate (RMR) was measured in 154 subjects, 76 non-obese men, 44 non-obese women and 34 obese women. Diet-induced thermogenesis (DIT) and in vivo fuel utilization rates were measured in a subgroup of 126 individuals, 55 men, 37 non-obese and 34 obese women. RMR was positively associated ($r = 0.80$) with the size of the fat-free mass (FFM), ie, RMR (kJ/d) = 1813 + 86*FFM (kg). The 5th and the 95th percentile values for the distribution of the RMR per kg fat-free mass (FFM) were 100 and 150 kJ/d, respectively. RMR per kg FFM was significantly lower in men compared to women, 115 $\pm$ 1 (mean $\pm$ sem) kJ/d versus 126 $\pm$ 2 kJ/d ($p<0.001$). RMR adjusted for FFM, age and fat mass was significantly ($p<0.05$) lower among obese women with a non-abdominal type of body fat distribution compared to non-obese men, non-obese women and obese women with an abdominal type of body fat distribution. Inter-individual variation in RMR, adjusted for FFM, age and fat mass, was similar among the various groups and averaged 10 percent.

DIT to a mixed test meal of 1775 $\pm$ 34 kJ was 7.5 $\pm$ 0.2 percent of the ingested energy. DIT was positively ($r = 0.52$) related to the energy content of the test meal. The 5th and 95th percentile points for the distribution of DIT were 4.0 and 12.0 percent (of energy content of test meal), respectively. DIT was significantly higher in men compared to women, 8.4 $\pm$ 0.3 versus 6.8 $\pm$ 0.3 percent ($p<0.001$). In men, on average 77 percent of DIT was calculated to be related to energy costs for processing the ingested nutrients, in women this was on average 99 percent. In each sex, DIT was not significantly associated with FFM, body weight or body fat content. Inter-individual variation in DIT among the various groups averaged 36 percent and was, respectively, 30 percent

in men, 43 percent in non-obese women and 26 percent among obese women. Postabsorptive and postprandial in vivo fuel utilization patterns were similar in men and women. After meal ingestion carbohydrate oxidation increased from on average 37 to 51 percent of total energy expenditure and fat oxidation decreased from on average 40 to 26 percent. On average less than 5 percent of the ingested glucose was converted to fat.
It is concluded that a relatively low RMR or DIT is not an exclusive characteristic of the obese.

keywords: indirect calorimetry, resting metabolic rate, diet-induced thermogenesis, fuel utilization, men, women, obesity, inter-individual variation

INTRODUCTION

Energy requirements for adults are assumed to be primarily dependent on age, sex, body weight and physical activity (1). The current theory on human energy requirements attributes variation in energy needs among individuals of given age, sex and body weight to differences in the degree of physical activity. It is assumed that age, sex and body weight determine maintenance requirements to which increments are added, depending on physical activity, to derive total energy needs. Maintenance requirements would be indicated by the level of the resting ("basal") metabolic rate (RMR). The RMR can be directly assessed using (in)direct calorimetry or it can be estimated from a variety of simple prediction equations (2-7). In addition to the energy expended for maintenance and physical activity, energy is dissipated by the body in the processing of ingested nutrients, ie, in diet-induced thermogenesis (DIT) (8).
The validity of the current theory on human energy requirements may, however, be questioned. At first, Sukhatme and Margen (9) have pointed out, that individuals can adapt to varying energy intakes not by alterations in the level of physical activity, but as a result of changes in the efficiency of energy utilization. These changes would manifest themselves in adaptive fluctuations of the level of energy expenditure in one or more components of daily energy output. Whether individuals may show the relatively large changes in efficiency of energy utilization as proposed by Sukhatme and Margen, is at present a matter of conjecture. Secondly, evidence is accumulating from metabolic studies for inter-individual differences in resting energy

expenditure among individuals of given sex, age, body weight and body composition (10,11). However, the nature and magnitude of these differences remain to be definitely assessed. Resolving this issue may be important for various reasons, ie, for the diagnosis of metabolic disorders as hypo- and hyperthyroidism, for the refinement of estimates of human energy requirements, and, for the identification, among the lean, of individuals, that would be at increased risk for body weight gain, and, among the obese, of individuals that would be particularly resistant to body weight reduction.
In the present study resting metabolic rate and diet-induced thermogenesis were measured in a group of 154 men and women, and in a subgroup of 126 persons, respectively. The aims of the study were to assess the magnitude and determinants of inter-individual differences in RMR and DIT. Information was also obtained in a subsample of 91 subjects on the intra-individual variation in RMR and DIT.

SUBJECTS AND METHODS

SUBJECTS

Between january 1986 and august 1988 resting metabolic rate (RMR) was measured in a group of 154 subjects; in 126 of these individuals diet-induced thermogenesis (DIT) was also assessed.
The subjects participated in ongoing studies on methodological aspects of the measurement of postprandial energy expenditure, or in studies on (non)-nutritional influences on RMR and DIT. During this period a total of 570 RMR measurements and a total of 360 DIT measurements were obtained in these subjects. Volunteers were invited to participate in the studies by advertisements in regional newspapers, the University weekly periodical, by poster or through informal contacts. After applying for participation, subjects received a letter informing them on the nature and purpose of the experiment. Subjects also received one or two questionnaire(s) for obtaining information on past and present health status, nutritional habits, smoking and (alcoholic beverages) drinking behaviour, habitual activity pattern and sporting activities. Subjects eligible for participation were invited for a first visit to the laboratory to familiarize them with the apparatus for measuring energy expenditure. After giving their informed consent subjects were allowed to participate. The protocol of each study was submitted to and

approved of by the Medical-Ethical Committee of the Wageningen Agricultural University. Subjects participating in the studies were all in apparent good health had no evidence of past or present thyroid disorders or diabetes mellitus, no glycosuria or proteinuria, were not using drugs known to affect energy metabolism, were all eating normal balanced diets, smoked less than 10 cigarettes/d, drunk no more than 5 alcoholic drinks/d, did not exercise more than 7 hours/week, and had had stable (within 2.5 kg of body weight at entry) body weights at least 6 months prior to the beginning of the study. Women were in the premenopausal state. In the obese fasting blood glucose values were all within the normal range, ie, between 3.9 and 5.6 mmol/l (12).

METHODS

Gas exchange measurements

Gas exchange measurements were performed with a ventilated hood system, which has been described in detail before (13,14). Briefly, a subject's head is covered with a transparent perspex hood with an air inlet on top and an air outlet on the side. The hood is made airtight around the neck with a cord. Fresh air is drawn through the hood by negative pressure created by a pump placed downstream. The fresh air mixes with the subjects expired air, which causes a decrease in oxygen content and an increase in carbondioxide content of the air leaving the hood, relative to the gas concentrations of the air entering the hood. Accurate measurement of the oxygen (Servomex 1100A, Zoetermeer, the Netherlands) and carbondioxide (Analytical Development Company SS100, Hoddesdon, UK) content of the inflowing and outflowing air combined with measurement of the volume of air (Brooks massflowmeter 5812N, Veenendaal, the Netherlands) flowing through the hood permits a calculation of the amount of oxygen used by the subject and the amount of carbondioxide produced. The analyzers and flowmeter voltage outputs were digitized (Hewlett Packard 3497A, Palo Alto, US) and connected to a desktop computer (Hewlett Packard 86B or 85, Palo Alto, US). Continuous integrated calorimetric measurements were recorded by the computer and printed over 2 or 5 minutes intervals. The rate of oxygen consumption and carbondioxide production serve as a basis for the calculation of metabolic rate. Metabolic rate was calculated by a computer using an equation published by Jéquier et al. (15). From repeated ethanol combustions

we calculated a random error of 2.8 percent of energy expenditure measurements with the ventilated hood system.

Resting metabolic rate

Resting metabolic rate (RMR) was generally measured in the morning between 7.30 hours and 9.00 hours, after an overnight fast, for a period of one hour continuously after a 30 minutes acclimatization period. Subjects were instructed to refrain from heavy physical exercise the evening before the RMR measurements. During the acclimatization period the hood was already placed, but subjects were still allowed to move in order to find a most comfortable position for the RMR measurement. Subjects were measured in a supine (semi-recumbent) position in a noise and temperature controlled (23-26°C) room during the RMR measurement. Subjects were asked to remain motionless, but awake. Subjects watched motion pictures during the measurement. In some subjects RMR was measured in the afternoon between 13.30 and 15.00 hours with the subjects in a 4.5 hours postabsorptive state after a standardized breakfast of about 2 MJ. We have previously shown that RMR's measured under these conditions in the morning do not differ systematically from RMR's measured in the afternoon (13). RMR was calculated omitting metabolic rate readings disturbed by spontaneous movements or hypo- or hyperventilation. For 103 subjects repeated RMR measurements were available to assess intra-individual variability in energy expenditure.

Diet-induced thermogenesis

In 126 individuals diet-induced thermogenesis (DIT) was measured. After an initial RMR measurement subjects received a mixed liquid meal composed of yoghurt, sucrose, orange juice, vegetable fat and a protein supplement with an average nutrient composition of 12 percent protein, 55 percent carbohydrates and 33 percent fat. The energy content of the test meal varied, depending on the type of study, between about 1300 kJ and 2600 kJ. Mean energy content was 1775 $\pm$ 34 kJ. The liquid meal was eaten within 5 minutes. The meal was sipped through a plastic tube which was passed through an opening in the hood. Fifteen subjects received a normal solid mixed meal which was eaten within 15 minutes. No systematic difference in DIT between a liquid or solid meal was observed (14). After the subjects had eaten their meal, energy expenditure was measured continuously for a period of 3-4 hours, depending on the type of

study. The duration of the postprandial measurements period was on average 224 ± 2 minutes. DIT was defined as the cumulative increase in energy expenditure after meal ingestion above the premeal energy expenditure, ie, the RMR. In some subjects energy expenditure at the end of the postprandial period had decreased to a level below the corresponding RMR. For these subjects a new baseline for the calculation of DIT was defined, ie, the mean of the RMR and the energy expenditure at the end of the postprandial period. The energy expended for the processing of the ingested nutrients (in kJ) was divided by the energy content of the test meal to express DIT (16). This allows a comparison of thermic responses to meals with differing energy contents (17). In 91 of the subjects repeated DIT measurements were obtained to assess intra-individual variability.
The energy content of the test meals was determined by chemical analyses of duplicate portions of the meals.

Substrate utilization rates

Pre- and postprandial substrate oxidation rates and nutrient balances were calculated as previously described (13,14), using the nonprotein respiratory quotient. The nonprotein respiratory quotient was calculated from oxygen consumption, carbondioxide production and total urinary nitrogen excretion. After the gas exchange measurements subjects voided for determination of total nitrogen excretion. Total nitrogen excretion was determined with the Kjeldahl method. The energy costs for processing the ingested nutrients were calculated using factors given by Flatt for the energy costs of processing the ingested nutrients through oxidation or storage pathways (18).

Body composition

Body weight was measured to the nearest 0.05 kg (Berkel ED-60T, Rotterdam, the Netherlands) with the subjects in an overnight fasting, or 4.5 hours postabsorptive state, after voiding and wearing underwear or swimming clothes only. Body composition was determined using densitometry. Subjects were measured underwater with a simultaneous assessment of residual lung volume according to a helium dilution method. Total body density was calculated and the size of the fat-free mass and the fat mass were estimated as previously described (13).

Statistics

Differences in continuous variables among groups were evaluated using analysis of variance or unpaired Student's t-tests. Adjusting group means for covariates was performed by analysis of covariance (19). Linear relationships between resting metabolic rate and body composition variables were assessed using univariate or multiple regression analyses (20). Correlation coefficients are Pearson's product-moment or partial correlation coefficients. Inter-individual variation in RMR and DIT was calculated after assessing linear effects on RMR or DIT of body composition (RMR) variables and energy intake of test meals (DIT) in multiple regression models. The residual mean square of these models was used as an estimator of the 'adjusted' between-person variation.

RESULTS

Table 1 shows physical characteristics, resting metabolic rate and respiratory quotients of subjects participating in the two studies. Besides systematic

Table 1. Physical characteristics and characteristics of postabsorptive resting energy expenditure of subjects in two studies.

	Study 1		Study 2	
	Inter-individual variation in resting metabolic rate		Inter-individual variation in diet-induced thermogenesis and in vivo fuel utilization	
	Men (n=76)	Women (n=78)	Men (n=55)	Women (n=71)
Variables studied				
age (yrs)	29.3 ± 0.4 (18–41)	31.7 ± 1.1 (18–49)	24.3 ± 0.5 (20–41)	32.3 ± 1.1 (20–49)
weight (kg)	73.9 ± 0.9 (58.4–102.0)	74.1 ± 2.0 (50.3–125.9)	73.4 ± 1.0 (58.4–101.8)	75.3 ± 2.2(50.3–125.9)
fat-free mass (kg)	62.7 ± 0.8 (48.8–91.4)	46.1 ± 0.5 (36.6–60.6)	62.4 ± 0.9 (48.8–91.4)	46.3 ± 0.6 (36.6–60.6)
fat mass (kg)	11.2 ± 0.4 (3.4–25.2)	28.0 ± 1.7 (9.1–70.4)	11.0 ± 0.5 (3.4–19.1)	29.0 ± 1.8 (9.1–70.4)
body fat content (%)	15.0 ± 0.5 (5.5–24.7)	35.5 ± 1.2 (14.6–55.9)	14.9 ± 0.5 (5.5–22.3)	36.3 ± 1.2 (14.6–55.9)
RMR (kJ/min)	4.99 ± 0.07 (3.93–7.29)	4.01 ± 0.06 (3.10–5.70)	5.00 ± 0.09 (3.93–7.29)	4.01 ± 0.06 (3.10–5.70)
RMR (kJ/kgFFM.d)	114.9 ± 1.3 (92.8–149.6)	125.8 ± 1.6(100.8–155.6)*	115.7 ± 1.6 (92.8–148.8)	125.1 ± 1.7(100.8–155.6)*
RQ – RMR	0.838 ± 0.005(0.746–0.940)	0.831 ± 0.004 (0.754–0.929)	0.836 ± 0.005(0.746–0.940)	0.833 ± 0.004(0.754–0.929)

All values are given as mean ± sem (range)

RMR = postabsorptive resting metabolic rate; kg FFM = fat-free mass; RQ = respiratory quotient

* significant difference between men and women at $p < 0.001$

differences between men and women in body composition variables, ie, in fat-free mass, fat mass and body fat content, RMR expressed on a fat-free mass basis was significantly higher in women compared to men. Women had on average 9 percent higher RMR's per kg fat-free mass than men did. Five percent of the total population had RMR's per kg fat-free mass below 100 kJ/d and five percent above 150 kJ/d. Inter-individual coefficients of variation in resting metabolic rate after adjusting for fat-free mass, age and fat mass were, respectively, 9.6 percent in men, 11.3 percent in non-obese women and 10.7 percent in obese women. Intra-individual variation in RMR was 5 percent in men and 6 percent in the non-obese and the obese women.

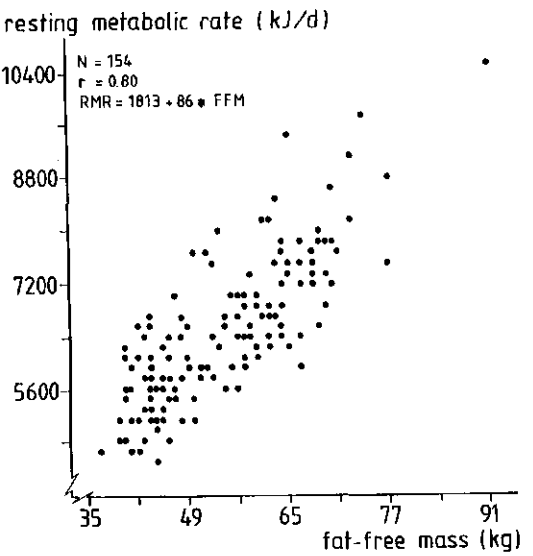

Figure 1: Relation between fat-free mass (FFM) and resting metabolic rate (RMR) in 154 subjects.

Figure 1 show the relationship between fat-free mass and RMR. No significant differences among men, non-obese women and obese women in slopes or intercepts of the regression equations were found. In the obese women, in addition to the size of the fat-free mass, the size of the fat mass was significantly positively related to RMR ($r=0.68$, $p<0.001$), even after adjusting for the

linear effects of the fat-free mass on fatmass ($r=0.44$, $p<0.01$). Comparing RMR's, adjusted for age, fat-free mass and fat mass, among men, lean women and obese women revealed significantly lower RMR's among the obese when compared to the non-obese women and men. Adjusted RMR's were, respectively, 4.59 ± 0.11 kJ/min (men), 4.59 ± 0.10 kJ/min (lean women) and 4.18 ± 0.18 kJ/min (obese women). A further analysis showed that the lower adjusted RMR's were, in particular, characteristic for the non-abdominal obese women, ie, for the women with a waist-to-hips girth ratio below 0.85. In these women adjusted RMR's were 4.13 ± 0.18 kJ/min, whereas in women with an abdominal type of body fat distribution adjusted RMR's were 4.52 ± 0.23 kJ/min.

Table 2. Resting metabolic rate, diet-induced thermogenesis and in vivo fuel utilization in men and women.

		Men (n=55)	Women (n=71)
RMR	(kJ/min)	5.00 ± 0.09 (3.93–7.29)	4.01 ± 0.06 (3.10–5.70)*
DIT	(kJ)	164 ± 7 (68–329)	109 ± 5 (–5–209)*
DIT – % energy content meal	(%)	8.37 ± 0.33 (4.02–14.39)	6.78 ± 0.31 (–0.35–15.49)*
DIT – % increase RMR	(%)	13.9 ± 0.7 (6.9–32.4)	12.3 ± 0.5 (–1.0–22.5)
basal fat oxidation	(10^{-3}.mg/min.kgFFM)	799 ± 40 (50–1623)	876 ± 37 (61–1623)
basal CHO oxidation	(10^{-3}.mg/min.kgFFM)	1748 ± 78 (262–3288)	1825 ± 67 (247–3300)
postprandial fat oxidation	(10^{-3}.mg/min.kgFFM)	594 ± 44 (–47–1466)	639 ± 35 (–254–1346)
postprandial CHO oxidation	(10^{-3}.mg/min.kgFFM)	3106 ± 89 (1569–4388)	3273 ± 87 (1326–4975)
protein oxidation	(10^{-3}.mg/min.kgFFM)	963 ± 24 (649–1364)	1069 ± 29 (538–1758)*
basal fat oxidation	(% RMR)	39.6 ± 1.8 (2.5–69.6)	39.9 ± 1.5 (2.9–71.2)
basal CHO oxidation	(% RMR)	37.6 ± 1.6 (5.4–67.9)	36.3 ± 1.2 (4.8–60.4)
basal protein oxidation	(% RMR)	22.5 ± 0.5 (14.3–34.5)	23.3 ± 0.7 (10.9–40.6)
postprandial fat oxidation	(% PEE)	26.3 ± 1.7 (–0.4–59.2)	26.3 ± 1.3 (–10.7–57.2)
postprandial CHO oxidation	(% PEE)	54.0 ± 1.5 (26.9–73.8)	53.3 ± 1.2 (23.3–70.8)
postprandial protein oxidation	(% PEE)	19.8 ± 0.5 (13.3–26.7)	20.8 ± 0.6 (9.1–35.7)

Values are given as mean ± sem (range)

RMR = postabsorptive resting metabolic rate; DIT = diet-induced thermogenesis; kgFFM = kg fat free mass; CHO = carbohydrate (glycogen or glucose); PEE = postprandial energy expenditure

* significant difference between men and women at $p < 0.001$

Table 2 shows RMR, DIT and in vivo fuel utilization rates in men and women. DIT was significantly ($p<0.001$) associated with the energy intake of the test meal ($r = 0.52$). The 5th and 95th percentile values of the DIT distribution in the total sample were 4 and 12 percent (of energy content of the test meal), respectively. A significant difference of 24 percent was observed in DIT between men and women. No significant difference was observed between lean

women and obese women in DIT. DIT was, respectively, 6.65 ± 0.56 percent in the lean and 5.71 ± 0.99 percent in the obese. DIT was not significantly affected in the obese women by type of body fat distribution. The differences in DIT between the sexes occurred during the entire first three hours of the postprandial period and parallelled differences in carbohydrate oxidation, but not in fat oxidation. Oxidation rates during the first three hours of the postprandial period were, respectively, for carbohydrates 217 ± 8 mg/min in men versus 163 ± 5 mg/min in women ($p<0.01$), and for fat 31 ± 3 mg/min in men versus 26 ± 2 mg/min in women. Protein oxidation differed also significantly between the sexes, ie, 60 ± 2 mg/min in men and 50 ± 2 mg/min in women. No differences in basal or postprandial fuel utilization rates expressed on a fat-free mass basis between the sexes was observed, except for protein oxidation, which was on average 11 percent higher in women compared to men. In both sexes, fat oxidation contributed in the basal state on average 40 percent to total basal energy expenditure. Carbohydrate oxidation contributed 37 percent to total basal energy expenditure. In the postprandial state, fat oxidation was reduced to 26 percent of total energy expenditure. A reciprocal change was observed in postprandial carbohydrate oxidation. In 23 persons there occurred during the postprandial period occasionally net transformation of glucose into fat. In the other 103 subjects no net lipogenesis occurred. On average, in men, 4 percent of the ingested glucose was converted to fat, 33 percent was stored as glycogen and 63 percent was oxidized. In women these figures were, respectively, 5, 39 and 56 percent. On average, 50 percent of the amount of ingested fat was oxydized in the postprandial period in both sexes.

The coefficients of inter-individual variation in DIT were 29 percent in men, 43 percent in non-obese women and 26 percent in obese women. Inter-individual variation in DIT was similar to intra-individual variation in DIT. The coefficients of inter-individual variation, adjusted for fat-free mass, of in vivo fuel utilization rates in the postabsorptive state were, respectively, 36 percent for fat, 30 percent for carbohydrates and 21 percent for protein. Inter-individual variation of fuel utilization rates in the postprandial state, adjusted for fat-free mass and nutrient intake with the test meal, was respectively, 50 percent for fat, 21 percent for carbohydrates and 20 percent for protein. Inter-individual variation in fuel utilization rates was similar among men, non-obese women and obese women.

Table 3 shows differences in body composition and energy metabolism between subjects characterized by relatively low (< 10th percentile of sex-specific

frequency distribution) or high (> 90th percentile) RMR per kg fat-free mass. Significant differences between both groups, separately for men and women, were observed in the size of the fat-free mass and in basal fat oxidation rates. In addition, in women a significant difference between both groups in DIT was observed. Women with relatively low RMR's were for the most part (n = 6) obese women characterized by a non-abdominal type of body fat distribution with waist-to-hips girth ratio of 0.75 ± 0.01.

Table 3. Body composition and energy expenditure in subjects characterized by relatively low or high resting metabolic rates per kg fat-free mass.

		Men		Women	
		low (n=7)	high (n=8)	low (n=8)	high (n=8)
weight	(kg)	78.2 ± 3.9	68.8 ± 2.9	85.1 ± 4.1	61.7 ± 3.4***
body fat content	(%)	14.9 ± 1.6	14.5 ± 1.4	40.4 ± 2.4	28.6 ± 2.7**
fat-free mass	(kg)	66.2 ± 2.1	58.7 ± 2.5*	50.3 ± 1.9	43.5 ± 1.3**
resting metabolic rate	(kJ/d.kgFFM)	98.2 ± 0.9	137.8 ± 2.8***	103.1 ± 0.8	152.4 ± 0.7***
diet-induced thermogenesis	(% Ein)	7.65 ± 1.90	7.99 ± 0.78	5.64 ± 0.59	10.15 ± 1.54*
fat oxidation	(10^{-3}.mg/min.kgFFM)	750 ± 71	1087 ± 105*	594 ± 83	1200 ± 97***
CHO oxidation	(10^{-3}.mg/min.kgFFM)	1298 ± 196	1784 ± 172*	1534 ± 150	2186 ± 271
protein oxidation	(10^{-3}.mg/min.kgFFM)	839 ± 54	1123 ± 71*	1113 ± 88	1047 ± 112

Values are given as mean ± sem

kgFFM = kg fat-free mass; Ein = energy content of test meal; CHO = carbohydrate (glycogen or glucose)

* significant difference between low and high groups at $p < 0.05$

** ibidem at $p < 0.01$

*** ibidem at $p < 0.001$

Table 4 shows some comparisons on variables between subjects characterized by a relatively high DIT and subjects with a relatively low DIT. For men, no significant differences between both groups in any of the variables was observed. Women with relatively high DIT's had higher postprandial carbohydrate oxidation rates and RMR's compared to women with relatively low DIT's.

In addition, we compared the measured RMR's in this study with RMR's predicted according to formulas recently published by Owen et al. (6,7). In men, the measured RMR's were 3.9 ± 1.1 percent higher ($p<0.001$) compared to predicted RMR's. Also in women, measured RMR's were systematically higher ($p<0.001$) compared to predicted RMR's, on average by 2.9 ± 1.3 percent.

Table 4. Body composition and energy expenditure in subjects characterized by relatively low or high thermic response to food.

		Men		Women	
		low (n=6)	high (n=6)	low (n=7)	high (n=8)
weight	(kg)	67.8 ± 2.2	73.0 ± 2.2	72.2 ± 7.6	75.0 ± 9.4
body fat content	(%)	12.0 ± 1.6	15.0 ± 1.6	33.1 ± 4.0	33.3 ± 5.0
fat-free mass	(kg)	59.8 ± 2.6	61.7 ± 1.9	46.6 ± 2.2	46.8 ± 2.0
resting metabolic rate	(kJ/d.kgFFM)	107.9 ± 5.9	110.7 ± 3.1	125.3 ± 5.5	142.6 ± 4.7*
diet-induced thermogenesis	(% Ein)	4.51 ± 0.20	12.21 ± 0.46	2.54 ± 0.55	12.06 ± 0.76
fat oxidation	(10^{-3}.mg/min.kgFFM)	568 ± 92	608 ± 186	762 ± 111	877 ± 81
CHO oxidation	(10^{-3}.mg/min.kgFFM)	2690 ± 131	3080 ± 358	1433 ± 237	1974 ± 222*
protein oxidation	(10^{-3}.mg/min.kgFFM)	886 ± 74	942 ± 80	1000 ± 127	1105 ± 90

Values are given as mean ± sem

kgFFM = kg fat-free mass; Ein = energy content of test meal; CHO = carbohydrate (glycogen or glucose)

* significant difference between low and high groups at $p < 0.05$

DISCUSSION

In this study we assessed the nature and magnitude of inter-individual differences in resting energy expenditure and in vivo fuel utilization rates. It was found that sex, the degree of body fatness and type of body fat distribution are important determinants of the amount of energy expended in the postabsorptive resting state, ie, in RMR. Body fatness or body fat distribution did not systematically affect DIT, in contrast to sex. Inter-individual variation in RMR was similar among non-obese men, non-obese women and obese women. Between-person variation in DIT was similar to the intra-individual variation, with slight differences among men, non-obese women and obese women.

The total amount of energy expended in RMR and DIT comprises in the average individual 70-85 percent of total daily energy output (8). The major determinants of resting metabolic rate are body weight and body composition (10), and for diet-induced thermogenesis, energy intake and nutrient composition (17,21).

Important questions to be answered in studies on problems of energy balance in humans are, whether obese individuals would have relatively lower rates of energy expenditure and, secondly, whether subjects susceptible to body weight

gain are also characterized by relatively low rates of energy expenditure. Such a characteristic would be indicative of an increased efficiency of energy utilization, sometimes referred to as an increased "metabolic" efficiency (22). An increased metabolic efficiency has been reported in various animal strains produced by selective genetic breeding (23-25). An increased efficiency of energy utilization among the obese could explain the seemingly anomalous finding of similar energy intakes among the obese and the lean (26-28). However, evidence supporting the hypothesis of increased metabolic efficiency among the obese is far from conclusive (8). This may be caused by a variety of reasons. First of all, it is possible that among lean subjects differences in the efficiency of energy utilization exist. Comparing obese with lean control subjects may then not reveal any differences in the rate of energy expenditure. Secondly, among the obese there may be variation in metabolic efficiency, and only specific groups of obese subjects may be characterized by an enhanced metabolic efficiency. Thirdly, the obese state may be an adaptation to a pre-obese state characterized by a relatively low rate of energy expenditure. Once obesity is established, the relatively low rate of energy expenditure is not longer manifest. The validity of the latter proposition can only be tested in prospective studies of lean individuals with relatively low rates of energy expenditure. Cross-sectional studies among lean and obese subjects, as the study here reported, may be used to assess the nature and magnitude of inter-individual variations in rates of energy expenditure. Such studies may answer the question whether there is variation among the lean and the obese in metabolic efficiency. Such studies may also be of great value for formulating hypotheses about longitudinal outcomes in individuals with differences in metabolic efficiency in terms of body weight gain.

This study shows that variation in indicators (RMR, DIT) of metabolic efficiency is as large among non-obese as among the obese. This means that relatively low rates of energy expenditure are not an exclusive characteristic of obese individuals. This finding confirms recent reports (6,7,10,11). The variation in RMR's per kg fat-free mass was 12 percent with 5 percent of the population having RMR's below 100 kJ/d.kg fat-free mass and five percent of the population with RMR's above 150 kJ/d.kg fat-free mass. These subjects may tentatively be characterized, respectively, as hypo- and hypermetabolic with respect to the RMR. It seems unlikely that differences in the composition of the fat-free mass, intra-individual variation in RMR or measurement error of the assessment of body composition could account for the differences in RMR

per kg fat-free mass between these subjects.

It was found that RMR per kg fat-free mass is significantly lower in men when compared to women. To the best of our knowledge this finding has not been reported before. This result could be caused by a higher proportion of metabolically less 'active' tissues in the total amount of fat-free mass in men when compared to women. RMR is determined for on average 65 percent (29) by organ activity, whereas the metabolic activity of skeletal muscles contributes on average 23 percent to RMR (29). A greater proportion of skeletal muscles in the total fat-free mass in men compared to women may explain the relatively lower RMR's among men. However, no accurate data are available on the proportion of skeletal muscle in the total fat-free mass in men and women to test this hypothesis. It would be interesting to relate RMR to fat-free mass in men or women with a relative hypertrophy of skeletal muscles, ie, in body builders. In addition, to differences in the composition of the fat-free mass between the sexes, it is possible that, at similar composition of the fat-free mass, men have lower rates of energy expenditure than women do. The present study gives no data to test this hypothesis.

In accordance with other findings (5-7,10,11), this study also shows a significant positive relation of the RMR to fat-free mass. On average 64 percent of the total variation in RMR was related to variation in the fat-free mass. The prediction equation of RMR from fat-free mass is very similar to equations published by other investigators (5-7,10,11). RMR's observed in this study were on average 3 percent higher in comparison to RMR's, predicted according to recently published equations of Owen et al. (6,7). Owen et al. show that most prediction equations overestimate RMR by 7-14 percent. Our study supports Owen et al.'s conclusions, although the overestimation of RMR appears to be slightly lower in our study in comparison with Owen et al's. study.

It was observed that in the obese women, in addition to fat-free mass, the size of the fat mass was positively related to RMR. This is in accordance with findings of Hofmans et al. (30). A positive association between the size of the fat mass and the RMR at a given fat-free mass, indicates that, at increased body fat masses, the proportion of metabolically 'active' tisues in the total fat-free mass is relatively enlarged, or that the rate of energy expenditure in tissues comprising the fat-free mass is relatively enhanced. It is possible that in obese individuals with a given fat-free mass, increasing amounts of total body fat cause a higher work load to various organs such as

the heart,lungs or kidneys, or that they cause a relative enlargement of these organs.

We also compared individuals with relatively low RMR's with individuals with relatively high RMR's. It was found, in men and women, that individuals with reduced RMR's were characterized by a relatively enlarged size of the fat-free mass. Again, this may indicate that in these individuals the proportion in the total fat-free body mass of skeletal muscles and other relatively less metabolically 'active' tissues, ie, bone or skin, is relatively increased. We observed that higher RMR's were in particular related to higher rates of fat oxidation per kg fat-free mass. The degree of fat oxidation is under hormonal control (12). Catecholamines increase lipolysis and enhance fat oxidation. Catecholamines may also increase thermogenesis through stimulatory effects on ion pumping or substrate cycling (8). It is possible that the enhanced basal fat oxidation rates in subjects with relatively low RMR's are a reflection of a higher sympathetic activity in these individuals. In women, it was found that the type of body fat distribution significantly affected the RMR. Obese women with a non-abdominal type of body fat distribution were characterized by reduced RMR's compared to abdominal obese women, lean men or lean women. We have recently reported in detail on the impact of body fat distribution in obese women on resting metabolic rate (14). In addition to this study, the present study shows that RMR's in women with non-abdominal obesity were reduced, not only in comparison with age-matched non-obese women, but also in comparison with younger non-obese men and women. The present study does not allow to infer on the mechanism behind this finding. Most likely the differences in adjusted RMR's among the various groups are related to a difference in the endocrinological state. Such a difference could, in its turn, affect directly or indirectly the rate of thermogenic processes in tissues. It is also possible that differences in the composition of the fat-free mass cause the differences in adjusted RMR's. The finding of relatively reduced RMR's among the obese in relation to type of body fat distribution is unique. Relatively reduced rates of energy expenditure have been reported for post-obese women (32). It has also been reported that in wrestlers repeated cycles of weight loss and regain are associated with changes in the RMR per kg fat-free mass (33). Wrestlers, who had experienced repeated cycles of weight gain and loss, had 14 percent lower RMR's per kg fat-free mass in comparison to wrestlers with relatively minor weight fluctuations. This suggests the possibility that periodic reductions in body weight combined with subsequent weight relapse can reduce RMR's. In this study

no difference was observed between abdominal and non-abdominal obese women in self-reported weight history. The majority of women reported to have had several unsuccesful attempts to reduce body weight. We found (14) that non-abdominal obesity is (pre)pubertal in onset, whereas abdominal obese obesity is of the adult-onset type. This suggests that the finding of relatively reduced RMR's in the non-abdominal obese may be of etiological significance for the obesity.

The second component of resting energy expenditure that was investigated in this study is DIT. Several investigators (34,35) have reported that in obese individuals DIT would be blunted in comparison with the non-obese. These findings have, however, not been corroborated by other investigators (5,6,17,22). In the present study no significant difference in DIT was found between non-obese women and obese women.

Variation in DIT for the total population was 36 percent, with five percent of the population having a DIT below 4 percent and five percent having a DIT greater than 12 percent (energy content of test meal). These subjects may tentatively be characterized as subjects with a hyper-efficiency or a hypo-efficiency of postprandial energy utilization, respectively. It seems unlikely that intra-individual variation in DIT can account for the differences in DIT between these subjects.

A further analysis of DIT among the obese in relation to type of body fat distribution revealed that body fat distribution was not associated with differences in DIT. An unexpected finding was the significant difference in DIT between the sexes. Men had a significant higher DIT than women did. This indicates that men have a lower efficiency of postprandial energy utilization compared to women. This finding may be explained by assuming differences in energy intake between the sexes. It has been customary to standardize the energy content of the test meals with respect to the size of the fat-free mass (17,34) or with respect to resting metabolic rate (36). In this study a higher DIT was observed among men compared to women, despite the fact, that men had on average 10 percent lower energy intakes from test meals than women did, either per kg fat-free mass or in percentage of resting metabolic rate. In men the average energy costs of absorbing and processing of the ingested nutrients were calculated at 126 kJ, which is 77 percent of the total thermic response. This figure is in good agreement with data on the relative contribution of the so called 'obligatory' component of the total DIT response (8,15). For women we calculated that the costs of processing the ingested nutrients averaged 108 kJ, which is 99 percent of the total DIT response. This suggests that the

difference between the sexes in DIT is caused by the presence of an additional thermic response in men to food, that is not directly related to the postprandial processing of the nutrients. This component has been referred to as the 'facultative' component of the DIT (8,15). The facultative component of the DIT is thought to be mediated by the activity of the sympathetic nervous system. Food ingestion would increase the activity of the sympathetic nervous system causing an increased turnover of catecholamines. This increase in catecholamines turnover would enhance the rate of energy expenditure of various metabolic processes (8).

These processes may, in particular, be stimulated in skeletal muscles. Recently, it was reported that in men about 50 percent of the increase in thermogenesis induced by the sympathomimeticum ephedrine took place in skeletal muscles (37). Since in men the proportion of skeletal muscles in total body weight is relatively higher than in women, this may offer some explanation for the mechanism behind the observed difference in DIT between the sexes. The difference in DIT between men and women suggests that in comparisons of DIT in lean and obese, analyses should be performed, stratified according to sex, or that groups should be matched in sex distribution.

We also compared individuals with relatively low DIT's with individuals with relatively high DIT's. In men no significant differences in body composition or variables of energy metabolism between both groups were found, suggesting that other factors were involved that caused the difference in DIT beteen both groups, for example, the degree of sympathetic nervous system activity. In women the rate of postprandial glucose oxidation per kg fat-free mass was significantly different between both groups, suggesting that part of the difference in DIT was related to differences in the processing of ingested carbohydrates.

The pattern of fuel utilization in the postabsorptive state and in the postprandial state was similar in men and women. In the basal state, fat oxidation contributed the greatest part to total energy dissipation. This is in accordance with general text book knowledge (12). After meal ingestion, there was a relative increase in glucose oxidation paralleled by a relative decrease in fat oxidation. After meal ingestion, insulin secretion rises rapidly causing a decrease in lipolysis and an increase in carbohydrate oxidation. The postprandial rise in insulin secretion and in carbohydrate oxidation generally lasts for a few hours. During this period the postprandial substrate utilization pattern gradually becomes more similar to the postabsorptive substrate utilization pattern. In accordance with recent

findings (38,39), the present study shows that postprandial net lipogenesis from glucose, ingested in moderate amounts from a mixed meal, is of negligible importance for the storing of ingested carbohydrates.

In conclusion, the present study shows that sex, body fat content and type of body fat distribution are important determinants of RMR. Sex also affected DIT. Inter-individual variation in RMR and DIT was comparable among the lean and the obese. This suggests that among the lean and among the obese there may be individuals with different efficiencies in the utilization of energy.

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Chapter 8.
Resting energy expenditure in women: impact of obesity and body fat distribution

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ABSTRACT

Resting metabolic rate (RMR) and diet-induced thermogenesis (DIT) were repeatedly assessed with an indirect calorimetric ventilated hood system in a group of 32 healthy premenopausal obese women, body fat percentage 46.4 ± 0.9 (mean ± sem), age 38.5 ± 0.9 years. RMR and DIT were also measured in a group of 10 healthy premenopausal non-obese women, body fat percentage 31.3 ± 1.7, age 37.7 ± 2.4 years.

The obese women were subdivided according to the waist-to-hips girth ratio (WHR) into three groups with a different type of body fat distribution. A gluteal-femoral obese group (n=10), WHR < 0.79, an intermediate obese group (n=10), 0,79 < WHR < 0.85, and an abdominal obese group (n=12), WHR >0.85. No significant differences were observed among the obese groups in age, body weight, body fat mass and fat-free body mass. Body fat distribution was not associated with differences in DIT, pre- and postprandial respiratory quotients and substrate oxidation rates. The abdominal obese women had significantly higher RMR's adjusted for age, fat mass and fat-free body mass (6075 ± 200 kJ/d) in comparison to the gluteal-femoral obese women (5502 ± 205 kJ/d), and in comparison to obese women with an intermediate body fat distribution (5517 ± 193 kJ/d), but not in comparison to a non-obese control group, 6790 ± 261 kJ/d. It is concluded that within the total group of obese women, the non-abdominal obese can be characterized by relatively reduced resting metabolic rates in comparison with either abdominal obese women or with non-obese women.

Keywords: indirect calorimetry, resting metabolic rate, diet-induced thermogenesis, obesity, women, body fat distribution.

INTRODUCTION

Obesity is the consequence of a prolonged imbalance between energy intake and energy expenditure, caused by either persistent hyperphagia or a reduced rate of energy expenditure. In spite of much research it has not been possible to define for a given obese individual the relative etiological importance of these factors (1-3). To determine the contribution of hyperphagia or a reduced rate of energy expenditure to the etiology of obesity, prospective studies are needed in which daily food intake and energy expenditure are accurately measured for prolonged periods of time in persons leading their normal lives. Such studies may answer the question whether individuals with similar body weights, body composition and degrees of physical activity have similar or different energy requirements, ie, rates of energy expenditure. There are, however, very few prospective data on energy expenditure (4). The majority of studies are of a cross-sectional nature (5-11), usually involving a comparison of obese individuals with non-obese subjects on one or more components of daily energy expenditure. Generally these studies show that the obese have an enlarged body fat-free mass and, consequently, elevated resting metabolic rates. Resting metabolic rate adjusted for fat-free body mass would, however, be similar among the obese in comparison with the non-obese. Controversy exists on the question whether the obese are characterized by a defective diet-induced thermogenesis. Some investigators (5,6,8,11) report a diminution in diet-induced thermogenesis in obese individuals in comparison with lean controls, whereas other investigators could not confirm these findings (7,9,10). The reason for the discrepancy in results is not clear. It could be that the obese can be subdivided into subgroups with and without reduced diet-induced thermogenesis or decreased resting metabolic rate. In this respect subdividing obesity according to type of body fat distribution may be important (12-14). It was shown that in obese individuals with a relative predominance of fat in the gluteal-femoral region the obesity was of earlier, ie, childhood, onset, whereas in individuals with a relatively greater storage of fat in the abdominal region, the obesity was of adult-onset (12-14). It might be hypothetized that childhood obesity, ie, gluteal-femoral obesity, is more closely associated with decreased energy

expenditure than adult-onset obesity, which might be more environmentally induced. This study was designed to test this hypothesis by assessing the impact of body fat distribution on resting metabolic rate and diet-induced thermogenesis. In addition, resting metabolic rate and diet-induced thermogenesis were measured in age-matched non-obese subjects. Resting metabolic rate and diet-induced thermogenesis were repeatedly assessed in each obese individual to allow for day-to-day within-person variation in diet-induced thermogenesis (13,15).
The results show a significant impact of body fat distribution on resting metabolic rate, but not on diet-induced thermogenesis.

SUBJECTS AND METHODS

SUBJECTS

The obese subjects for this study were invited for participation in the study by advertisements in regional newspapers. Thirty-two of the forty-five obese women, who responded were selected for participation in the study on the basis of their body fat percentage (>35 percent as assessed by densitometry), age (between 20-45 years), premenopausal state, smoking behavior (smoking less than 10 cigarettes/d), health status (no evidence of past or present hypothyroidism, hyperthyroidism, diabetes mellitus) and drug use. Subjects taking medicines known to affect energy metabolism were excluded from the study. Health status was evaluated using a medical questionnaire by a physician (JGAJH). Only those subjects were selected who had not experienced greater weight changes than 2,5 kg in the half year preceding the start of the study. By questionnaire information was obtained in the obese women on nutritional habits, weight history, onset of obesity and family history of obesity. The ten non-obese subjects were members of the University staff, who met the same entry criteria as the obese subjects except for body fat percentage, which had to be under 35 procent. Each individual was fully informed on the nature and purpose of the study and all women gave their written informed consent.
The protocol of the study was submitted to and accepted by the Medical Ethical Committee of the Department of Human Nutrition, of the Wageningen Agricultural University.

METHODS

Experimental design

Resting metabolic rate (RMR) was measured in the morning after an overnight fast, on average three times in the obese individuals (in fifteen obese subjects RMR was measured in duplicate, in seventeen obese subjects RMR four times) and once in the non-obese subjects. It should be noted that the control group was added to the study after the study on the obese subjects was finished. Gas exchange measurements were performed in the obese during spring and in the non-obese during summer. The methodology and procedures of the gas exchange measurements were, however, similar for the obese and the non-obese group. Between each measurement were at least two days, all obese subjects completed the study in three weeks. No attempt was made to measure RMR and DIT in a specific phase of the menstrual cycle. All gas exchange measurements were, however, made at least 3 days after the onset of menstruation. For twelve obese subjects a balanced number (n=2) of gas exchange measurements was available for each period of the menstrual cycle (pre- and post-ovulary). Ovulation was estimated to occur 14 days before the onset of menstruation. No significant differences in RMR and DIT between the two phases of the menstrual cycle were found. Therefore, all RMR and DIT measurements were pooled for each individual and the mean values were used in the analysis. The women were instructed to keep to their normal eating and drinking pattern three to four days preceding the study and to abstain from strenuous physical activity the day immediately before the tests. A dietary history was obtained from each obese woman to assess habitual food consumption. Subjects who ate less than 200 g/d of carbohydrates were instructed to increase the consumption of carbohydrate-rich products, since a relatively low carbohydrate consumption may decrease diet-induced thermogenesis (16). In seventeen (4 gluteal-femoral, 5 intermediate, 8 abdominal) obese subjects diet-induced thermogenesis (DIT) was measured four times for four hours after ingestion of a mixed liquid meal (meal A) on the morning after a corresponding preprandial resting metabolic rate measurement. In fifteen (6 gluteal-femoral, 5 intermediate, 4 abdominal) obese subjects DIT was measured in duplicate for three hours after ingestion of a solid mixed meal (meal B). In the non-obese subjects DIT was measured for three hours after ingestion of a liquid mixed meal (meal C). The cumulative postprandial increase of the premeal RMR was defined as diet-induced

thermogenesis. During the experiment fifteen testmeals of type A, 6 of type B and 5 of type C were chemically analyzed for energy and nutrient content. Meal A was composed of a yoghurt-based liquid meal containing on average 1810 kJ of metabolisable energy (11.3 percent protein, 34.1 percent fat, 54.6 percent carbohydrates). Meal B was composed of a normal Dutch breakfast (bread, margarine, orange juice, cheese, apple syrup) containing on average 1315 kJ (12.4 percent protein, 33.2 percent fat, 54.4 percent carbohydrates). Meal C contained on average 1310 kJ (12.2 percent protein, 30 percent fat, 57.8 percent carbohydrates). Besides differences in the metabolisable energy content, meal A, B and C were almost identical in composition. Meal A, meal B and meal C represented similar caloric loads for the postprandial period, ie, about 450 kJ/h for meal A and about 440 kJ/h for meal B and meal C. The results showed no difference in DIT, expressed as a percentage of the energy content of the test meal between the test meals A and B, ie, 7.03 $\pm$ 0.30 (mean $\pm$ sem) percent for meal A and 6.69 $\pm$ 0.57 percent for meal B. Therefore in the analyses the DIT results of the obese subjects were pooled.
In the obese subjects receiving meal A, every morning before the start of the gas exchange measurements an overnight-fasting venous blood sample of 10 ml was taken for determing serumlipids and blood glucose levels. In the obese subjects receiving meal B every first test morning of the DIT measurement an overnight-fasting venous blood sample of 10 ml was taken. No bloodsamples were taken in the non-obese subjects.

Gas exchange measurements

Resting metabolic rate

On the day of each RMR measurement the subject was taken to the laboratory by car. After voiding they lay supine but awake and motionless on a hospital bed. The subjects wore light indoor clothing. After a 30 minutes rest period with the hood placed over the subjects head RMR was continuously measured between 08.00 and 09.00 hours at an environmental temperature of 23-25°C. During the RMR measurement subjects watched romantic family type motion picture films on video.

Postprandial energy expenditure

After the RMR measurement the subjects were given the test meal. Meal A and

meal C were given through a straw over 3-5 minutes after which postprandial energy expenditure was measured continuously for, respectively, 3 or 4 hours. Meal B was eaten within 10-15 minutes after removement of the hood. Five or three minutes after the meal had been eaten and the hood had been replaced postprandial energy expenditure was measured continuously for three hours. During the postprandial energy expenditure measurement the subjects were awake, remained motionless and watched video tapes. After the gas exchange measurements, urine was collected for determining total urinary nitrogen.
All gas exchange measurements were performed with a ventilated hood system. A transparent perspex ventilated hood was placed over the head of the subject and made airtight around the neck. A pump (Ocean SCL210, Dieren, The Netherlands) drawed fresh outside air through the hood. The flow rate through the hood was measured by a thermal mass flowmeter (Brooks,model 5812 N, Veenendaal, The Netherlands). The flow rate was kept between 30 and 40 l/min by an electrically operated control valve (Brooks, model 5837, Veenendaal, The Netherlands). A small sample of 0.4 l/min of the air leaving the hood was analyzed for oxygen (O_2) and carbondioxide (CO_2) content by, respectively, a paramagnetic O_2-analyzer, adjusted to a full scale range of 20-21 percent (Servomex 1100A, Zoetermeer, The Netherlands) and an infrared CO_2-analyzer, full scale range 0-1 percent (Analytical Development Company SS100, Hoddesdon, UK). The analyzers were calibrated each morning using dried gas mixtures with known O_2 and CO_2 content. O_2 and CO_2 concentrations in dried fresh outside air were measured for five minutes every 30 minutes during the RMR measurements and every hour during the postprandial energy expenditure measurements. Air flow rate, O_2 and CO_2 concentration of the inflowing and outflowing air were computed on line by an automatic data acquisition unit (Hewlett Packard 3497A, Palo Alto, USA) interfaced to a microcomputer (Hewlett Packard 86B or 85, Palo Alto, USA). The outputs of the gas analyzers and the flowmeter were also continuously recorded by a multi-pen recorder (Kipp&Zonen BD101, Delft, The Netherlands). O_2 and CO_2 concentration of the outflowing air and air flow rate through the hood were measured every 30 seconds0 and integrated over five minutes interval periods to obtain O_2 consumption, CO_2 production,
respiratory quotients and metabolic rate according to equations described by Jéquier et al. (17).

Calculation of DIT and substrate oxidation rates

To obtain the mean energy expenditure for each hour after ingestion of the meal, the O_2 consumption and CO_2 production were averaged over each hour using the five minutes interval data. These values were used to calculate the average DIT for the individual hours after the test meal and for the total mean DIT over four or three hours. DIT was calculated by subtracting the RMR value from the respective average hourly or total mean postprandial energy expenditure (PEE), ie, DIT=PEE-RMR. The DIT is expressed in kJ, in a percentage of the increase in the premeal RMR and in a percentage of the energy content of the test meal.
Protein oxidation was calculated from total urinary nitrogen excretion. Carbohydrate and fat oxidation during RMR and the postprandial energy expenditure were calculated using the nonprotein respiratory quotients. Oxygen and carbon dioxide equivalents for substrate oxidation before and after the ingestion of the test meal were obtained from Lillioja et al. (18). A nonprotein respiratory quotient exceeding 1.00 indicated net de novo lipogenesis, ie, lipogenesis exceeding concomitant fat oxidation. Carbohydrate oxidation was corrected for net de novo lipogenesis from glucose as previously described (13).

Body composition and body fat distribution

Fat-free mass was estimated by densitometry. Total body density of each subject was measured by the under water weighing method with simultaneous measurement of residual lung volume by a helium dilution technique. Fat-free body mass was calculated from total body density as previously described (13).
Body fat distribution was assessed in the obese women using the waist-to-hips girth ratio (19). Three categories of body fat distribution were defined, ie, a gluteal-femoral type of body fat distribution when the WHR < 0.79, an intermediate type of body fat distribution with the WHR between 0.79 and 0.85 and an abdominal type of body fat distribution with the WHR > 0.85.

Blood analysis

Blood variables were measured in postabsorptive venous blood samples. Blood glucose was determined by the glucose 4-amino-antipyrene (GOD-PAP) method

with a kit supplied by Boehringer Mannheim GmBH (Almere, the Netherlands). Total serum cholesterol was obtained by the cholesterol-oxidase 4-aminoantipyrene (CHOD-PAP) high performance method with a kit also supplied by Boehringer Mannheim. Serum high density lipoprotein (HDL) cholesterol was determined by a precipitation technique (20). Serum triglycerides were assayed as described by Sullivan et al. (21).

Statistics

The groups of obese individuals subdivided according to type of body fat distribution and the control group of the non-obese subjects were compared on continuous variables using analysis of variance. Analysis of covariance was used to compare among groups RMR and DIT adjusted for fat-free body mass, age and fat mass. Two-sided Student's t-tests were used to evaluate differences in several variables between obese subjects from the highest and lowest quintile of the distribution of DIT (percentage of energy content of test meal) and RMR (kJ/d.kg fat-free mass). All results are expressed as mean $\pm$ sem values.

RESULTS

Table 1 shows some characteristics of the obese women according to type of body fat distribution and for the non-obese women. No significant differences, except in the WHR, were observed among the obese subgroups. On average the abdominal obese had higher body fat and fat-free body masses in comparison to subjects from the other groups, but the differences were not significant. The non-obese subjects had lower values for all variables, except for age, in comparison with the obese.
Table 2 shows serum lipids and blood glucose levels in the obese women according to type of body fat distribution. With increasing WHR total serum cholesterol, serum triglycerides and blood glucose levels increased, whereas the reverse was found for the level of serum HDL-cholesterol. The difference in blood glucose levels among the groups was the most marked.

Table 1. Some characteristics of participating obese and non-obese women.

		Obese			Non-obese	P-value
		Body fat distribution				F-ratio
		Gluteal-femoral	Intermediate	Abdominal		
		WHR* < 0.79	0.79<WHR < 0.85	WHR > 0.85		
		n=10	n=10	n=12	n=10	
Age	(yr)	35.3 ± 2.2	40.7 ± 0.7	39.4 ± 1.4	37.7 ± 2.4	0.19
Body weight	(kg)	89.6 ± 4.6	84.6 ± 4.6	97.7 ± 4.0	64.4 ± 2.5	< 0.0001
Body mass index†	(kg/m^2)	32.2 ± 1.4	31.6 ± 1.1	35.4 ± 1.4	21.9 ± 0.7	< 0.0001
Fat-free mass	(kg)	47.6 ± 1.2	46.6 ± 1.9	50.2 ± 1.5	44.1 ± 1.4	0.05
Fat-mass	(kg)	42.0 ± 3.7	38.0 ± 2.9	47.5 ± 3.1	20.4 ± 1.6	< 0.0001
Fat percentage	(%)	46.2 ± 1.6	44.5 ± 1.3	48.1 ± 1.4	31.3 ± 1.7	< 0.0001
WHR		0.744 ± 0.011	0.821 ± 0.005	0.902 ± 0.012	not assessed	< 0.0001

Values are given as mean ± sem

* waist-to-hips girth ratio

† body weight (kg)/body height^2(m)

Table 2. Serumlipids and blood glucose in obese women subdivided according to body fat distribution.

		Obese			
		body fat distribution			
		Gluteal-femoral	Intermediate	Abdominal	P-value
		n = 9	n = 10	n = 12	F ratio
Cholesterol	(mmol/l)	5.04 ± 0.24	5.34 ± 0.23	5.45 ± 0.23	0.47
HDL-cholesterol	(mmol/l)	1.31 ± 0.11	1.16 ± 0.07	1.07 ± 0.06	0.13
HDL-c/total-c*		0.26 ± 0.02	0.22 ± 0.01	0.20 ± 0.01	0.03
Triglycerides	(mmol/l)	0.91 ± 0.08	1.59 ± 0.18	1.71 ± 0.26	0.03
Blood glucose	(mmol/l)	4.46 ± 0.19	4.82 ± 0.12	5.22 ± 0.16	0.009

Values are given as mean ± sem

* HDL-cholesterol-total cholesterol ratio

No differences in self-reported weight history or family history of obesity were found among the various groups of the obese. However, 80 percent of the gluteal-femoral obese reported an onset of obesity before the age of 20 years in comparison with 50 percent of the subjects with an intermediate type of body fat distribution and 25 percent of the abdominal obese women.
Table 3 shows resting metabolic rate (RMR) diet-induced thermogenesis (DIT) and respiratory quotients (RQ) for the different obese groups and the non-obese group. RMR and postprandial energy expenditure (PEE) (data not shown) differed significantly among the groups, the abdominal obese and the

non-obese subjects had on average higher RMR's and PEE's than subjects with a non-abdominal type of body fat distribution. The RMR expressed in kJ/d.kg fat-free mass, was also significantly different among the groups, but this was not found when the non-obese subjects were excluded from the analysis. Variation among the non-obese subjects in RMR per kg fat-free mass was relatively larger compared to the variation among the other groups. This was due to the fact that three non-obese women had relatively large RMR's per kg fat-free mass (FFM), ie, between 150 and 155 kJ/d.kg FFM. In the other non-obese women RMR's per kg fat-free mass were all between 115 and 140 kJ/d.kg FFM. DIT and RQ's, including the nonprotein RQ's (data not shown), did not differ either among the groups. Pre- and hourly postprandial RQ's were not significantly different among the four groups. At the end of the postprandial measurement period energy expenditure was on average still elevated with about 7-8 percent above the premeal RMR. This was not different among the four groups.

The mean coefficient of variation (CV) for the RMR measurements in the obese was 6.0 $\pm$ 0.5 percent (range, 0.7 - 12.7 percent). The intra-individual day-to-day variation in the obese in DIT of 31.9 $\pm$ 2.7 percent (range, 0.1 to 60.7 percent) was much larger than the within-person variation in RMR. No significant sequence effect, ie, time of measurement effect, on RMR or DIT was observed in the obese subjects for whom repeated measurements were available. The average difference between the mean first RMR, or DIT, and the mean last RMR, or DIT, measurements were, respectively, 2, ie, 4.05 (first RMR) versus 4.13 (last RMR) kJ/min, and 4 percent, ie, 105 (first DIT) versus 109 (last DIT) kJ.

Figure 1 shows the increase in resting energy expenditure in the obese subjects after ingestion of the test meal in comparison with the premeal RMR. The major part of the DIT occurred in the first two hours of the postprandial period, ie, about 70 percent of the total calculated response. No significant differences were observed in response pattern among the various groups.

Table 4 shows pre- and postprandial substrate oxidation rates and nutrient balances for the various groups. None of these variables, except the glucose balance differed significantly among the groups.

Obese individuals who were either in the highest quartile of the distribution of RMR (kJ/d.kg fat-free mass) or in the highest quartile of the DIT (percentage of the energy content of the test meal) distribution were compared on various variables with subjects who were in the lowest quartiles of the corresponding distributions. Table 5 lists the results of these comparisons.

Individuals with a relatively high RMR were characterized by a more abdominal type of body fat distribution (p=0.05).

Table 3. Resting metabolic rate, diet-induced thermogenesis and pre-and postprandial respiratory quotients in obese and non-obese women.

		Obese			Non-obese	P-value
		Body fat distribution				F ratio
		Gluteal-femoral	Intermediate	Abdominal		
		n=10	n=10	n=12	n=10	
RMR	(kJ/min)	3.95 ± 0.14	3.78 ± 0.13	4.46 ± 0.19	4.35 ± 0.14	0.01
RMR	(kJ/d.kgFFM)	119.5 ± 3.7	117.8 ± 3.7	128.4 ± 3.8	140.2 ± 5.5	0.0005
RQ - preprandial		0.83 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	0.84 ± 0.01	0.62
RQ - postprandial		0.86 ± 0.01	0.87 ± 0.01	0.88 ± 0.01	0.87 ± 0.02	0.86
DIT	(kJ)	109 ± 15	108 ± 6	110 ± 11	112 ± 27	0.99
DIT (%ME-intake)		7.0 ± 0.	7.2 ± 0.5	6.6 ± 0.6	8.3 ± 2.0	0.70
DIT (%RMR)	(%)	13.2 ± 1.3	13.9 ± 0.9	11.3 ± 1.0	12.1 ± 2.6	0.62

Values are given as mean ± sem
RMR = resting (preprandial) metabolic rate; RQ = respiratory quotient;
DIT = diet-induced thermogenesis; ME-intake=metabolisable energy intake;
kgFFM = kg fat-free mass

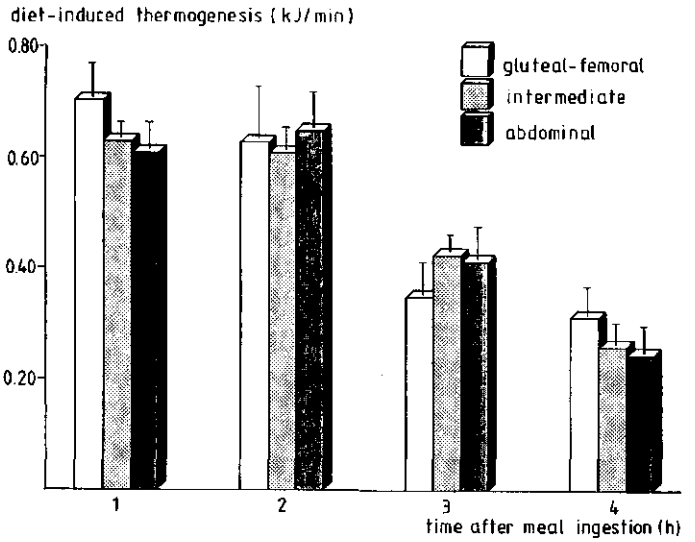

Figure 1. Diet-induced thermogenesis (mean ± sem) in obese women subdivided according to body fat distribution.

Table 4. Pre- and postprandial substrate oxidation rates and postprandial nutrient balances in obese and non-obese women.

		Obese			Non-obese	P value F ratio
		Body fat distribution				
		Gluteal-femoral n = 9	Intermediate n = 10	Abdominal n = 11	n = 10	
- Preprandial oxidation:						
Protein	(mg/min.kgFFM*)	1.17 ± 0.09	1.19 ± 0.03	1.26 ± 0.06	1.18 ± 0.13	0.85
Fat	(mg/min.kgFFM)	0.74 ± 0.10	0.84 ± 0.08	0.97 ± 0.09	0.97 ± 0.17	0.44
Glucose	(mg/min.kgFFM)	1.76 ± 0.19	1.46 ± 0.15	1.57 ± 0.15	2.09 ± 0.26	0.11
- Postprandial oxidation:						
Fat	(mg/min.kgFFM)	0.49 ± 0.09	0.60 ± 0.07	0.58 ± 0.09	0.80 ± 0.16	0.24
Glucose	(mg/min.kgFFM)	3.26 ± 0.20	2.95 ± 0.21	3.33 ± 0.26	3.46 ± 0.40	0.61
- Nutrient balance†:						
Protein	(g)	- 1.0 ± 0.8	- 1.2 ± 0.6	- 2.9 ± 0.9	- 0.6 ± 1.3	0.27
Fat	(g)	8.1 ± 0.7	7.3 ± 1.0	8.0 ± 1.2	5.4 ± 1.3	0.31
Glucose	(g)	22.4 ± 2.5	25.1 ± 2.2	23.8 ± 2.4	13.9 ± 3.5	0.03

Values are given as mean ± sem

* equals postprandial protein oxidation

† intake (g) - cumulative oxidation (g)

In addition, RMR's and DIT's adjusted for fat-free body mass, age and fatmass, were compared among groups differing in body fat distribution. The RMR, extrapolated to 24-hour and adjusted for these covariates was significantly higher in the abdominal obese when compared to the gluteal-femoral obese, respectively 6075 ± 200 versus 5502 ± 205 kJ/d ($p=0.04$). Also in comparison with subjects with an intermediate type of body fat distribution, the abdominal obese had higher adjusted RMR's, ie, 6075 ± 200 versus 5517 ± 193 kJ/d ($p=0.05$). The adjusted RMR in the non-obese group was 6790 ± 261 kJ/d, which was significantly ($p<0.01$) higher than the adjusted RMR's in the obese with either a gluteal-femoral or intermediate type of body fat distribution. No significant difference in adjusted RMR was found between the non-obese and the abdominal obese. Excluding the three control subjects with relatively high RMR's per kg fat-free mass from the analysis did not produce a significant change in results. After the exclusion of these subjects from the analysis, adjusted RMR's were respectively, 5399 ± 201 kJ/d in the gluteal-femoral obese, 5455 ± 190 kJ/d in the obese with an intermediate type of body fat distribution, 5995 ± 180 kJ/d in the abdominal obese and 6329 ± 311 kJ/d in

the control women (n=7). The differences between, on the one hand, the abdominal obese and non-obese women, and, on the other hand, the other two obese groups, remained significant at the $p < 0.05$ level. No differences were found among the groups in DIT, adjusted for these covariates.

Table 5. Resting metabolic rate, diet-induced thermogenesis and various other variables in obese women categorized in the highest and lowest quartiles of the distribution of parameters indicative of efficiency of energy metabolism.

		RMR - kgFFM *				DIT-% ME-INTAKE †			
		Lowest n=8		Highest n=9		Lowest n=8		Highest n=8	
RMR	(kJ/d/kgFFM)	106.6	1.4	138.4	2.1 ‡	113.2	2.8	125.2	5.5
DIT (% ME intake)	(%)	6.57	0.59	7.58	0.57	4.50	0.38	9.49	0.34 ‡
Fat-free mass	(kg)	50.0	1.5	47.0	2.1	51.5	1.5	47.6	1.7
Fat mass	(kg)	41.3	2.9	43.5	4.4	44.1	3.1	46.5	4.6
WHR §		0.81	0.01	0.86	0.02‡	0.84	0.03	0.81	0.03
- Preprandial oxidation:									
Fat	(mg/min.kgFFM)	0.63	0.09	1.04	0.07‡	0.76	0.11 ‖	0.87	0.14
- Postprandial oxidation:									
Glucose	(mg/min.kgFFM)	2.81	0.20	3.79	0.17‡	2.62	0.30 ‖	3.34	0.24

Values are given as mean ± sem

* resting metabolic rate expressed per kg fat-free mass

† diet-induced thermogenesis expressed in percentage of metabolisable energy content of test meal

‡ significant ($p<0.05$) difference among groups

§ waist-to-hips girth ratio

‖ fat and glucose oxidation rates assessed in 7 subjects from the lowest quartile.

DISCUSSION

Obesity is a complex disorder, whose etiology is incompletely understood in the absence of clear hormonal or genetic disorders. It is difficult to characterize the obese as individuals with reduced energy requirements or energy expenditure. Some investigators report a defective diet-induced thermogenesis in obese individuals, whereas others could not observe a reduced diet-induced thermogenesis in obese individuals in comparison with lean controls (5-11). The controversy on defective thermogenesis in the obese might be due to variation among the obese in energy expenditure. The study here reported has investigated this issue. It is unique in two respects. In the first place, it studies the impact of body fat distribution on variation in energy expenditure in a relatively large group of obese women, and, secondly,

in each obese individual, energy expenditure was repeatedly assessed to obtain a more precise estimate of the individuals 'true' DIT and RMR. In addition, energy expenditure was measured in age-matched non-obese women.

The results show no clear differences among the obese and non-obese women in diet-induced thermogenesis. Resting metabolic rate, adjusted for fat-free body mass, age and fatmass was, however, significantly lower in obese women with a gluteal-femoral or intermediate type of body fat distribution in comparison with obese women with an abdominal type of body fat distribution and the non-obese women. The results of this study support the hypothesis that body fat distribution affects energy expenditure in obesity.

Currently, only one study has investigated the relation between body fat distribution and energy expenditure in obesity (13). The study of Den Besten et al. (13) showed in a limited number of subjects that body fat distribution did not significantly influence diet-induced thermogenesis and postabsorptive resting metabolic rate. However, a trend was observed toward higher RMR's (kJ/d.kg fat-free mass) in the abdominal obese in comparison with the non-abdominal obese. The present study uses a considerable larger group of subjects and more measurements of energy expenditure in each subject than did the study of Den Besten et al (13). In addition, values for RMR and DIT were obtained in a non-obese control group.

The average within-person day-to-day variation in RMR in the obese was relatively small in comparison to the variation in DIT. The within-person variation in RMR observed in this study was comparable to values found by investigators who studied RMR in respiration chambers in individuals on controlled diets (14). Suprisingly few studies report on reproducibility of DIT measurements (13,15). Both studies report high within-person day-to-day variation in DIT, ie, of about 30-60 percent. A high within-person variation in DIT means that studies comparing relatively small groups of randomly selected obese individuals with randomly selected lean controls might have had insufficient statistical power to detect meaningful physiological differences in DIT between obese and lean subjects.

Pre-, and total postprandial energy expenditure were on average significantly lower in the non-abdominal obese in comparison with the abdominal obese or the non-obese subjects. Also when the RMR (kJ/d.kg fat-free mass) was compared among the groups it was found that the abdominal obese and the non-obese had still higher pre- and postprandial rates of energy expenditure.

Respiratory quotients showed a characteristic pattern. Fasting RQ's were between 0.80 and 0.85, which is in good agreement with findings from other

studies (5,6,13). In the fasting state about 40 percent of the energy dissipated was derived from fat oxidation, and about 25 percent from protein oxidation. In the postprandial state about 60 percent of the energy expended was derived from glucose oxidation. This is in agreement with findings of Flatt et al. (22) who showed that carbohydrate intake elicits a prompt increase in the rate of glucose oxidation.

Nutrient balances were on average also similar among the different obese groups, indicating no differences among the groups in oxidative and non-oxidative storage of ingested carbohydrates and fat.

A comparison of obese subjects characterized by clear differences in DIT and RMR's revealed that it was difficult to characterize subjects with a relatively high DIT from subjects with a relatively low DIT. Age, body composition and body fat distribution did not differ significantly among these subjects. Obese individuals with a higher DIT had on average slightly, but not significantly, greater postprandial glucose oxidation rates than obese subjects with a lower DIT. Increased cellular oxidation of glucose might be associated with increased transport of glucose into the cells for nonoxidative storage, ie, formation of glycogen or fat. These latter two processes require more energy than oxidation of glucose and could, thereby, contribute to an increased DIT (23). In addition, it has been reported that DIT is only partly determined by the metabolism of the ingested macronutrients. Activation of the sympathetic nervous system by ingestion of nutrients might also be important in determining the level of a subject's DIT (2). No information was obtained in the present study on activation of the sympathetic nervous system by food ingestion.

Obese individuals with relatively high RMR's were characterized, in particular, by relatively high preprandial fat oxidation rates, high postprandial glucose oxidation rates and a more abdominal type of body fat distribution. When RMR's adjusted for age, fat mass and fat-free mass (4) were compared among the different groups of body fat distribution, it was found that with an increasing waist-to-hips girth ratio these adjusted RMR's also increased. Thus, RMR's adjusted for age, fat mass and fat-free mass were on average 11 percent (about 0.6 MJ/d) lower in the gluteal-femoral obese and the obese with an intermediate type of body fat distribution when compared to the abdominal obese. The DIT adjusted for these covariates did not differ significantly among the three groups of obese individuals. No differences in adjusted DIT's were found between the obese and the non-obese subjects, whereas adjusted RMR's were highest in the non-obese subjects. The relatively

high adjusted RMR's among the non-obese can be partly attributed to the fact that three of the non-obese had relatively high RMR's per kg fat-free mass. It is possible that RMR in these women was elevated, for example, due to anxiety caused by the RMR measurement or due to some undetected minor spontaneous movements during the RMR measurement. However, excluding these women from the analysis, did not produce a significantly different result.

The reasons for the difference in RMR adjusted for age, fat mass and fat-free mass among the various groups are not directly clear. Fasting energy expenditure at rest is determined largely by the metabolic activity of the organs and to a smaller extent by the metabolic activity of skeletal muscles (22). It could be that the proportion of organs in the total fat-free mass is higher in the non-obese in comparison with the obese, and that among the obese this proportion is higher in the abdominal obese than in the obese with a non-abdominal type of body fat distribution. It is also possible that the metabolic activity of the organs differs among the various groups, for example, by differences in rates of ion pumping, protein turnover or various substrate cycles, which could be related to differences in hormonal status or activity of the sympathetic nervous system. However, information on the metabolic activity of these processes in relation to body fat percentage and body fat distribution is lacking or scarce.

It is well known from in vitro studies (25) that the metabolic activity of abdominal fat is higher than the activity of gluteal-femoral fat, but whether this difference is associated in vivo with higher rates of energy expenditure in abdominal fat than in gluteal-femoral fat is unknown. Even when the activity of the triglyceride-free fatty acid substrate cycle is relatively elevated in abdominal fat, it seems unlikely that this could explain the total difference of more than 10 percent in adjusted RMR's among the obese subgroups. It has also been found that skeletal muscles in abdominal obese contain relatively more glycolytic fast twitch fibers (26). This might, even in resting conditions, lead to an increased output of lactate from skeletal muscles in the abdominal obese than in the non-abdominal obese. Lactate may then be converted by the liver into glucose at the expense of 4 moles of ATP for 1 mole of glucose recirculated in this way (23). This recycling of lactate could also elevate resting metabolic rate. Finally, it has been reported that the abdominal obese have higher levels of plasma free testosterone in comparison with non-abdominal obese (27). This difference in hormonal profile may cause the difference in RMR's adjusted for age, fat-free mass and fat-mass among the various subgroups of the obese.

This study joins others (25,26) in demonstrating a relationship between body fat distribution and serum lipids and blood glucose. With increasing waist-to-hips girth ratio serum triglycerides, serum cholesterol and blood glucose levels increased, whereas HDL-cholesterol levels decreased. Fasting blood glucose levels indicated no major perturbations in glucose tolerance or insulin resistance in the different obese groups. However, to evaluate insulin resistance and glucose tolerance it would have been better to measure insulin and glucose levels in response to the test meals.

In conclusion, the present study shows that DIT may show considerable intra-individual day-to-day variation suggesting that in studies of small groups of obese individuals it may be necessary to assess DIT repeatedly in each individual. Body fat distribution did not have an impact on DIT. However, RMR's adjusted for age, fat-free body mass and fat mass were significantly higher in the abdominal obese in comparison with the non-abdominal obese. This study also showed that adjusted RMR's in the abdominal obese were not abnormally high in comparison with the adjusted RMR's in a non-obese control group. Our findings suggest that the non-abdominal obese are the obese with reduced resting metabolic rates and, as a corollary, energy requirements. These individuals, in particular the gluteal-femoral obese, also reported a significant earlier onset of obesity than the abdominal obese. It is not clear from this study whether the reduced resting metabolic rates in the non-abdominal obese women are related to their earlier onset of obesity in comparison with the abdominal obese.

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Chapter 9.
Alcohol and its acute effects on resting metabolic rate and diet-induced thermogenesis

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ABSTRACT

In two studies the impact of alcohol (ethanol) on resting energy expenditure was determined. In a first study the thermic effect of ethanol on resting metabolic rate (RMR) was assessed in ten non-obese men, age 26.5 ± 1.7 (mean ± sem) years. Energy expenditure was measured with a ventilated hood system. After an initial RMR measurement, subjects received in random order 20 g of ethanol in three concentrations, respectively, of 7.5, 18 and 30 ml/100 ml water. The thermic effect of ethanol was then continuously measured for 90 minutes. Ethanol had a significant thermic effect, which varied, depending on its concentration, between 0.22 kJ/min (7.5%) to 0.30 kJ/min (30%). However, no significant differences in thermic effect among the three concentration were observed. In a second study the impact of ethanol on diet-induced thermogenesis (DIT) was assessed in twelve non-obese men, age 27.3 ± 1.5 years. After an initial RMR measurement, subjects received in random order, in duplicate, either a meal of food (2.00 MJ, 13 percent protein, 30 percent fat, 57 percent carbohydrates) plus an alcoholic aperitif (20 g in 18 ml/100 ml) or an iso-energetic meal of food alone (2.55 MJ, 13 percent protein, 30 percent fat, 57 percent carbohydrates) plus a placebo aperitif, similar in taste and appearance to the alcohol aperitif. DIT was then continuously measured for a period of 4 hours. No significant differences in DIT between the treatments were observed. After the meal plus alcohol, substrate oxidation rates of glucose and fat were significantly reduced in comparison with the meal

alone. At the postprandial period during which ethanol oxidation was at its maximum, there was a slight potentiation of DIT by ethanol.

Keywords: indirect calorimetry, alcohol, ethanol, thermic effect, resting metabolic rate, diet-induced thermogenesis, substrate oxidation, human

INTRODUCTION

In most Western countries total per capita alcohol consumption has increased dramatically over the last few decades (1,2). A recent nation-wide food consumption study in the Netherlands showed that alcohol contributes on average 3 percent to total daily energy intake in women and 5.4 percent in men aged 22-49 years (3). Similar figures have been reported for men and women in other Western countries (4,5).
The health effects of excessive alcohol intake are well documented (6-8), but the metabolic consequences and relation to the incidence of disease of moderate alcohol use remain to be definitely assessed. In this respect there is a general interest in the utilization of alcohol as an energy source. The issues that receive most attention are whether alcohol calories are added to or substituted for non-alcoholic calories in the diet and whether energy intake from alcohol is related to increased levels of adiposity in the same way as energy intake from non-alcoholic sources.
The majority of observational studies show a striking phenomenon. Alcohol usually provides additional energy to the diet, but its use is not associated with higher levels of adiposity (9-13). In concordance with these findings are the results of various experimental studies (14-16). In these studies it was found that iso-energetic substitution of non-alcoholic calories by alcohol resulted in weight loss in volunteers and that alcohol added to the diet did not result in weight gain. These findings suggest that alcohol is used less efficient as an energy source than, for example, fats and carbohydrates, or that alcohol interferes with the efficient utilization of these substrates.
However, results from metabolic studies assessing the impact of alcohol ingestion on energy expenditure give equivocal support for the latter proposition. In the classical report of Atwater and Benedict it was

shown that alcohol use did not increase energy expenditure in man relative to fats or carbohydrates (17). More recent studies did observe either an increase in, or no effect on oxygen consumption after alcohol use (18-21). The reasons for these discrepant findings are unclear, but in general the experimental design, ie, number and sex of subjects studied, duration of study, amount of alcohol given and way in which alcohol was provided and the technique used to measure energy expenditure differ considerably among the various studies. Thus, in spite of its importance as an energy source in the average adult individual, little is known with certainty on the impact of alcohol on overall energy metabolism. In particular, there is little data on the effect of alcohol on diet-induced thermogenesis (19,21).
In this report we used the ventilated hood technique to assess the thermic effect of ethanol in varying concentrations. In addition, we investigated the impact of ethanol on diet-induced thermogenesis. The results show a significant thermic effect of ethanol on fasting energy expenditure, but no systematic potentiation of diet-induced thermogenesis by ethanol.

SUBJECTS AND METHODS

The present study consisted of two parts. In the first study the impact of ingestion of ethanol on postabsorptive resting metabolic rate (RMR) was studied. In a second study we assessed the effect of ethanol on diet-induced thermogenesis (DIT).

SUBJECTS

Eighteen male subjects were invited for participation in the studies by an advertisement in the weekly periodical of the University and through friends and relatives. Four subjects participated in both experiments. Subjects were all, except one, students or members of staff of the University. After applying for participation subjects received a letter in which the purpose and nature of the experiment(s) were explained. They also received two questionnaires, one for assessing past and present health status and one for obtaining information on habitual eating, drinking, smoking and exercise habits. The medical questionnaire

was evaluated by a physician (JGAJH). After the subjects gave their written consent to participate and when they fulfilled the entry criteria, subjects could participate in the experiments. Subjects had to be apparently healthy (no past or present evidence of hypo- or hyperthyroidism or diabetes mellitus), non-obese (body fat percentage below 25 percent) and had to have moderate smoking (less than 10 cigarettes/d) or drinking habits (no more than 5 alcoholic consumptions/d). None of the subjects used drugs known to affect energy metabolism, none was on a diet, all subjects were consuming normal balanced meals and all subjects had had weight fluctuations within 2.5 kg for at least six months prior to the start of the study. The protocol of the studies was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

Thermic effect of ethanol

In this study ten subjects received on three occasions at different days an oral dose of 20 g ethanol dissolved in either 335 ml water (7.5 percent solution), 140 ml water (18 percent solution) or 85 ml water (30 percent solution) after an initial RMR measurement. To these solutions were, respectively, added 1.2 g of a nonnutritive sweetener (Natrena, Bayer GmbH, Germany) and 0.012 mg of aniseed oil (to the 7.5 percent solution) or 0.8 g of the sweetener and 0.006 mg of aniseed oil (to the 18 and 30 percent solutions) to improve sensory characteristics (mimicking Anisette). The ethanol solutions were drunk at a temperature between 10-15°C within 10 minutes and with the ventilated hood removed from the subjects heads. After the alcohol ingestion the hood was replaced and energy expenditure was measured continuously for another 90 minutes. Energy expenditure was measured with a ventilated hood system as previously described (22). Subjects received the three ethanol solutions either early in the morning after an overnight fast or, early in the morning after a fasting period of at least 4.5 hours after a standardized breakfast of about 2 MJ. In a separate study in 5 subjects

(data not shown) the thermic effect of drinking 350 ml water plus 1.2 g of the sweetener was assessed. Water plus sweetener did not induce a thermic effect. Between each session was a time-interval of at least two days. Subjects received the three ethanol solutions in randomized order.

Impact of ethanol on diet-induced thermogenesis

In this study twelve subjects received on four different days in the afternoon two treatments in duplicate, ie, either a test treatment consisting of the ingestion of an alcoholic aperitif (containing 20 g of ethanol in an 18 percent solution (v/v)) and a yoghurt-based mixed liquid test meal (containing 1.96 MJ, 13 percent protein, 27 percent fat, 60 percent carbohydrates) or a placebo aperitif (containing zero calories) and an iso-energetic yoghurt-based mixed liquid test meal (containing 2.55 MJ, 13 percent protein, 27 percent fat, 60 percent carbohydrates). To both aperitifs flavorings were added to mimick Anisette. To the alcoholic aperitif was added 0.8 g of a nonnutritive sweetener (Natrena, Bayer GmbH, Germany) and 0.06 mg of aniseed oil. To the placebo aperitif were added 0.8 g of the sweetener, 0.06 mg of aniseed oil, 0.03 mg of kinin and 0.007 mg of maltol. In a separate control experiment in 8 subjects (data not shown) the thermic effect of kinin in a much larger dose (220 mg in 350 ml water) was tested. Kinin did not induce a significant thermic effect. The impact of maltol on energy expenditure was not studied. The quantity used was extremely low and no effects on energy expenditure were expected. The aperitifs were given at a temperature between 10-15°C after RMR had been measured for at least 1 hour, and with the hood removed from the subjects. After ingestion of the aperitif (within 10 minutes), the hood was replaced over the subject's head. Fifteen minutes later subjects received the liquid test meal through a straw which was passed through the hood. The meals were given at room temperature and were eaten within 5 minutes. Energy expenditure was then continuously measured for 4 hours. Control and test treatments were assigned to the subjects according to a Latin square design.
Between treatments was a time-interval of at least two days.

Energy exchange measurements

Energy expenditure of subjects resting in a hospital bed in a supine position was measured with a ventilated hood system. Details of the measurement procedure and conditions have been previously published (22,23). Spontaneous movements of the subjects were unobtrusively registered by means of a load cell (Tokyo Sokki Kenkyujo, TKA-200A, Tokyo, Japan) placed under one of the legs of the hospital bed. Once every hour, during calibration of the gas analyzers with fresh outside air, subjects were allowed to move briefly for 2-3 minutes.
Subjects whose energy expenditure was measured in the afternoon were instructed to have a small breakfast (< 2 MJ) before 8.00 hours and to avoid coffee drinking, sleeping and moderate or heavy exercise on the morning before the gas exchange measurements. Under these conditions, RMR and DIT measured in the afternoon do not differ systematically from RMR and DIT measured in the morning (22). After the gas exchange measurements urine was collected for determining urea-nitrogen excretion.
Energy expenditure was calculated according to Jéquier et al. (24). The thermic effect of ethanol was calculated as the difference between the average energy expenditure over a period of 90 minutes and the corresponding baseline RMR. DIT with or without ethanol was calculated as the difference between the average hourly and total (4 hours) postprandial energy expenditure and the corresponding baseline RMR.

Substrate oxidation rates and nutrient balances

Substrate oxidation rates and nutrient balances were calculated as previously described (22-24). In addition, the contribution of ethanol oxidation to total oxidation was estimated. Alcohol oxidation was assumed to be equal to the average elimination rate of alcohol from the blood, ie, 100 mg/kg body weight/hour (25).
Generally about 93-98 percent of an ingested dose of alcohol is eliminated from the body by oxidation and negligable amounts leave the body by respiration and in the urine (25). We calculated the amount of oxygen needed to oxydize 1 g of ethanol (1.4594 l) and the amount of carbondioxide thus produced (0.973 l). Total oxygen consumption and carbondioxide production were subsequently corrected for the volumina of

oxygen and carbondioxide associated with ethanol oxidation. These corrected gasvolumina were used in the calculation of the oxidation rates of fats and carbohydrates. It should be noted that there may be considerable inter-individual difference in the elimination rate of alcohol from the blood (25). However, since in this study each person is measured repeatedly under different conditions such variation will probably not bias conclusions on within-person comparisons of treatments.

Body composition

Body fat mass and body fat-free mass were estimated from total body density using Siri's formula (26). Total body density was measured in each subject by hydrostatic weighing with simultaneous determination of residual lung volume by a helium dilution technique.

Statistics

The thermic effect of ethanol was evaluated using two-sided paired t-tests.
Analysis of variance was used to test differences among the three treatments (7.5, 18 and 30 percent solutions) in thermic effect. In the second study, results of the duplicate measurements were pooled and averaged for both treatments. Analysis of differences between both treatments were performed using two-sided paired t-tests. Correlation coefficients were calculated as Pearson's product-moment correlation coefficients.
Results are expressed as mean $\pm$ sem.

RESULTS

Table 1 gives some characteristics of the subjects participating in the two experiments.

Table 1: Age, body weight, body fat percentage and habitual alcohol use of the subjects.

	Study 1 Thermic effect of alcohol n = 10			Study 2 Effect of alcohol on diet-induced thermogenesis n = 12		
	Mean	sem	Range	Mean	sem	Range
Age (yrs)	26.5	1.7	22 - 41	27.3	1.5	21 - 41
Body weight (kg)	76.8	3.2	66.2 - 102.0	78.3	3.0	58.8 - 101.8
Body fat content (%)	17.0	1.1	13.0 - 24.7	16.0	1.2	7.9 - 22.5
Habitual alcohol use *	1.2	0.2	0.0 - 3	1.4	0.4	0.0 - 5

* number of alcoholic beverages habitually used/d

The average was 27 ± 1.5 years (range: 21-41 years), body fat percentage was on average 16.5 ± 1.2 percent (range: 7.6-24.7 percent). Three subjects had a body fat percentage between 20 and 25 percent, but none was obese. Habitual use of alcoholic beverages was on average 1-2 units/d (range: 0-5).

Table 2 shows metabolic rate, oxygen consumption, carbondioxide production and respiratory quotients in subjects before and after ethanol ingestion in three different concentrations. None of the variables was significantly different among the three treatments. Alcohol ingestion induced a significant increase in metabolic rate and in oxygen consumption, but not in carbondioxide production. As a consequence respiratory quotients decreased significantly after alcohol use. On average, metabolic rate increased 4.4 percent after intake of 7.5 percent ethanol solution, 4.8 percent after ingestion of the 18 percent ethanol solution and 6.2 percent after use of alcohol in a 30 percent solution. These values did not differ significantly.

Since we did not observe significant differences in thermic effect among iso-energetic ethanol solutions of varying concentration, the effect of ethanol on DIT was studied in the second study using the moderately concentrated solution of 18 percent.

Table 3 gives pre- and postprandial metabolic rate, oxygen consumption and carbondioxide production in subjects who received in duplicate either a meal with or without ethanol as an aperitif.

Metabolic rate, oxygen consumption and carbondioxide production increased significantly after ingestion of the meal either with or without alcohol. No significant difference was found between both treatments in the increase in metabolic rate or in oxygen consumption. The postprandial rise in carbondioxide was, however, significantly smaller on the test treatment in

Table 2: Metabolic rate, oxygen consumption, carbondioxide production and respiratory quotients in subjects before and after ingestion of a 20 g ethanol dosis in three different concentrations.

	ETHANOL					
Volume percent	7.5		18		30	
	Before	After	Before	After	Before	After
Metabolic rate (kJ/min)	4.98 ± 0.13	5.20 ± 0.16*	4.95 ± 0.21	5.19 ± 0.24†	4.85 ± 0.22	5.15 ± 0.24†
O_2 Consumption (ml/min)	247 ± 7.0	261 ± 8.3*	245 ± 11.3	260 ± 12.5†	241 ± 11.0	258 ± 12.1†
CO_2 production (ml/min)	206 ± 5.7	204 ± 6.1	206 ± 6.5	203 ± 7.4	201 ± 9.3	200 ± 10.3
Respiratory quotient	0.84 ± 0.02	0.78 ± 0.01‡	0.85 ± 0.02	0.78 ± 0.01‡	0.84 ± 0.02	0.77 ± 0.01‡

Values are given as mean ± sem

* Difference between 'before' and 'after' significantly different from zero at $p < 0.05$

† Ibidem at $p < 0.01$

‡ Ibidem at $p < 0.001$

Table 3: Metabolic rate, oxygen consumption, carbondioxide production, respiratory quotient and diet-induced thermogenesis in subjects before and after ingestion of a test meal with or without a dose of 20 g ethanol given as an aperitif.

	Meal + ethanol*		Control - meal†	
	Before	After	Before	After
Metabolic rate (kJ/min)	5.34 ± 0.23	6.25 ± 0.25	5.34 ± 0.26	6.12 ± 0.27
O_2 Consumption (ml/min)	267 ± 11	312 ± 12	267 ± 13	300 ± 13
CO_2 Production (ml/min)	216 ± 9	251 ± 11	216 ± 11	263 ± 11
Respiratory quotient	0.81± 0.01	0.80 ± 0.01	0.88 ± 0.01	0.88 ± 0.01‡
DIT (kJ)	-	219 ± 14	-	185 ± 20
DIT-% ME (%)	-	8.6 ± 0.6	-	7.2 ± 0.8

Values are given as mean ± sem

DIT = diet-induced thermogenesis; % ME = percentage of metabolisable energy intake

* Energy intake: 2.545 ± 0.005 MJ (22.7 percent derived from ethanol)

† Energy intake: 2.551 ± 0.004 MJ

‡ Postprandial respiratory quotients significantly different between treatments ($p = 0.002$)

comparison to the control treatment. Respiratory quotients remained at their preprandial level after ingestion of a meal with ethanol, whereas after ingestion of a meal without ethanol respiratory quotients showed a significant increase. On average, DIT was about 16 percent higher on the test treatment in comparison with the control treatment. The difference in DIT between the treatments was, however, not significant.

Figure 1 shows average hourly DIT values separately for both treatments.

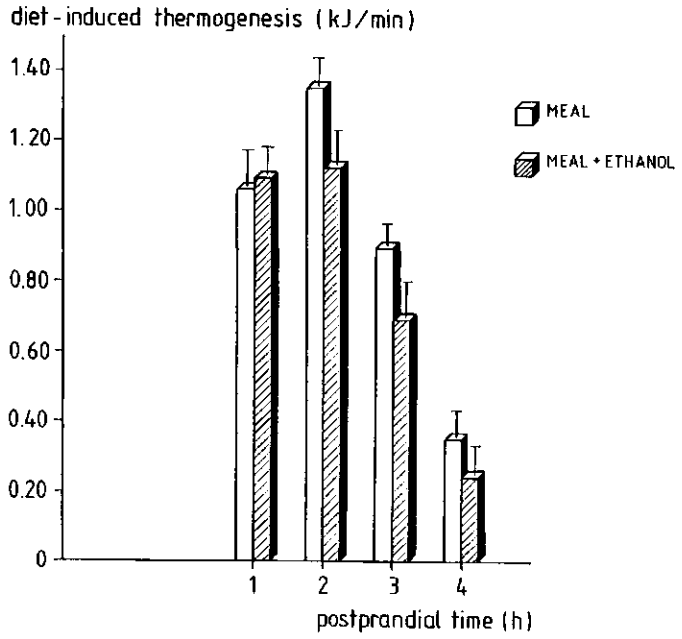

Figure 1. Diet-induced thermogenesis (mean ± sem) in subjects after ingestion of iso-energetic meals with or without ethanol.

During the second, third and fourth hour, DIT was higher on the test treatment compared to the control treatment, but only for the second hour the difference reached statistical significance ($p=0.03$).

Figure 2 gives respiratory quotients for RMR and for the course of the DIT measurement separately for both treatments.

Significant differences were found between both treatments in respiratory quotients during the first, second and third hour of the postprandial period.

Table 4 gives energy and nutrient intake, and postprandial metabolism of the ingested nutrients for both treatments. Glucose and fat oxidation rates were significantly lower ($p < 0.001$) on the test treatment in comparison with the control treatment. Postprandial protein oxidation was not systematically affected by ethanol ingestion. On the control treatment subjects oxydized on average about 50 percent of the carbohydrate and fat intake. During a 4 hours postprandial period, when food was eaten with ethanol, about 40 percent of the

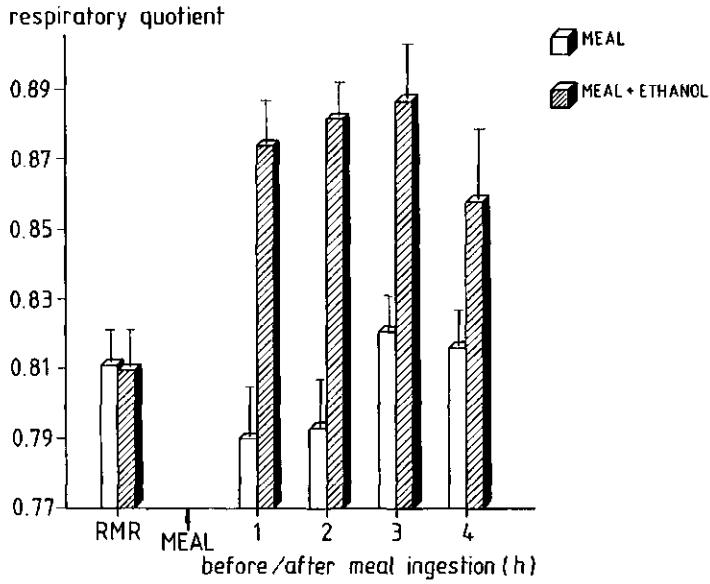

Figure 2. Respiratory quotients (mean ± sem) in subjects before and after ingestion of iso-energetic meals with or without ethanol.

glucose intake and about 20 percent of the fat intake were subsequently oxydized. Total energy retention was not significantly different between both treatments.

On the test treatment, net lipogenesis occurred in nine subjects during the first postprandial hour (1.9 ± 0.7 g) and in eight subjects during the second hour (1.9 ± 0.7 g). Net lipogenesis was of negligable importance in the subjects on the control treatment.

Table 4: Nutrient and energy intake, utilization and storage in the body over a period of 4 hours in subjects after ingestion of a test meal with or without a dose of 20 ethanol given as an aperitif.

	Energy		Carbohydrate		Fats		Protein		Ethanol
	M+E	M-E	M+E	M-E	M+E	M-E	M+E	M-E	M+E
	kJ	kJ	g	g	g	g	g	g	g
Intake	2543 ± 5	2550 ± 4	68.7 ± 0.1	90.8 ± 0.1	14.1 ± 0.0	18.6 ± 0.0	15.0 ± 0.0	19.8 ± 0.0	19.9 ± 0.1
Oxidation			29.8 ± 3.5	47.5 ± 3.3*	3.1 ± 1.5	9.2 ± 1.6†	17.5 ± 1.5	18.8 ± 1.4	19.8 ± 0.3
Expenditure	1500 ± 60	1469 ± 65							
Storage	1093 ± 61	1081 ± 66	38.9 ± 3.5	43.3 ± 3.3	11.0 1.5	9.4 ± 1.6	-2.6 ± 1.5	1.0 ± 1.4*	0.1 ± 0.0

Values are given as mean ± sem

M+E = Meal with ethanol; M-E = meal without ethanol

* Significant difference between M+E and M-E at $p < 0.01$

† Significant difference between M+E and M-E at $p < 0.05$

DISCUSSION

Thermic effect of ethanol

The results of this study show that ethanol in a moderate amount of 20 g induces in fasting individuals a significant increase in oxygen consumption and metabolic rate, but not in carbondioxide production. The increase in metabolic rate was not significantly related to the concentration of the ethanol solution.

Ethanol induced an increase in metabolic rate of about 4-6 percent of the ingested caloric load (about 0.6 MJ). This is similar or slightly lower to the thermic effect of fats or carbohydrates (27). The results of this study support the findings of Perman (18) and Rosenberg and Durnin (19), but are in contrast with the observations of Stock and Stuart (21) and Barnes et al. (22). It is, however, difficult to compare these studies, because of differences in experimental design and in the technique used for assessing alcohol-induced thermogenesis. In contrast to the present study, none of the aforementioned studies employed the ventilated hood technique to assess energy expenditure. This technique is particularly suitable for measuring energy expenditure accurately over prolonged periods of time, ie, over several hours, and has been recommended for use in metabolic studies assessing thermogenesis (28).

In the present study we gave 20 g of ethanol dissolved in varying amounts of water with added flavorings. This enabled us to study the impact of ethanol in

different concentrations on energy expenditure. Perman (18) and Rosenberg and Durnin (19) gave ethanol, respectively, in a dosis of 0.29 g/kg dissolved in 150 ml of chilled fruit juice or in a dosis of 23 g as 230 ml of red wine. Barnes et al. (22) and Stock and Stuart (21) used whisky in a dosis of 1.5 ml/kg and in a quantity of about 80 ml, respectively. Besides differences among these studies in the amount of ethanol given, the concentration of the ethanol solutions and the amount of non-alcoholic calories varied. For example, red wine and fruit juice may contain carbohydrates, which can provoke an increase in energy expenditure (27).

For the present study we first tested a quantity of 30 g ethanol (18 percent) and found that this amount, drunk within 10 minutes, induced drowsiness and symptoms of mild intoxication in fasting individuals. Since drowsiness may interfere with a correct assessment of metabolic rate, we opted for the smaller dosis of 20 g. In fasting subjects such an amount of ethanol should quickly raise the blood ethanol concentrations to values between 5 and 15 mmol/l (29). It was expected that the more concentrated solutions would lead to higher maximal blood alcohol curves (29). At higher blood alcohol levels an increased metabolism of ethanol by the microsomal ethanol oxydizing system (MEOS) occurs (14). Oxidation of ethanol by MEOS will result in a less efficient coupling (about 20 percent reduction in net ATP production) of oxidation to ATP synthesis and an increased demand for NADPH compared to oxidation by the alcohol-dehydrogenase (ADH) pathway. The latter pathway operates fully at lower blood alcohol concentrations (14). This may affect thermogenesis in two ways. A relative increase in ethanol oxidation by MEOS may increase the rate of ethanol oxidation to meet the tissues demand for ATP, secondly, the eventual NADPH repletion may increase energy, ie, ATP demand, from other metabolic pathways. Both processes could lead to higher oxygen consumption and hence increased thermogenesis. However, we did not find a significant relation between the thermic effect of ethanol and its concentration. Nonetheless, at lower ethanol concentrations (7.5 and 18 percent) the thermic effect of ethanol was on average 4.6 percent versus 6.2 percent at the highest ethanol concentration. This is a relative difference of about 30 percent and could be partly attributed to an increase in rate of ethanol oxidation at the highest ethanol concentration to compensate for the less efficient metabolism of ethanol, ie, a relatively greater part by MEOS than by the ADH pathway. However, the increase in the thermic effect of ethanol at the highest ethanol concentration was not a systematical phenomenon and did not reach statistical significance. It is also possible that the

different solutions did not result in clear differences in blood ethanol concentrations and hence in ethanol metabolism. Since blood alcohol curves were not measured we can not put this explanation to empirical test.
In this study it was also found that ethanol ingestion and its subsequent oxidation significantly reduced basal respiratory quotients. It is well known that ethanol oxydizes with a respiratory quotient of 0.666, which is much lower than respiratory quotients usually found in overnight fasting individuals (30).

Impact of ethanol on diet-induced thermogenesis

Very few studies have assessed the effect of ethanol on diet-induced thermogenesis (19,21). Rosenberg and Durnin (19) found no significant difference between the average increase in metabolic rate over 3 hours after an iso-energetic meal of food or of food plus alcohol. However, in the latter study, during the last 30 minutes of the 3 hours postprandial period the increased oxygen consumption was significantly greater after the meal including the alcohol. Stock and Stuart (21) found that whisky (837 kJ) taken with a meal produced a 22 percent increase in oxygen consumption which was significantly greater than the 13 percent increase caused by consuming an iso-caloric non-alcoholic meal. This effect was observed during the entire postprandial period. In both studies, however, at the end of the postprandial period energy expenditure was still elevated with more than 20 percent above the premeal baseline energy expenditure, indicating that the total thermogenic response was not completely measured.
In this study we compared thermogenesis induced by a iso-energetic meal of food alone and of food plus alcohol. Alcohol was given as an aperitif to minimize effects of food on the absorption of ethanol from the gastro-intestinal tract. Control and test treatments were carried out in duplicate to obtain a more precise estimate of an individuals response of energy metabolism to food. In previous studies it was shown that diet-induced thermogenesis is prone to considerable within-person variation (22,31). Postprandial measurements were carried out over a period of 4 hours, which was sufficiently prolonged in duration to allow an almost complete assessment of the thermogenic response. Thus, energy expenditure averaged over the fourth hour of the postprandial period was on average 4.5 percent elevated above the corresponding premeal baseline energy expenditure on the control treatment and 6.5 percent on the test treatment.

In the present study overall diet-induced thermogenesis was not potentiated by ethanol ingestion. On average, in particular during the last three hours of the postprandial period, DIT values were, however, slightly higher on the test treatment than on the control treatment. The maximum potentiation of DIT was observed during the second and third hour of the postprandial period. After ethanol consumption it takes about 30-45 minutes in fasting subjects to reach the maximal blood ethanol concentrations (29). Depending on the total amount of ethanol ingested ethanol, elimination will take several hours. It can be calculated that in this study most of the ethanol will have been eliminated between a period of 30-150 minutes after ingestion of the meal, which was given 15 minutes after the aperitif. During this period ethanol oxidation contributed on average 3.8 kJ/min to total energy expenditure. On average between 55 and 65 percent of total energy expended was derived from ethanol oxidation.

Analysis of the substrate oxidation rates revealed that ethanol was preferentially oxydized in comparison with fats and carbohydrates, but not in comparison with proteins. In the first 180 minutes of the postprandial period, glucose and fat oxidation were markedly reduced, respectively, on average by about 22 g and 7 g after ingestion of a meal of food plus alcohol in comparison with a meal of food alone. This means that during this period more fats and carbohydrates were stored on the test treatment when compared to the control treatment. Increased storage of nutrients prior to oxidation after a meal will increase postprandial energy expenditure (30). When fats and carbohydrates are oxydized without prior storage, about 2 percent of the energy content of these substrates has to be expended to provide ATP for handling costs. In contrast, if fats are stored prior to oxidation total energy dissipated for handling this process is about 4 percent of the energy content of fat. For glucose stored as glycogen or as fat the costs for processing are, respectively, about 7 and 24 percent of the energy content of glucose (30). The average difference in handling costs of the postprandial processing of the nutrients was calculated at about 0.2-0.3 kJ/min for the first, second and third hour of the postprandial period. Figure 1 shows that for this period differences were found between both treatments in energy expenditure on average of -0.03, 0.23 and 0.21 kJ/min, respectively. In particular, the values for the second and third hour are close to what was expected. Probably, over the first hour of the postprandial period differences in storage of nutrients between both treatments are small. This could be due to a relatively low ethanol oxidation especially in the first half of this

period. It has also been found (27) that nutrient-induced thermogenesis cannot solely be explained in terms of the biochemistry of energy expenditure associated with the processing of ingested nutrients (30). Part of nutrient- or diet-induced thermogenesis, ie, about 30-40 percent, is thought to be mediated by an increased activity of the sympathetic nervous system after food or nutrient ingestion (32). The latter part of the total thermogenic response is usually designated with the term facultative thermogenesis, indicating that this part of thermogenesis might not be operating at the same level in each individual, or that it might be susceptible to environmental stimuli. It is possible that nutrient-induced facultative thermogenesis is effectively operative in the period immediately after food ingestion (0-60 minutes) and that ethanol reduces the amount of energy expended by the facultative mechanism.

The results of this study on the thermic effect of ethanol and the impact of ethanol on DIT do not indicate that moderate amounts of ethanol are less efficiently used as an energy source in comparison to fats or carbohydrates. We therefore tentatively conclude that the observation that alcohol provides additional energy to the diet, but that its use is not associated with increased levels of adiposity, is due to methodological problems of accurately quantifying energy and alcohol intake in free-living humans, or of the accurate quantition of other factors than diet and alcohol that affect energy balance or body weight in man, for example, of the degree of physical activity.

In conclusion we can state that ethanol consumed in a moderate amount has a significant thermic effect, probably, similar to fats or carbohydrates. No potentiation by ethanol ingestion of diet-induced thermogenesis was found. Ethanol fed in combination with a mixed liquid meal had a substantial impact on substrate oxidation and storage rates, in particular during the period at which ethanol oxidation was at its maximum. During this period ethanol ingestion slightly increased diet-induced thermogenesis. Overall postprandial energy retention was, however, similar between a iso-energetic meal of food plus alcohol and of food alone. Substituting moderate amounts of calories from fats and carbohydrates by alcohol in non-alcoholics is not expected to decrease energy retention significantly or to be useful as an adjuvant in weight reducing regimes.

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Chapter 10.
Does palatability effect the postprandial response of energy expenditure?

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ABSTRACT

The impact of palatability on diet (DIT)- and sucrose (SIT)-induced thermogenesis was studied in two experiments with 24 healthy young normal-weight subjects, 12 men and 12 women. In the first study, subjects received at random, in duplicate, either a normal liquid test meal (2000 kJ, 12 percent protein, 33 percent fat, 55 percent carbohydrates) or an iso-energetic unpalatable test meal with kinin. Palatability did not have a significant impact on postprandial metabolism. Integrated DIT values were, respectively 165 $\pm$ 15 (mean $\pm$ sem) kJ for the control treatment and 185 $\pm$ 14 kJ for the test treatment. In the second study, subjects received at random either a palatable sucrose solution (900 kJ), an iso-energetic standard sucrose solution or an iso-energetic unpalatable sucrose solution. Kinin and a citrusflavor were used to vary palatability. Postprandial energy expenditure over a period of 150 minutes was not significantly affected by differences in palatability. In both studies, men had a higher rise in postprandial energy expenditure than women did, in particular, after the control meal and after the unpalatable sucrose solution. A separate control experiment to assess the effect of kinin on energy expenditure was carried out in 8 subjects. Kinin had no significant effect on energy expenditure over a period of 120 minutes after ingestion.

Keywords: indirect calorimetry, energy expenditure, palatability, diet-induced thermogenesis, postprandial, sucrose, human.

INTRODUCTION

Food intake evokes a prompt increase in energy expenditure, known as diet-induced thermogenesis, the thermic effect of food or postprandial thermogenesis. The postprandial rise in energy expenditure depends largely on the amount of energy and type of nutrient ingested (1,2). Generally, the thermic effect of food increases linearly with energy intake (2) and is larger for proteins and carbohydrates than for fats (1). In the average individual about 10 percent of the total daily energy output would be associated with food ingestion (3). This represents a considerable loss of energy for the individual. Therefore it is not surprising that interest arose in diet-induced thermogenesis, in particular, at the suggestion that obese individuals would expend less energy after meal ingestion when compared to lean subjects (4). Postprandial energy expenditure can be largely attributed to the energy needed for processing the ingested nutrients (1). However, the total observed thermic response to food or nutrients can not completely be understood in terms of the biochemistry of energy expenditure of the postprandial processing of nutrients (1,5). Part of the response, about 30-40 percent, is thought to be mediated by a diet-induced increase in the activity of the sympathethic nervous system (5). This latter part of the thermogenic response involves facultative thermogenic mechanisms, which may vary between individuals or in response to environmental stimuli. The determinants of diet-induced facultative thermogenesis in man are largely unknown. Degree of adiposity (6), aerobic capacity (7), type of nutrient (8) and sensory characteristics (9) of food may be important.

Information on the impact of sensory characteristics of food on diet-induced thermogenesis is scarce. Meal ingestion is usually associated with olfactive, gustative and cognitive stimulation (10), which may affect the activity of the sympathetic nervous system. It is possible that meals differing in palatability result in different sensory stimulation and, hence, induction of metabolic processes involved in facultative thermogenesis.

Recently, LeBlanc et al. (9) reported that diet-induced thermogenesis was significantly elevated on an iso-energetic palatable meal in comparison with an unpalatable meal. In this study, however, the palatable and unpalatable meal varied extremely in water content, which could have caused the observed difference in dietary thermogenesis (11). Differences in diet-induced thermogenesis between palatable and unpalatable meals have been associated with differences in insulin responses (9). After ingestion of an unpalatable

meal the insulin response was significantly reduced in comparison with a palatable meal (9). This phenomenon may be related to differences in the early, ie, pre-absorptive or cephalic insulin response (10,12-14). After ingestion of an unpalatable meal the cephalic insulin response would be reduced or absent. Blocking of the early insulin response was found to reduce the thermic effect of infused glucose (15).
In this report we describe two studies assessing the impact of palatability on diet-induced thermogenesis. In the first study, iso-energetic meals identical in energy content, nutrient composition and water content, but differing in palatability were compared. In the second study, iso-energetic sucrose solutions of varying palatability were tested. This study was performed to investigate differences in postprandial thermogenesis between a palatable and unpalatable nutrient solution with a particular strong impact on postprandial insulin and glucose homeostasis.
The results of these studies show that palatability did not significantly affect postprandial energy expenditure.

SUBJECTS AND METHODS

The total experiment consisted of several studies, two metabolic studies for determining the impact of palatability on postprandial energy expenditure and a sensoric study to assess the palatability of the meals and sucrose solutions to be used in the energy expenditure studies. In addition, a control study was performed to measure the impact of kinin on energy expenditure. Kinin was used for decreasing palatability.

SUBJECTS

Subjects were recruited for participation in the studies by an advertisement in the weekly periodical of the University. In addition, some subjects were contacted for participation through student organisations. In total 36 subjects applied for participation. Subjects received a letter informing them on the nature and purpose of the experiments. In addition, they received two questionnaires, one for obtaining information on past and present health status and one for getting information on eating, drinking, smoking and exercise habits. The medical questionnaire was evaluated by a physician

(JGAJH). For both studies 24 subjects were needed, 12 women and 12 men. Subjects were selected for participation when they had a body fat percentage less than 25 percent for men or less than 35 percent for women, did not smoke or smoked less than 10 cigarettes/d, were apparently healthy, were consuming normal balanced diets and had had no major weight fluctuations (more than 2.5 kg) within a period of six months prior to the study. In addition, the selected subjects had to show consistent preference ratings in the sensory evaluation of the test meals and solutions. This means that they had to be able to discriminate consistently between the various meals and solutions. All subjects were students at the University, none of the subjects was on a diet or used drugs known to affect energy expenditure. All subjects participated in only one energy expenditure study. Before the start of the studies subjects gave their written informed consent to participate. The protocol of the study was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

Sensory evaluation

Before the start of the energy expenditure studies, the taste preference of each subjects for the various test meals and sucrose solutions was evaluated in a sensoric study.

Three different test meals were prepared, identical in energy content, nutrient composition, texture and water content. Liquid yoghurt-based test meals were used with an energy content of 4.12 MJ/kg (12 percent protein, 33 percent fats, 55 percent carbohydrates). Meals were varied in palatability either by adding 0.10 g/kg of a coloring agent (E-151, brillant black) or by adding the coloring agent plus 0.79 g/kg of a 10 percent solution of kinin in ethanol. A meal without these added substances served as a reference meal. Sucrose solutions (143 g/l) were varied in palatablity either by adding 0.63 g/kg of a 10 percent kinin solution in ethanol or by adding 0.63 g/kg of a commercially available citrusflavor on ethanol basis (Baukje, Rijssen, the Netherlands). A standard sucrose solution was used for reference purposes. After being instructed in the standard sip-and-spit procedure, subjects

received the various stimuli for pairwise comparison in small plastic cups (approximately 15 ml) at room temperature. Tests were carried out in the afternoon with the subjects in a 5-6 hours postabsorptive state. Thorough rinsing between the samples was emphasized. For each study all 6 permutations of the 3 items were used and the order of presentation of stimulus pairs was randomized across subjects. In addition, to the pairwise comparisons of the various stimuli subjects had to rank their preference for each item on a standard nine-point hedonic preference scale that ranged from 'dislike extremely' (1) to 'like extremely' (9). Results of the sensory evaluation showed that the meal with added coloring agent was not sufficiently disliked in comparison with the standard meal. Therefore, the impact of palatability on meal-induced thermogenesis was investigated with two meal types only, ie, the standard meal and the meal with coloring plus kinin. Differences in palatability among the three sucrose solutions were statistically significant.

Impact of palatability on meal-induced thermogenesis

In this study 12 subjects, 6 males and 6 females, received in the morning, after an overnight fast, on four different days two times an iso-energetic meals in duplicate, either the palatable standard test meal with an energy content of 2.00 MJ (12 percent protein, 33.1 percent fat, 54.9 percent carbohydrates) or the unpalatable test meal. Meals were given at room temperature after an initial measurement of resting metabolic rate of at least one hour. Energy expenditure was assessed with a ventilated hood system as previously described (16). Meals were given through a straw which was passed through the ventilated hood. Meals were ingested within five minutes. Energy expenditure was then continuously measured for 3.5 hours. Meals were given to the subjects according to a Latin square design to balance the order of treatment with the type of treatment. No attempt was made to measure the women in a specific phase of the menstrual cycle. In a previous study (17) we had not found a significant impact of the stage of the menstrual cycle on postprandial energy expenditure. Measurements were, however, always carried out at least three days after the onset of menstruation. Between the measurements was an interval of at least two days.

Impact of palatability on sucrose-induced thermogenesis

In this study 12 subjects, 6 men and 6 women, received on three days in the afternoon three iso-energetic solutions consisting of a standard sucrose solution (0.90 MJ), a highly unplalatable sucrose solution or a highly palatable sucrose solution. Subjects were measured in the afternoon for practical reasons. Subjects were instructed to have a small breakfast (< 2 MJ) before 8.00 hours and to avoid coffee drinking, smoking, sleeping and moderate or heavy exercise on the morning before the energy expenditure measurements. Under these conditions resting metabolic rate and diet-induced thermogenesis do not show significant diurnal variation (16). Sucrose solutions were given at room temperature after an initial measurement of baseline postabsorptive energy expenditure of at least one hour. Sucrose solutions were given through a straw, which was passed through the ventilated hood and were drunk within 5 minutes. Energy expenditure was then continuously measured for 2-2.5 hours. Because of the relatively low energy content of the sucrose solutions (< 1MJ) a shorter duration of the postprandial measurements was chosen. Similar to the other study, subjects recieved sucrose solutions in a random order according to a Latin square design. Also no attempt was made to measure the women in a particular phase of the menstrual cycle. Between the tests was a time interval of at least two days.

Control study using kinin

In 8 subjects, 3 men and 5 women, recruited from students and staff working in the laboratory, the impact was assessed on energy expenditure of 0.22 g of a 10 percent (v/v) kinin solution in ethanol, given either in 350 ml of water or in a gelatin capsule ingested with 350 ml of water. Resting metabolic rate was measured after an overnight fast for at least one hour. After the RMR measurement subjects received the kinin solution or capsule with water in a random order. Energy expenditure was then assessed continuously for 120 minutes. All subjects considered the kinin solution as extremely unpleasant. No sensory stimulation was induced by the ingestion of the kinin capsule. Thus the impact of kinin on energy expenditure could be assessed controlling for its sensory-mediated effects. Between the tests was an interval of at least two days.

Gas exchange measurements

Energy expenditure was measured with a ventilated hood system in subjects reclining in a supine position in a hospital bed. Details of the measurement procedure and conditions have been published previously (16). In addition, movements were objectively registered using a load cell (TKA-200A, Tokyo Sokki Kenkyujo Co Ltd, Tokyo, Japan) connected to one of the legs of the bed. Once every hour during calibration of the oxygen analyzer with fresh outside air, subjects were allowed to move briefly for 2-3 minutes. After the gas exchange measurements for assessing the meal- or sucrose-induced thermogenesis in relation to palatability, urine was collected for determining urea-nitrogen excretion.

Calculations of energy expenditure and substrate oxidation

Energy expenditure was calculated according to Jéquier et al. (18). The thermic effect of the various test meals and solutions was defined as the integrated increase in postprandial energy expenditure above the corresponding preprandial baseline energy expenditure after ingestion of the test food. Substrate oxidation rates and postprandial nutrient balances were calculated as previously described (16).

Body composition

Body fat content was estimated from total body density using Siri's formula (19). Total body density was measured in each subject participating in the two major studies by hydrostatic weighing with simultaneous assessment of residual lung volume with a helium dilution technique.

Statistics

Pairwise comparisons of test stimuli were statistically evaluated using the chi-square statistic. Preference ratings for each stimuli were compared using paired t-tests with a two-tailed region of rejection and a confidence level of 95 percent. Treatments were compared in the meal study using paired t-tests on the pooled average results of the duplicate measurements. In the sucrose study, treatments were compared using analysis of variance.

RESULTS

Table 1 gives age and some characteristics of the subjects participating in the various experiments. Subjects participating in the palatability studies

Table 1. Age, body weight and body fat percentage in subjects participating in studies.

	Study 1 palatability - DIT		Study 2 palatability - SIT		Study 3 Control - kinin	
	men n = 6	women n = 6	men n = 6	women n = 6	men n = 3	women n = 5
Age (yrs)	22.7±0.8	21.2±0.8	22.2±0.3	21.8±1.1	35.3±6.8	23.4±0.8
Body weight (kg)	71.8±2.9	60.6±2.0	74.2±1.6	61.4±2.1	71.8±3.1	60.0±0.8
Body fat content (%)	12.0±1.0	29.0±1.1	12.4±1.5	28.0±2.5	16.5±2.8	29.1±2.1

Results are given as mean ± sem
DIT = diet-induced thermogenesis; SIT = sucrose-induced thermogenesis

had ages between 18 and 25 years, normal body weights and body fat percentages. The men who participated in the kinin study were on average slightly older compared to the other men, ages were, respectively, 28, 29 and 49 years, but none of these men was obese.

Table 2. Preference rating of the various test meals and sucrose solutions.

Meal type	Study 1 palatability - DIT n = 12		Solution type	Study 2 palatability - SIT n = 12	
1. standard meal	6.4 ± 0.2	p<0.001	1. standard sucrose	5.5 ± 0.3	p<0.001
2. standard + color	6.6 ± 0.3		2. standard + flavor	6.6 ± 0.3	
3. standard + color + kinin	1.8 ± 0.3	p<0.001	3. standard + kinin	1.8 ± 0.2	p<005, p<0.001

Results are given as mean ± sem
DIT = diet-induced thermogenesis; SIT = sucrose-induced thermogenesis
Preferences were rated on a standard nine-point hedonic scale ranging from 'dislike extremely (1) to 'like extremely' (9)

Table 2 gives the results of the sensory evaluation of the various test meals and sucrose solutions. For the meals a significant difference in preference rating was found between the standard meal and the meal with added coloring plus kinin only. Therefore, it was decided to study the impact of palatability on diet-induced thermogenesis with these meal types only. Preference ratings differed significantly among the three sucrose solutions. The largest differences occurred between the standard solution and the solution with citrusflavoring on the one hand, and the solution plus kinin on the other hand.

Table 3. Diet-induced thermogenesis, postprandial respiratory quotients and substrate oxidation rates in subjects receiving meals of differing palatability.

		palatable meal	unpalatable meal
		n = 12	
DIT	(kJ)	166 ± 15	185 ± 14
DIT - %/ME	(%)	8.3 ± 0.7	9.0 ± 0.7
RQ 0 - 60 min		0.90 ± 0.01	0.89 ± 0.01
60 - 120 min		0.93 ± 0.01	0.93 ± 0.01
120 - 180 min		0.86 ± 0.01	0.84 ± 0.01
180 - 210 min		0.83 ± 0.01	0.81 ± 0.01
Glucose oxidation	(mg/min.kgFFM)	3.4 ± 0.17	3.7 ± 0.23
Fat oxidation	(mg/min.kgFFM)	0.7 ± 0.06	0.8 ± 0.09
Protein oxidation	(mg/min.kgFFM)	1.4 ± 0.09	1.5 ± 0.09

Results expressed as mean ± sem
DIT = diet-induced thermogenesis; %ME = percent of energy intake;
RQ = respiratory quotients; kgFFM = kg fat-free mass

Table 3 gives diet-induced thermogenesis, postprandial respiratory quotients and substrate oxidation rates in subjects after the palatable and unpalatable test meal. None of the variables differed significantly between the treatments. Baseline energy expenditure rates (resting metablic rate) and respiratory quotients did not differ significantly between the treatments. Resting metabolic rates and respiratory quotients were, respectively, 4.58 ± 0.25 kJ/min and 0.83 ± 0.01 before ingestion of the palatable meal and 4.68 ± 0.25 kJ/min and 0.82±0.01 before the unpalatable meal.
Figure 1 gives the time course in DIT after ingestion of a palatable or an unpalatable meal. No significant differences were observed in integrated hourly DIT values between both treatments.

There was a surprising sex difference in DIT. On both treatments DIT was higher in men than in women, respectively, 203 ± 11 kJ compared to 128 ± 16 kJ ($p=0.005$) for the palatable meal and 210 ± 16 kJ versus 159 ± 18 kJ ($p=0.06$) for the unpalatable meal. The difference between men and women occurred entirely in the first two hours of the postprandial period.

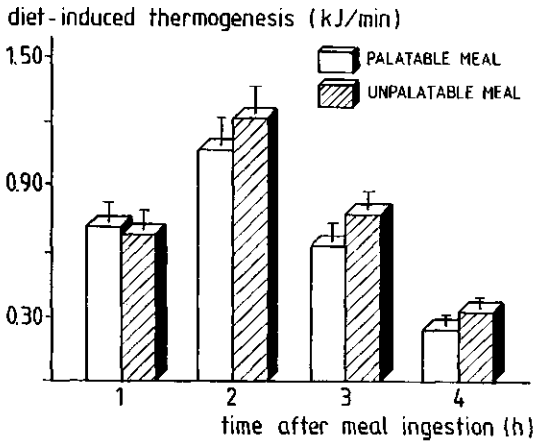

Figure 1. Diet-induced thermogenesis (mean ± sem) over 4 hours in subjects after ingestion of a palatable or unpalatable meal.

Table 4 gives sucrose-induced thermogenesis, postprandial respiratory quotients and substrate oxidation rates in the subjects after ingestion of the three sucrose solutions. None of the variables differed significantly among the treatments. Baseline energy expenditure rates and respiratory quotients were similar for the three treatments, respectively, 4.81 ± 0.26, 4.95 ± 0.33 and 5.03 ± 0.30 kJ/min, and 0.81 ± 0.01, 0.80 ± 0.01 and 0.80 ± 0.01. Figure 2 gives the time course of the sucrose-induced thermogenesis for the various treatments. No differences in time course among treatments were observed. SIT was reduced to zero for most subjects after two hours. A significant sex difference in SIT was observed only for the unpalatable sucrose solution, 43 ± 5 kJ in men versus 26 ± 4 kJ in women ($p=0.03$).

Table 4. Sucrose-induced thermogenesis, postprandial respiratory quotients and substrate oxidation rates in subjects receiving sucrose solutions of differing palatability.

		Most palatable	Standard solution n = 12	Unpalatable solution
SIT	(kJ)	44 ± 9	42 ± 12	34 ± 4
SIT-%ME	(%)	4.9 ± 1.0	4.6 ± 1.3	3.8 ± 0.5
RQ 0 - 60 min		0.88 ± 0.02	0.93 ± 0.01	0.91 ± 0.02
60 - 120 min		0.85 ± 0.01	0.87 ± 0.01	0.88 ± 0.01
120 - 150 min		0.84 ± 0.02	0.86 ± 0.01	0.86 ± 0.01
Glucose oxid	(mg/kgFFM.min)	3.0 ± 0.26	3.6 ± 0.26	3.2 ± 0.23
Fat oxid	(mg/kgFFM.min)	0.9 ± 0.12	0.6 ± 0.12	0.6 ± 0.06
Protein oxid	(mg/kgFFM.min)	1.6 ± 0.14	1.5 ± 0.14	1.5 ± 0.09

Results are expressed as mean ± sem
SIT = sucrose-induced thermogenesis; %ME = percent of energy intake;
RQ = respiratory quotients; kgFFM = kg fat-free mass

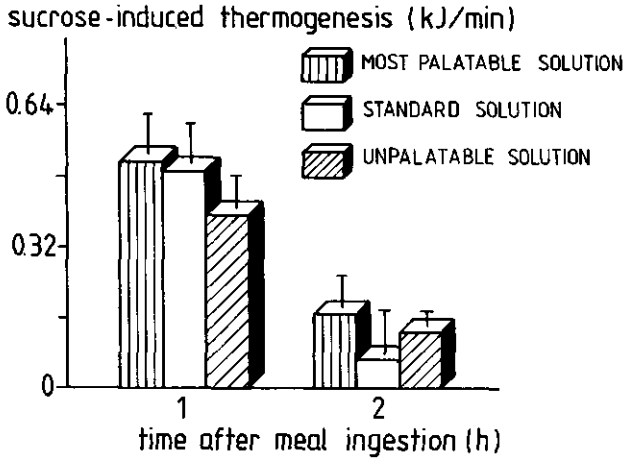

Figure 2. Sucrose-induced thermogenesis (mean ± sem) in subjects after ingestion of sucrose solutions of different palatability.

Table 5. Resting metabolic rate and respiratory quotients in subjects before and after kinin ingestion.

	Kinin solution * n = 8		Kinin capsule † n = 8	
	before	after	before	after
Resting metabolic rate (kJ/min)	4.23 ± 0.36	4.32 ± 0.36	4.05 ± 0.35	4.17 ± 0.32
Respiratory quotient	0.81 ± 0.01	0.79 ± 0.02	0.85 ± 0.03	0.81 ± 0.02‡

Results are expressed as mean ± sem

*** Kinin was given in a dosis of 0.22 g (10% ethanol solution v/v) in 350 ml water**

† Kinin was given in gelatin capsule in a dosis of 0.22 g (10% ethanol solution v/v)

‡ Significant difference at $p < 0.01$ between 'before' and 'after'

Table 5 gives the results of the impact of kinin, either in solution or in capsule on resting metabolic rate and respiratory quotient. On average, resting metabolic was not significantly affected by kinin ingestion. There was, however, a small increase in resting metabolic rate of on average 2.3 percent. Respiratory quotients declined significantly after kinin ingestion, in particular after ingestion of the kinin capsule with water.

DISCUSSION

This study did not show a significant impact of palatability on diet-induced thermogenesis. The thermic effect of sucrose was, albeit not significantly, slightly increased following ingestion of a palatable sucrose solution when compared to an unpalatable sucrose drink. Postprandial respiratory quotients and substrate oxidation rates were very similar following ingestion of either a palatable or unpalatable meal or sucrose solution. A surprising difference was found between men and women in diet-induced thermogenesis. Men had a significantly higher rise in postprandial energy expenditure than women did, in particular following ingestion of the palatable meal and the unpalatable sucrose solution.

Our findings with respect to the effect of palatablity on dietary thermogenesis are in contrast with observations of LeBlanc et al. (9). The experimental design used in this study differs considerably from the one employed by LeBlanc et al. (9). LeBlanc et al. (9) prepared the unpalatable

meal by mixing together in a blender all the ingredients served in the palatable meal and presenting it to the subjects in the form of a desiccated biscuit. The palatable meal was composed of a parmesan fondue, spaghetti with meat balls, a chocolate eclair and a bottle of soft drink. Although no quantitative data on the mass of both meals are given in LeBlanc et al.'s report, it seems likely that the unpalatable meal had a much lower weight than the palatable meal. This may seriously affect the interpretation of their results. Recently, Blondheim and Hirt (11) reported that diet-induced thermogenesis was considerably smaller after an isocaloric meal of small mass than after a meal of a larger size. Over a period of 75 minutes the difference between these meals in rise in oxygen consumption after ingestion of the meal was on average about 8.4 percent. This figure is very similar to the difference between the palatable and unpalatable meal in postprandial oxygen consumption, ie, 8 percent, LeBlanc et al. report. It is not clear, therefore, whether the difference in diet-induced thermogenesis between meals of varying palatablity LeBlanc et al. found, has to be ascribed to the impact of palatability per se, or to the difference in mass between these meals.
In this study meals identical in caloric content, nutrient composition, water content and mass, but different in palatability were investigated on their differential effect on diet-induced thermogenesis. Palatability of the test meals was varied with kinin and a coloring agent. Sensory ratings, obtained in 5-6 hours postabsorptive subjects, showed that these meals were significantly different in pleasantness. For each meal type, postprandial measurements were carried out in duplicate for a period of 2.5 hours. Total and hourly integrated values for diet-induced thermogenesis were not significantly different between the two meal types. Since an effect of kinin on energy expenditure could not be excluded, a control experiment was performed for assessing the impact of kinin on energy expenditure. Kinin ingestion was associated with a slight but non-significant increase in resting metabolic rate. This increase was not mediated by sensory stimulation since kinin encapsulated caused a similar increase in energy expenditure as kinin in solution. When the observed integrated diet-induced thermogenesis is corrected for the slight effect of kinin on baseline energy expenditure, the resulting average value of 163 kJ is very similar to the thermic effect observed after ingestion of the palatable test meal, ie, 166 kJ. The results clearly refute

the hypothesis that palatability significantly affects diet-induced thermogenesis.

It was expected that after ingestion of a palatable meal the sympathetic nervous system activity would be more stimulated than after ingestion of a revolting meal. A palatable meal was also expected to induce a significantly higher cephalic insulin response, and, hence, a total insulin response in comparison to an unpalatable meal (10,12-14). Both effects could increase postprandial thermogenesis. Firstly, through stimulation of facultative thermogenic mechanisms, and, secondly, by stimulation of postprandial glucose metabolism, ie, oxidation and storage. Our results suggest that these processes were not sufficiently stimulated by ingestion of the palatable meal, or, alternatively, suppressed by the unpalatable meal, to have a major impact on the overall rate of energy expenditure. In addition, it is possible that ingestion of a revolting meal may provoke a stress reaction, which, in its turn, could increase the turnover of catecholamines and the rate of energy expenditure. Consequently, a masking of a difference in diet-induced thermogenesis between meals of differing palatability would occur. More research is necessary to investigate these possibilities.

In agreement with the findings on diet-induced thermogenesis, sucrose-induced thermogenesis was not significantly affected by palatability either. There was a trend toward a lower thermic effect of sucrose following ingestion of the unpalatable sucrose solution compared to the more palatable solutions. The total thermic effect of sucose was low, ie, about 4 percent of the ingested caloric load. This is probably related to the relatively low energy content of the test drinks, ie, of less than 1 MJ (about 55 g of sucrose). In most subjects the thermogenic response was completed after 2 hours. The response we found of about 40 kJ is in accordance with data given by Welle et al. (20). The observed thermic effect is in good agreement with the energy expended in the postprandial oxidation and storage of the sucrose (21). At the end of the experiment about half of the ingested dose was oxydized at the expense of about 10 kJ and the other half was stored, predominantly as glycogen, at an energy cost of about 30 kJ. When the integrated sucrose-induced thermogenesis is corrected for the impact of kinin on energy expenditure the thermic effect is reduced to about 20 kJ. This value is lower than the thermic effect calculated on the basis of the energy costs of the postprandial processing of sucrose. This may be indicative of a diminution of the thermogenic response to sucrose when sucrose is given as an unpalatable solution. A replication of the

experiment with sucrose solutions of higher energy content is necessary to test this hypothesis.

There was a clear effect of gender on the rise in postprandial energy expenditure. To our knowledge this effect has not been described before. Owen et al (22) report differences between men and women in postprandial metabolism, but give no data on energy expenditure. The difference between the sexes in rise in postprandial energy expenditure following meal ingestion was present only during the first two hours of the postprandial period. During this period, in particular during the first hour of the postprandial period, men had significantly higher respiratory quotients than women did. The mechanism underlying the difference in diet-induced thermogenesis between men and women is not clear. Various factors may be involved. A higher rate of digestion and absorption of the ingested nutrients may result in a greater flux of nutrients from the gastrointestinal tract into the bloodstream per unit of time in men when compared to women. The relatively large availability of nutrients may exceed the capacity for immediate oxidation causing nutrients to be directed into 'energetically wasteful' (21) storage processes. In this respect, the postprandial processing of glucose is interesting. The respiratory quotient indicates that the rate of glucose digestion and absorption was higher in men than in women, in particular in the first hour of the postprandial period. Most of the absorbed glucose will be oxydized or stored in the body as muscle- or hepatic-glycogen. It is well known that men, at similar body weights, have a larger muscle mass than women. Hence, men have a larger storage capacity for glucose than women. The relatively larger storage capacity for glucose combined with a relatively greater influx of nutrients from the gastrointestinal tract into the circulation may cause the rise in postprandial energy expenditure to be relatively enhanced in men in comparison to women. In addition, it is possible that in men stimulation by food ingestion of facultative thermogenic mechanisms is relatively larger than in women.

In conclusion we can state that in this study no significant impact of palatablity on meal-or sucrose-induced thermogenesis was found. However, the thermic effect of sucrose was slightly reduced following ingestion of an unpalatable sucrose solution. Men had on average a greater rise in postprandial energy expenditure compared to women as well as after ingestion of mixed meal as after ingestion of an unpalatable sucrose solution.

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Chapter 11.
Unexpected interaction between the effects of short-term carbohydrate overfeeding and prior exercise on resting metabolic rate and diet-induced thermogenesis

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ABSTRACT

In 10 young normal weight subjects, 5 men and 5 women, the effects were investigated of carbohydrate overfeeding and prior exercise on resting metabolic rate (RMR) and diet-induced thermogenesis (DIT). Subjects were kept on controlled diets in a cross-over design for two periods of 8 days, with a one-week interval in between. During the last 4 days of each period subjects received additional carbohydrates on top of their diet. The carbohydrate overfeeding started at 15 percent in excess of the estimated energy requirements on day 1 increasing to 60 percent on day 4. At the beginning and at the end of the 4-days carbohydrate overfeeding periods RMR and DIT were measured, respectively, for 1 and 3.5 hours using a ventilated hood system. In one of the 8-days periods, on evenings before the energy expenditure measurements, subjects performed a maximum work capacity test on a cycle ergometer. Subsequently, they cycled for a total of about 80 minutes at fixed percentages (between 30-80%) of their maximum work capacity. RMR and DIT on control treatment were 4.56 $\pm$ 0.22 kJ/min and 7.6 $\pm$ 0.9 percent (of energy content of test meal (Ein)), respectively. After exercise, RMR and DIT were 5.10 $\pm$ 0.25 kJ/min and 10.1 $\pm$ 0.8 percent (Ein), respectively. Prior exercise on days before the measurements increased RMR and DIT significantly, respectively, by 11.9 $\pm$ 2.6 percent and 32.2 $\pm$ 13.7 percent. RMR after carbohydrate overfeeding was 4.50 $\pm$ 0.28 kJ/min; in combination with prior exercise, RMR was 4.82 $\pm$ 0.17 kJ/min. DIT was 11.5 $\pm$ 1.2 percent (Ein) after carbohydrate overfeeding and 13.2 $\pm$ 1.4 percent (Ein) after carbohydrate overfeeding with prior exercise. Carbohydrate overfeeding systematically reduced the impact of prior exercise on RMR and DIT. Carbohydrate overfeeding

did not significantly influence RMR, but increased DIT systematically by, respectively, 50 ± 18.6 percent without prior exercise and by 31.1 ± 8.7 percent with prior exercise.
It is concluded that prior exercise and an antecedent diet high in carbohydrates affects RMR and DIT, and that these effects are interactive.

Keywords: indirect calorimetry, carbohydrates, overfeeding, exercise, resting metabolic rate, diet-induced thermogenesis, dietary trial, human

INTRODUCTION

An important area in research on human energy metabolism pertains to the quantitation of the response of energy expenditure to thermogenic stimuli, in particular, to food. It is now generally assumed, that in the average sedentary individual, food intake contributes about 10 percent to total energy expenditure (1,2). However, the nature and determinants of inter-individual differences in the proportion of daily energy output depending on food intake remain to be definitely assessed (1,2).
The thermic effect of food, also referred to as diet-induced thermogenesis (DIT), is primarily a function of the amount of energy and type of nutrients ingested (3,4). In addition, the antecedent diet may affect DIT as well (5). Recently, it was shown that a diet high in carbohydrates increased DIT (5). It was suggested that in this respect the state of the body's glycogen stores would be of pivotal importance. On a high carbohydrate diet the amount of glycogen stored in liver and skeletal muscles increases (6-8). In this state a relatively larger part of ingested carbohydrates would be converted into fat, resulting in a less efficient energy utilization compared to storage of the ingested carbohydrates as glycogen. Thus, the energy expended when a given amount of glucose is transformed to lipid and is subsequently oxydized is substantially greater than if the glucose is converted into glycogen before being oxidized (9).
In addition to diet, physical activity can have a particular strong impact on the state of the body's glycogen stores (6,7). Prolonged high intensity exercise is well known to deplete liver and muscle- glycogen (6,7).
Whether prior glycogen-depleting exercise affects DIT is unclear. Since prolonged exercise reduces the body's glycogen stores, it might be hypothesized that DIT would be reduced. Alternatively, DIT might be increased,

since sustained heavy exercise may induce an excess postexercise oxygen consumption of relatively high magnitude and duration (10-12).
In the present study the state of the body's glycogen stores was modulated by short-term (4-days) carbohydrate overfeeding or by prolonged prior heavy exercise bouts. The purpose of the study was to assess the effects of these two treatments, and their interaction, on postabsorptive - and postprandial resting energy expenditure and substrate oxidation rates.

SUBJECTS AND METHODS

SUBJECTS

Subjects were recruited for participation in the experiment by posters placed in University buildings and student dormitories.
After applying for participation, subjects received a letter with detailed information on the nature, purpose and possible inconveniences of the experiment. They also received a questionnaire for obtaining information on past and present health status, sporting activities, smoking habits, habitual use of alcoholic beverages, food aversions and allergies, and, for women only, use of oral contraceptives. The medical part of the questionnaire was evaluated by a physician (JGAJH). Subjects were selected for participation when they were non-obese (body fat percentage below 25 percent in men and below 35 percent in women), apparently healthy (no past or present evidence of diabetes mellitus and thyroid disorders), non-smokers, consumed less than 20 alcoholic beverages/week and when they had moderate sporting activities (between 1 and 7 hours/week). A total of 10 subjects, 5 men and 5 women, were selected for participation. These subjects were not on a diet, had had no major, ie, greater than 2.5 kg, body weight fluctuations within a period of 6 months prior to the beginning of the study and were not using any drugs. Two women used oral contraceptives. Before the start of the study subjects gave their written informed consent to participate. The protocol of the study was submitted to and approved of by the Medical-Ethical Committee of the Wageningen Agricultural University.

METHODS

Experimental design

The study used a crossover design and consisted of two periods of 8 days with a one week interval between both periods to prevent carry-over effects (13). The total study lasted 24 days. During both periods of 8 days subjects were on controlled experimental diets. In the week between the experimental periods subjects consumed their habitual diets. At fixed time intervals during the study, ie, on the morning of day 5, day 9, day 20 and day 24, energy expenditure was measured in subjects in the fasting and postprandial state. Between day 1 and day 5, and between day 16 and 20 subjects were kept on a normal mixed weight maintenance diet. Between day 5 and day 9, and between day 20 and day 24 subjects received additional carbohydrates in their diet. Carbohydrate overfeeding was started at 15 percent of the energy intake during the weight maintenance period. Carbohydrates were added cumulatively to the diet, starting with 15 percent on the first day and increasing to 60 percent on the fourth day. Half of the subjects, randomly chosen, had exercise bouts on two evenings before the energy expenditure measurements in the first period of the experiment. The other half of the subjects performed the exercise bouts in the second period. On the evening (between 19.30 and 22.30 hours) of day 4 and day 8, in the first or second period subjects performed a maximum work capacity test on a cycle ergometer. After the determination of the maximum work capacity test subjects cycled at fixed percentages of their maximum work capacity for a maximum of two periods of 45 minutes with a fifteen minutes resting period in between.

Experimental diets and carbohydrate overfeeding

Before the subjects started in the study their energy requirements were estimated. Energy requirements were estimated on the basis of a duplicate resting metabolic rate (RMR) measurement. Overnight fasting subjects were taken in the morning by car to the laboratory for a measurement of their RMR. RMR was assessed with a ventilated hood system as previously described (14). After the RMR measurement, subjects received a small breakfast tailored to their individual energy needs but not exceeding 2.0 MJ. Subjects either stayed at the laboratory or were brought home by car. On the same day in the afternoon the RMR was measured again, now with the subjects in a 4.5 hours

postabsorptive state. No systematic diurnal variation in RMR occurred. Therefore, the mean of the two RMR measurements was taken as the most precise estimator of a subject's RMR. It was then assumed, that in subjects with 1-3 hours/week sporting activities the RMR represented 70 percent of the total daily energy expenditure, ie, requirements. In subjects with 3-5 hours/week sporting activities, the RMR was taken to represent 67 percent of the total daily energy output and for subjects with 5-7 hours/week the RMR was taken as 64 percent of the daily energy output.

It is generally assumed that the RMR represents on average between 60 and 75 percent of total daily energy output (1,2). This assumption has been corroborated by several respiration chamber studies and studies using the doubly labelled water method in free-living individuals (15,16). Subsequently, energy requirements were calculated for each individual. Energy was provided in the diet in 0.5 MJ/d modules, starting with a basal diet of 7.0 MJ/d. For each indivual the experimental diets were weighed out according to the estimated energy requirements. The experimental diets consisted of normal food stuffs and provided breakfast, lunch and dinner as well as snacks and drinks. Subjects were instructed to consume each day only the food items that they were provided with. Subjects were also allowed to have one free alcoholic consumption each day or one glass of orange juice. Coffee, tea (both without sugar and milk) and mineral water or water could be consumed ad libitum. Sugar use in tea or coffee was compensated for by reducing the amount of sugar used in other foodstuffs. Subjects kept a daily record of all food items and drinks consumed in excess of the food and drinks they received. Sporting activities, intercurrent illnesses and menstruation dates were also recorded in this notebook.

A 4-days rotating menu was given to the subjects to have a balanced number of consumptions of each daily menu over the entire experimental period. For practical reasons it was not possible to provide the subjects with an 8-days rotating menu, a 2-days rotating menu was considered to be too monotonous for the subjects and to cause problems with dietary compliance. The diets were prepared by a trained dietitian and nutritionist and consisted of on average 14 percent protein, 35 percent fat (11 percent saturated fats, 12 percent mono-unsaturated fat, 12 percent poly-unsaturated fat), 51 percent carbohydrates (28 percent mono - and disaccharides, 23 percent polysaccharides), 5 mg/MJ cholesterol and 5 g/MJ dietary fiber. Energy and nutrient intake were calculated using the 1985 edition of the Dutch computerized food table (17). Subjects consumed their food at home, hot meals

were provided as take-away deepfrozen meals.
Energy intake was adjusted according to the subject's wishes in the beginning of the study when subjects had problems eating all the food, when they experienced chronic hunger feelings or when weight changed with more than 0.5 kg during the first four days of the experiment. Seventy percent of the carbohydrate overfeeding was performed with normal food stuffs (fruit juice, raisins, apple-sauce, marsh-mallows, English licorice), predominantly containing mono- and disaccharides. Thirthy percent of the carbohydrate load was given as a commercially available maltodextrins supplement (Fantomalt, Nutricia, Zoetermeer, The Netherlands).
Duplicate portions were taken of three complete 4-days menus, respectively, for a hypothetical subject with an energy intake of 8-8.5 MJ/d, 8.5-9 MJ/d or of 9-9.5 MJ/d. The duplicate portions were stored at -20^{o}C before analysis of energy and nutrient content. The duplicate portions of each 4-days menu were mixed thoroughly and then freezedried. The ash content and moisture level were determined (18) and then the material was stored at -20 C. Aliquots were analyzed for protein (19), total fat (20), the proportion of individual fatty acids after saponification and methylation (21), dietary fiber (22) and cholesterol (23). Mono-, di- and polysaccharides were enzymatically determined (24). The mean composition of the diets was calculated from the duplicate portion analysis. No chemical analysis was performed of the foodstuffs used for the carbohydrate overfeeding treatment. The mean composition of the diets during the carbohydrate overfeeding periods was calculated from the duplicate portion analyses plus the calculated contribution of the extra carbohydrates from foodtables.

Maximum work capacity and exercise tests

On two occasions during the experiment subjects were requested to perform a maximum work capacity test on a cycle ergometer and, subsequently, to cycle for a maximum of 90 minutes at three fixed percentages of the maximum work capacity. Half of the subjects, randomly chosen, had their exercise and work capacity test on the evening of day 4 and day 8, ie, on the evening before the energy exchange measurements. The other half on the evening of day 19 and day 23. Subjects came to the laboratory at least 2 hours after their last meal. Subjects then performed a maximum work capacity test on a cycle ergometer. The first fifteen minutes period of each test was used for a warming-up. During this period women cycled equal periods of time at 100, 125 and 150 W. Men

followed the same procedure but at work loads of 150, 175 or 200 and 200 or 250 W, depending on estimated physical fitness. After this period the work load was gradually increased every three minutes with either 10 or 25 W steps until exhaustion occurred. The complete test was always finished within 25 minutes. After the assessment of the maximum work capacity subjects had a fifteen minutes rest period during which they could drink water or tea, or on the second occasion only, could eat some of their extra carbohydrates. After this rest period, subjects had to cycle for 45 minutes, for equal periods of time at a more or less fixed percentage of their maximum work capacity, starting with 70-80 percent, followed by 50-60 percent and finally by 30-40 percent. Some subjects could not sustain cycling at 80 percent for the entire period of 15 minutes. For these individuals the highest work load was slighlty decreased, but never below 70 percent. After the first cycling period of 45 minutes subjects could rest for 15 minutes during which water or tea were drunk. Subjects then started a second period of 45 minutes (or less if exhaustion occurred prematurely) cycling according to the same protocol used in the first period. The protocol followed on the first cycling evening was repeated on the second evening. After the exercise tests (between 22.00 and 22.30 hours) the exhausted subjects received 4 (women) or 8 (men) g of glucose before they went home (by bike mostly). They were instructed to fast until the next morning, not to smoke and to drink water only.

Energy exchange measurements and calculations

Energy expenditure was measured with a ventilated hood system on mornings. Subjects reclined in a supine position in a hospital bed. Details of the measurement procedure and conditions have been published before (14). Movements of the subjects were registered by means of a load cell (TKA-200A, Tokyo Sokki Kenkyujo, Tokyo, Japan) placed under one of the legs of the hospital bed. Once every hour during calibration of the oxygen analyzer with fresh dried outside air, the subjects were allowed to move briefly for 2-3 minutes.

Resting metabolic rate (RMR) or premeal baseline energy expenditure, was continuously measured in overnight fasting individuals for at least 60 minutes. After the RMR measurements subjects received a liquid yogurt-based test meal, containing on average 1.34 MJ (13 percent protein, 34 percent fat, 53 percent carbohydrates). The liquid test meal was given through a straw which was passed into the ventilated hood. The meal was eaten within 5

minutes. After ingestion of the test meal postprandial energy expenditure was measured continuously for 210 minutes. After the gas exchange measurements urine was collected for determining urea-nitrogen excretion. Energy expenditure was calculated according to Jéquier et al. (25). Diet-induced thermogenesis was calculated as the increase in postprandial energy expenditure above the premeal baseline energy expenditure (RMR) after ingestion of the test food. Substrate oxidation rates and postprandial nutrient balances were calculated using the nonprotein respiratory quotient and Lusk's equations (26). Net lipogenesis was quantitated as previously described (14).

Body composition

Body fat content was estimated from total body density as previously described (14).

Statistics

Analysis of variance was used to evaluate the effects of the exercise treatment and the carbohydrate overloading on the RMR and the thermic effect of food (27). In addition, pairwise comparisons were performed between different treatments. Pairwise comparisons were statistically evaluated using the within-person t-test with a two-tailed region of rejection at a confidence level of 95 percent. Data are given as mean values $\pm$ sem.

RESULTS

Table 1 shows some characteristics of the subjects at entry of the study. All subjects had a body fat content within a normal range.
Morning RMR's did not differ significantly from afternoon RMR's. Energy requirements estimated on the basis of the RMR measurements were on average close to energy requirements, observed during the control period. Two subjects, no 2 and 6, experienced problems eating all their food. For these subjects energy intake was slightly reduced on the fourth day of the study with 0.5 MJ/d. In one subject, no 5, energy requirements were underestimated with about 20 percent. This subject's energy intake was increased on the third day of the experiment with 1.0 MJ/d and on the fifth day with another 1.0

MJ/d. In this subject the first energy expenditure measurements were made on the morning of day 8.

Table 1: Some characteristics of subjects at the beginning of the study.

Subject No	Sex M/F	Age Years	Weight Kg	Fat %	Sport Hrs/W	RMR morning kJ/min	RMR Afternoon kJ/min	RMR Mean kJ/min	Estimated Energy requirement MJ/d
1	M	29	66.1	14.5	1 - 2	4.33	4.48	4.41	9.0 - 9.5
2	M	22	65.5	20.1	1 - 2	4.68	4.68	4.68	9.5 - 10.0
3	M	22	83.6	17.5	3 - 4	5.14	5.31	5.22	11.0 - 11.5
4	F	24	62.1	14.6	4 - 5	4.43	4.40	4.42	9.0 - 9.5
5	M	23	69.0	8.8	6 - 7	4.93	4.35	4.64	10.0 - 10.5
6	M	22	81.4	13.8	5 - 6	6.22	6.05	6.14	13.5 - 14.0
7	F	20	51.8	18.6	1 - 2	3.97	4.25	4.11	8.0 - 8.5
8	F	22	53.1	20.0	1 - 2	3.39	3.43	3.41	7.0 - 7.5
9	F	24	55.0	20.8	3 - 4	4.21	4.17	4.19	9.0 - 9.5
10	F	22	54.1	25.1	1 - 2	4.02	4.06	4.05	8.0 - 8.5
Mean ± sem		23 ± 0.8	64.2±3.6	17.4±1.5		4.53±0.25	4.52±0.23	4.53±0.23	

RMR = resting metabolic rate

Table 2: Mean daily energy and macronutrient intake in subjects before and during periods of carbohydrate overfeeding.

	Energy MJ/d	Protein g/d	Fat g/d	Carbohydrates g/d
Before CHO overfeeding:				
day 1 - 4 / 1 - 19	9.9 ± 0.6	85 ± 5	93 ± 6	293 ± 17
During CHO overfeeding:				
day 5/20	11.4 ± 0.7	85 ± 5	93 ± 6	382 ± 23
6/21	12.9 ± 0.8	85 ± 5	93 ± 6	470 ± 28
7/22	14.4 ± 0.8	85 ± 5	93 ± 6	559 ± 33
8/23	15.9 ± 1.0	85 ± 5	93 ± 6	652 ± 38

Values are given as mean ± sem
CHO: carbohydrate

Body weight did not show marked changes during the course of the experiment. Body weights were, respectively, 64.2 ± 3.6 kg (control), 64.5 ± 3.6 kg (carbohydrate), 63.8 ± 3.5 kg (exercise) and 64.2 ± 3.6 kg

(carbohydrate/exercise).

Table 2 gives energy and macronutrient intake before and during the carbohydrate overloading periods.

Mean energy intake was 9.9 ± 0.6 MJ/d (14.6 percent protein, 34.9 percent fat and 50.5 percent carbohydrates) during the four days before the carbohydrate suppletion. Energy intake increased during the carbohydrate overfeeding from a mean of 11.4 MJ/d (day 1) to 15.9 MJ/d (day 4). Carbohydrate intake increased during this period from an average of 293 g/d to 652 g/d. On the first day of the carbohydrate suppletion periods subjects consumed on average 90 g/d (1.5 MJ/d) in excess of their intake of carbohydrates on the first four days of the treatment period requirements. On the last day of the suppletion periods the excess carbohydrate intake amounted on average to 359 g/d (6.1 MJ/d). Most subjects experienced some problems in ingesting such large amounts of carbohydrates on top of their daily diet.

Table 3: Maximum work capacity, average work load and exercise duration during a cycle ergometer test in subjects before and at the end of a carbohydrate overloading period.

Subject No	Sex M/F	Maximum work capacity		Average work load		Average exercise duration	
		Start CHO	End CHO	Start CHO	End CHO	Start CHO	End CHO
		W	W	W	W	Min	Min
1	M	270	270	140	140	65	70
2	M	225	225	130	135	65	65
3	M	290	280	170	190	90	90
4	F	220	220	130	130	90	90
5	M	240	245	149	150	68	90 *
6	M	315	330	170 *	240	90	90
7	F	200	200	120	120	90	90
8	F	205	205	135	138	80	80
9	F	200	210	125	130	90	90
10	F	185	190	110	120	75	75
Mean ± sem		235 ± 13.7	238 ± 13.9	137.9 ± 6.3	149.3 ± 11.9	80.3 ± 3.5	83 ± 3.1

* both subjects had additional exercise before cycle test (running). This was compensated for on the second exercise test by increasing the duration (no 5) or the work load (no 6) of the cycle test

CHO = carbohydrate

Table 3 shows maximum work capacity, average work load and exercise duration obtained on two cycle ergometer tests, one at the start of the carbohydrates

suppletion period and one at the end of this period. At the start of the suppletion period subjects cycled on average for 80 minutes at 59 percent of their maximum work capacity. At the end of the carbohydrate suppletion period subjects exercised on average for 83 minutes at 63 percent of their maximum work capacity. On both occasions a maximum work capacity test was performed prior to the exercise test. During the assessment of the maximum work capacity the men cycled on average 22 min at 200-205 W and the women cycled 20 min at 140 W. In total, subjects cycled on both exercise tests on average for more than 100 minutes.

Table 4: Resting metabolic rate and respiratory quotients in postabsorptive subjects at different periods in the study.

Subject	Sex	Control		CHO		Exercise		Exercise + CHO	
		RMR	RQ	RMR	RQ	RMR	RQ	RMR	RQ
No	M/F	kJ/min		kJ/min		kJ/min		kJ/min	
1	M	3.97	0.85	3.75	0.90	4.17	0.83	4.70	0.85
2	M	5.33	0.83	4.94	0.92	5.60	0.79	4.98	0.97
3	M	5.39	0.85	5.04	0.81	5.85	0.77	5.40	0.84
4	F	4.35	0.84	4.89	0.91	5.16	0.79	4.69	0.86
5	M	5.01	0.80	5.90	0.90	5.06	0.74	4.71	0.87
6	M	5.46	0.81	5.37	0.89	6.40	0.77	5.86	0.83
7	F	4.58	0.83	4.18	0.99	5.65	0.77	4.73	0.89
8	F	3.75	0.83	3.05	0.93	4.01	0.79	3.75	0.88
9	F	3.61	0.86	3.46	0.95	4.65	0.75	4.80	0.85
10	F	4.16	0.83	4.42	0.94	4.45	0.82	4.60	0.87
Mean ± sem		4.56 ± 0.2	0.83 ± 0.01	4.50 ± 0.28	0.91 ± 0.01	5.10 ± 0.25	0.78 ± 0.01	4.82 ± 0.17	0.87 ± 0.01

RMR = resting metabolic rate; RQ = respiratory quotients; CHO carbohydrate overloading

Table 4 shows RMR's and RQ's in fasting subjects at different periods in the study. Prior exercise on the day before the measurements significantly increased RMR ($p=0.02$). No systematic effect of the carbohydrate suppletion on the RMR was observed. Both treatments had a significant impact on fasting RQ's. Postabsorptive RQ's were on average 10 percent higher at the end of the carbohydrate suppletion period compared to the RQ's observed at the start of the suppletion period. In contrast, RQ's were on average 12 percent lower after prior exercise compared to the RQ's measured in the non-exercise state. Not all subjects showed the same relative magnitude in response of RMR to exercise. Subject no 5 did not respond at all, whereas subject no 6 increased her RMR 29 percent in response to prior exercise.

Table 5 gives DIT's and postprandial RQ's in subjects at different periods in the study. There was a significant effect of carbohydrate overfeeding on DIT. Carbohydrate suppletion without prior exercise was associated with a mean increase in DIT of 50 percent. Prior exercise did not systematically affect DIT. DIT values were only significantly increased by 33 ± 14 kJ , ie, 0.16 ± 0.07 kJ/min (p=0.035) in response to exercise, when subjects did not receive extra carbohydrates. DIT values expressed as a percentage of the ingested energy content were, respectively, 7.6 ± 0.9 percent (control), 11.5 ± 1.2 percent (carbohydrate), 10.1 ± 0.8 percent (exercise) and 13.2 ± 1.4 percent (carbohydrate/exercise). The inter-individual difference in response of the postprandial rise in energy expenditure to prior exercise or carbohydrate overloading was considerable. Differences in response were not systematically associated with the sex of the subject or with the maximum work capacity.

Table 5: Diet-induced thermogenesis and postprandial respiratory quotients in subjects at different periods in the study.

Subject		Control		CHO		Exercise		Exercise + CHO	
		DIT	RQ	DIT	RQ	DIT	RQ	DIT	RQ
No	M/F	kJ		kJ		kJ		kJ	
1	M	63	0.90	174	0.89	142	0.82	268	0.93
2	M	131	0.83	145	0.96	188	0.77	176	0.96
3	M	80	0.88	148	0.93	113	0.81	103	0.89
4	F	94	0.90	151	0.91	186	0.79	226	0.89
5	M	146	0.89	280	0.93	130	0.76	178	0.86
6	M	97	0.85	170	0.89	95	0.79	219	0.88
7	F	139	0.85	119	0.99	143	0.79	155	0.93
8	F	44	0.85	118	0.97	127	0.80	164	0.85
9	F	71	0.87	133	0.99	96	0.80	77	0.91
10	F	156	0.92	94	0.97	128	0.82	200	0.92
Mean ± sem		102 ± 12	0.87 ± 0.01	153 ± 16	0.94 ± 0.01	135 ± 10	0.79 ± 0.01	177 ± 18	0.90 ± 0.01

DIT = diet-induced thermogenesis; RQ = respiratory quotient; CHO = carbohydrate overloading

Prior exercise and carbohydrate suppletion affected postprandial RQ's in a similar way as they influenced the postabsorptive RQ's. Figure 1 shows the time course of the postprandial rise in energy expenditure for each of the four treatments. The highest response was observed in the period between 30

and 120 minutes of the postprandial period. After 210 minutes the thermic effect of food was negligible for all treatments, except for the carbohydrate/ exercise treatment.

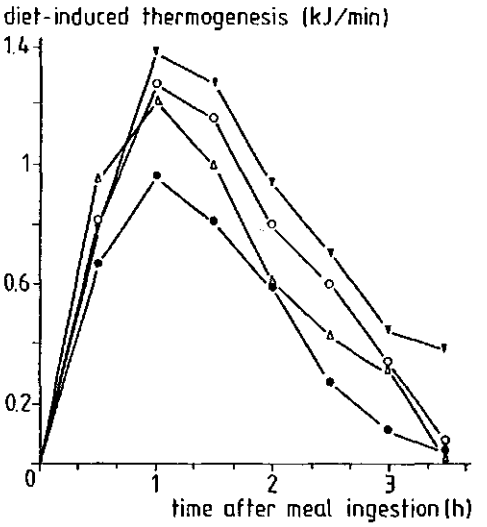

Figure 1. Diet-induced thermogenesis in subjects during control treatment (●), after prior excerise (△), after short-term carbohydrate overfeeding (○), after short-term carbohydrate overfeeding plus prior exercise (▲).

Table 6 give pre-and postprandial substrate utilization rates at different periods in the study. Protein oxidation rates were significantly reduced by carbohydrate overfeeding. Also fat oxidation rates, both pre- and postprandially, were systematically decreased by carbohydrate suppletion. Glucose oxidation rates were significantly increased after carbohydrate overloading. Exercise impacted significantly on pre-and postprandial glucose and fat oxidation rates. Postprandial glucose and fat balances were significantly affected by both exercise and carbohydrate overfeeding. Postprandial fat balances were, respectively, 5.2 ± 1.0 g (control), 9.9 ± 1.1 g (carbohydrate), -4.5 ± 1.2 g (exercise) and 6.4 ± 0.9 g (carbohydrate/exercise). Postprandial glucose balances were, respectively, for the different treatments, 8.2 ± 2.1 g, -8.0 ± 3.1 g, 22.9 ± 1.6 g and -4.8 ± 3.6 g.

Table 6. Pre- and postprandial substrate utilization rates in subjects at different periods in study.

		Preprandial Period in study				Postprandial Period in study			
		1	2	3	4	1	2	3	4
Protein	(mg/min)	46 ± 4	37 ± 3	51 ± 4	45 ± 4	46 ± 4	37 ± 3	51 ± 4	45 ± 4
Fat	(mg/min)	49 ± 4	22 ± 6	79 ± 6	37 ± 4	37 ± 4	13 ± 5	84 ± 6	30 ± 4
Glucose	(mg/min)	100 ± 6	165 ± 12	58 ± 7	14 3 ± 11	169 ± 11	237 ± 15	93 ± 8	223 ± 16
Lipogenesis	(mg/min)	0	0	0	0	1.1 ± 0.9	6.2 ± 18	0	2.8 ± 0.8

Results are given as mean ± sem

Period 1 = control

2 = carbohydrate overloading

3 = exercise

4 = carbohydrate overloading + exercise

DISCUSSION

In recent years there has been considerable interest in defining the determinants of an individual's resting metabolic rate (RMR) and thermic response to food, ie, diet-induced thermogenesis (DIT) (3,4,28-31). The RMR of a subject is primarily depended on the size of his or her fat-free body mass (28-31). The thermic effect of food is largely depended on the amount of energy and type of macronutrients ingested (3,4). In addition, to assessing the size and nature of inter-individual variation in RMR and DIT, it is important to investigate within-person responses of RMR and DIT to various stimuli. Such studies may give information on the biological capacity in humans of modifying energy use through metabolic processes in response to changing environmental factors.

In this study we investigated the impact on RMR and DIT of an antecedent hyper-energetic diet high in carbohydrates and of prolonged intense prior exercise bouts. The present study also confirmes a previous study of this laboratory on the absence of significant diurnal variation in RMR (14). Exercise and carbohydrate overfeeding were chosen for studying their impact on RMR and DIT, since they are known to have large effects on the state of the body's glycogen stores (6-8). The state of the body's glycogen stores seems of pivotal importance in determining the metabolic routing of ingested carbohydrates (5,32,33). Thus, with boosted glycogen stores ingested glucose will, in addition to immediate oxidation, be stored as lipid rather than as

glycogen (5,32,33). In contrast, in a glycogen-deprived state ingested glucose will be stored rather as glycogen than as fat (5,32,33). Storage of ingested glucose as glycogen or as fat involves a higher ATP use than direct oxidation of the absorbed glucose (9). This implicates that a metabolic state favoring storage of dietary carbohydrates either as glycogen or as fat prior to oxidation will increase the thermic effect of food, compared to a metabolic state where most of the ingested carbohydrates are oxydized without prior storage.

The results of the present study show an increased DIT in response to carbohydrate overloading either with or without prior exercise. We also found that prior exercise increased DIT. The systematic effect of prior exercise on DIT was, however, lacking during the carbohydrate overloading. Furthermore, prior exercise did increase the RMR, but, again, the effect of exercise on RMR was modulated by carbohydrate overloading. Both carbohydrate overloading and prior exercise had significant effects on postabsorptive and postprandial substrate utilization rates. Exercise increased fat oxidation rates and reduced glucose oxidation, whereas the opposite effect was observed for the carbohydrates overloading, confirming the findings of previous studies (5,10-12,32,33).

The effect of prior exercise on RMR has been observed in other studies (10-12,33-35) as well. Generally this phenomenon is designated excess postexercise oxygen consumption (EPOC) (36). Several studies have, however, not observed a significant after-effect on RMR of prior exercise (37-39). The reasons for these discrepant findings are not clear. Generally, there is considerable variation among the studies in the type, the intensity and duration of the applied exercise tests. In addition, in some studies no strict control over the antecedent diet was performed (33-35,38). It is likely that only prolonged intense glycogen-depleting exercise affects the body's energy economy sufficiently well to cause an enhanced thermogenesis in the postexercise period. Furthermore, as this study shows, the effect of exercise on RMR can be affected by the antecedent diet.

The biochemical basis of the excess postexercise oxygen consumption is not yet elucidated (36). Probably excess energy is expended in the postexercise period for replenishing the body's glycogen stores, for example, in converting glyconeogenic precursors into glycogen. Exercise may also increase various other energy-demanding processes in the postexercise period such as increased substrate cycling and sodium pumping (36). The finding that excess carbohydrates reduced the after-effect of exercise on RMR suggests that a

relatively large part, ie, about 0.30 kJ/min (5.10 - 4.80 kJ/min) of EPOC might be related to energy expended for refilling the body's glycogen stores. The latter process is likely to be diminished after prolonged exercise with previous carbohydrate overloading compared to prolonged exercise without previous carbohydrate suppletion.

The effect of prior exercise on DIT has not been studied before. Only one study assessed the impact of prior exercise on the thermic effect of glucose (40). In the study of Young et al. (40) it was found that prior exercise potentiated the thermic effect of glucose by about 65 percent. The effect of exercise was found to be independent of the state of training of the subjects. In this study exercise potentiated DIT on average by 33 percent. No significant relation was found between the response to exercise of DIT and the maximum work capacity. Part of the potentiation of DIT by prior exercise can be attributed to the increased energy costs for storing the ingested glucose as glycogen. Postprandial nutrient balances showed that on the morning after exercise without previous carbohydrate overloading on average 22.9 g of glucose were retained in the body, presumably as glycogen. The cost of this nonoxidative storage of glucose adds up to 20 kJ. This is almost 60 percent of the total increase in DIT after prior exercise. The remaining part of the increase should be attributed to stimulation by prior exercise of thermogenic processes, such as increased sodium pumping and substrate cycling (36).

Similar to the effect of prior exercise on RMR the effect of exercise on DIT was modulated by carbohydrate overloading. Carbohydrate overfeeding caused an increase in DIT of on average 50 percent (without prior exercise) and of 31 percent (with prior exercise). These results are in contrast with findings of Welle et al. (41). However, as pointed out by Welle et al. (41), the relatively short duration of DIT measurements in their study precludes any major conclusions on the impact of short-term carbohydrate overfeeding on DIT. The result of the present study are concordant with the findings of Acheson et al. (5). Acheson et al. observed a significant increase in DIT in subjects on a high carbohydrate compared to the DIT on iso-energetic diets high in fat or of normal composition. The relative differences among the various diets in DIT were, respectively, 60 percent (high carbohydrate versus high fat) and 32 percent (high carbohydrate versus mixed diet) in the study of Acheson et al. These differences agree well with the relative differences in DIT observed among the various treatments in this study.

The reason for the potentiation of the DIT by the carbohydrate overloading is not directly clear. Postprandial nutrient balances showed that all the

ingested carbohydrates were oxydized and that no major net storage occurred. There was some net lipogenesis, ie, 6 mg/min on the morning without prior exercise and 3 mg/min on the morning with prior exercise. We calculated that at the most 5-10 percent of the total thermic response was due to the net conversion of ingested carbohydrates into lipid. It should be realized, however, that indirect calorimetry is not a suitable technique for measuring total lipogenesis (25). Indirect calorimetry gives an estimate of the amount of lipid synthetized from glucose in excess of the amount of fat concomitantly oxydized (25). This means that if substantial fat synthesis occurs with concomitant fat oxidation, the simultaneous occurrence of these two processes would cause an increase in energy expenditure of about 20-25 percent of the energy contained in the glucose channeled into lipogenesis. Calculating the amount of energy expended for the conversion of glucose into net lipogenesis would, in that case, underestimate the energy costs made for total lipogenesis. It is not known whether the body would simultaneously synthetize and oxydize large quantities of fat. When it is assumed that postprandial glucose oxidation rates in excess of the rate observed at the control treatment, represent glucose oxydized as lipid, then, the energy costs made for lipogenesis could easily explain the observed differences in DIT among the treatments. The amount of glucose oxydized as lipid corresponds to 14.3 g (control versus carbohydrate) and 11.3 g (exercise versus carbohydrate/exercise). The energy expended for converting these amounts of glucose into fat are, respectively, 54 kJ and 42 kJ. This is equal to the difference in DIT between the control and carbohydrate treatment and to the difference in DIT between the exercise and carbohydrate/exercise treatment. It represents, however, only 56 percent of the difference in DIT between the control and carbohydrate/exercise treatment. This suggests that the combination of exercise and carbohydrate overloading resulted in a stimulation of additional thermogenic mechanisms in the postprandial period. Ivy et al. (42) suggest that a diet high in carbohydrates following exercise causes an increased insulin response. Insulin has been shown to increase circulating norepinephrine concentrations, ie, to activate the sympathethic nervous system activity, independent of changes in blood glucose (43). Both insulin and SNS activity could affect the rate of influx of sodium into cells and thereby stimulate the use of ATP to extrude the sodium from cells (44,45). Catecholamines may also increase the rate of substrate cycling (46) and the recycling of glucose through 3-carbon compounds (47). That prior exercise can

potentiate the thermic effect of insulin was recently reported by Devlin and Horton (39).

In conclusion, the results of the present study show that intense prolonged prior exercise increases energy expenditure both in the postabsorptive and the postprandial phase. Short-term carbohydrate overfeeding enhances the thermic response to food considerably, but does not increase resting metabolic rate. Carbohydrate suppletion seems to reduce the excess loss of energy from the body after exercise. The results indicate that in assessing the thermic response to food control over prior exercise may be warranted. The results also underscore the role of the antecedent diet in determining an individual's thermic response to food.

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Chapter 12.
General discussion

The studies described in this thesis were conducted to answer the following questions:

1. How large is the intra-individual variation in resting metabolic rate and diet-induced thermogenesis? Do sex, degree of body fatness and control of the antecedent diet affect the size of the intra-individual variation in resting energy expenditure?

2. What is the nature and magnitude of inter-individual differences in resting metabolic rate and diet-induced thermogenesis in lean and obese individuals? Does the type of body fat distribution in obesity affect these differences?

3. To what extent is resting metabolic rate affected by non-nutritional factors, ie, previous exercise, psychological stress and time of the day, and to what degree by nutritional factors, ie, ethanol ingestion and short-term carbohydrate overfeeding?

4. To what extent is diet-induced thermogenesis affected by non-nutritional factors, ie, previous exercise, psychological stress, time of the day and palatability of food, and to what degree by nutritional factors, ie, ethanol ingestion and short-term carbohydrate overfeeding?

AD 1

Two components of daily energy expenditure were investigated, resting metabolic rate (RMR) and diet-induced thermogenesis (DIT). These components comprise 70-85 percent of total energy expenditure in the average individual (1). It is not surprising that research on human energy expenditure has focused on studying these components in relation to the pathogenesis of

obesity. They are quantitatively important for overall energy expenditure and can be assessed relatively easy with the use of indirect calorimetry.

In the studies described in this thesis the ventilated hood technique was used to measure RMR and DIT in humans. Before the start of the experiments much effort was spent in the development and construction of a ventilated hood system. There was no experience in our Department with the assessment of energy expenditure using the ventilated hood technique. Therefore, extensive studies were carried out to test the accuracy and the reproducibility of measurements of energy expenditure. These studies are described in chapter 3. A salient finding of these studies is the relatively low reproducibility of measurements of DIT. Intra-individual coefficients of variation were around 30 percent. When a characteristic shows a relatively large intra-individual variation, this may pose serious problems for studies that aime to determine within-person responses to treatments (2). Also, distinguishing groups of individuals from another may become difficult (2).
Reproducibility data, obtained from the studies described in chapter 3, were used to determine the experimental designs of the studies reported in subsequent chapters of this thesis. In assessing the effect on DIT of non-nutritional and nutritional factors, a minimum sample size of 10 subjects was used. In most studies repeated measurements per subject were obtained for each treatment condition. Experimental designs were devised to have a power of 80 to 90 percent in detecting treatments effects on DIT of about 30 percent. Chapter 7 reports on between-group comparisons of DIT. Data on between-person variation in DIT, as described in chapter 3, indicate that sample sizes per group were sufficiently large to detect, with a power between 80 to 90 percent, real between-group differences in DIT of about 25 percent. This means that the failure to reach statistical significance in treatment effects or between-group differences, as reported in some of our studies, is probably related to the actual lack of effect of treatment or between-group differences.

In the study of human energy expenditure one of the more controversial issues is, whether obese persons have a defective thermogenic response to food in comparison to their lean counterparts (3). A defective thermogenic response to food could be of significance for the pathogenesis of human obesity (3). Whether a relatively high intra-individual variation in diet-induced thermogenesis has contributed to the genesis of this controversy, can not be

ascertained from our studies. After scrutinizing the literature for data of other groups on the reproducibility of DIT, only one reference was found containing this information (4). Ravussin et al. (4) conclude that DIT shows low reproducibility. Our results confirm their conclusions. A survey of the various studies on DIT shows, that most of the studies reporting no systematic difference in DIT between the lean and the obese had sample sizes probably too small to detect even relatively large differences of about 50 percent (5-17). The absence of data on reproducibility of DIT measurements is puzzling. Reproducibility of RMR or 24-hour energy expenditure measurements is frequently reported (4,18-21). Whatever the reason is for the lack of data on reproducibility of DIT measurements, this thesis shows that, before concluding on the existence or absence of systematic treatment effects on DIT or between-group differences in DIT, investigators should obtain information on within-person and between-person variation in DIT.

The specific cause for the relatively high within-person variability in diet-induced thermogenesis was difficult to determine. Coefficients of variation were not affected by sex, degree of body fat content or oral contraceptive use. Control of the antecedent diet did not seem to affect the size of the intra-individual variation. It was concluded that, in particular, variation in subject's behavior and in measurement circumstances, ie, in motion-pictures, attributed to the high within-person variability. To assess the effect of variation in motion-pictures on RMR and DIT, two extreme types of motion-pictures were compared, the romantic-family type versus the horror type. DIT increased significantly on horror films compared to romantic-family type of films. This effect was due to a synergistic effect of meal ingestion and exposure to horror motion-pictures. The results suggest that part of the within-person variation in diet-induced thermogenesis could have been related to variation among the repeated measurements in the type of motion-picture that was shown to the subjects. Under normal measurement circumstances, however, variation in motion-pictures was not as extreme as applied in the study described in chapter 6. The synergistic interaction between the exposure to a stressor and food ingestion on DIT is difficult to explain. The results of chapter 5 suggest the existence of facultative diet-induced thermogenesis after food ingestion under exposure to a relatively mild stressor. From the absence of an effect of psychological stress on fasting energy expenditure, it may be concluded that food triggers sympathetic nervous system activity (22), and that additional exposure to a stressor may enhance this process and thereby facultative thermogenesis.

The extent and nature of inter-individual variation in RMR and DIT were determined in a group of 154 obese and nonobese persons.
An important outcome of this study was the lack of a systematic difference between non-obese and obese females in the efficiency of energy utilization, either in fed or fasted conditions. This confirms recent reports (4,17), but is in contradiction with reports of various other groups (3,23-25). We have no explanation for this discrepancy. As stated before, the present study had sufficient power to detect even relatively minor differences in DIT between the non-obese and the obese females. Since no obese men were studied in this thesis, inferences on differences in DIT between obese men and non-obese men should be done cautiously.
A surprising finding was the lower DIT of men compared to women. Around 25 percent of the thermogenic response to food in men was unaccounted for by the energy expended for nutrient oxidation and retention. This indicates that men have a facultative thermogenic response of significant magnitude after mixed meal ingestion.
Do these observations fit in with the 'model' on the nature of DIT? Men had in particular higher postprandial glucose oxidation rates when compared to women. Postprandial glucose oxidation is likely to be a function of the amount of carbohydrates ingested and subsequently digested, the insulin response to ingested carbohydrates and the mass of glucose-metabolizing cells. Under normal resting conditions after carbohydrates ingestion, insulin determines largely the intensity of cellular glucose metabolism. I hypothetize that the difference in DIT between the sexes is related to a difference in insulin response. Men may have a relatively larger insulin response, ie, characterized by relatively large peak heights or postprandial surface areas. A higher insulin response could increase the cellular metabolism of glucose and, in addition, activate the sympathetic nervous system (22). This thesis offers no hard data to test this hypothesis. A difference in insulin response between men and women could partly be a function of differences in the rate of digestion and absorption of carbohydrates. A higher rate of stomach emptying is expected to result in an increased flux of carbohydrates into the small intestine, resulting in a higher absorption rate of carbohydrates, and, consequently, in a higher insulin response. This hypothesis could be tested by monitoring and varying the rate of stomach emptying in response to different meals in men and women. It predicts that, at given oral doses of

carbohydrates, increased rates of stomach emptying and carbohydrate absorption will result in a higher DIT. When this hypothesis can not be refuted, it would indicate that the model of the current theory on DIT should be expanded by incorporating in the model of DIT the **rate** of nutrient digestion and absorption as a determinant of DIT.

A second surprising finding was the significantly lower RMR per unit weight of fat-free mass in men when compared to women. This does not necessarily indicate a difference in basic energy-demanding processes at the cellular level between the sexes as explained in chapter 2. Men may have a relative hypertrophy of fat-free tissues with low metabolic activity compared to women. A combination of accurate in vivo imaging techniques for assessing body composition, direct or indirect macrocalorimetry and microcalorimetry may elucidate the precise nature of our finding.

Whereas the data on RMR indicate that men are more efficient users of energy compared to women, the results of the postprandial energy expenditure measurements suggest the opposite. A similar anomaly between trained and untrained males has recently been published by Poehlman et al. (26).

The clinically most relevant finding of the studies described in chapters 7 and 8 was the observation that non-abdominal obese women had relatively lower RMR's when compared to abdominal obese women, young lean men, young women and non-obese women matched in age. The impact of the type of body fat distribution on metabolic complications of overweight and obesity is currently the object of intense investigations (27). A relative predominance of fat in the abdominal region has conclusively been shown to be associated with an increased risk on metabolic complications. Whether the type of body fat distribution affects energy expenditure was unknown when we started our studies. The results of our studies suggest that the type of body fat distribution might be a predictor of long-term body weight gain.

The question how to interprete the relatively lowered RMR's among the non-abdominal obese is intrigueing. Did these lowered rates of energy expenditure contribute to the obesity in these women or are they secondary to the obese state? Only prospective studies with pre-obese persons subdivided according to type of body fat distribution and according to rate of energy expenditure can give a definite answer to this question. I think, however, that the reduced rates of energy expenditure among the non-abdominal obese contributed to their obesity rather than being caused by it. The arguments for this opinion are based on three observations. Firstly, the non-abdominal obese women, in particular the gluteal-femoral obese, reported a childhood

(prepubertal) history of obesity, whereas the abdominal obese reported an adult-onset obesity. The association of an early onset of obesity in the non-abdominal obese and the occurrence, later in adulthood, of reduced rates of energy expenditure suggests a causal relationship between the reduced rates of energy expenditure and the obesity. Secondly, in an earlier report of our group (28) it was observed that during weight loss RMR's reduced significantly in abdominal obese women but not in non-abdominal obese women. RMR's in the non-abdominal obese did not adapt to the chronically reduced energy intake. This indicates that the reduced RMR's in the non-abdominal obese did contribute to the obese state. Thirdly, the abdominal obese, in contrast to the non-abdominal obese, are frequently characterized by hyperinsulinemia and hypertension (27). Insulin may increase the sympathetic nervous system activity (22). An increased sympathetic nervous system activity may increase thermogenesis, for example, in processes contributing to RMR. In that case, the relatively increased RMR's in the abdominal obese could be viewed as an adaptation in the abdominal obese to excessive energy intake. The increased thermogenesis would antagonize further weight gain. This means that the relatively increased RMR's in the abdominal obese would be a consequence of the obese state, implying that the relatively reduced RMR's in the non-abdominal obese have not adapted to the obese state, ie, are constitutional.

When the reduced RMR in the non-abdominal obese are constitutional, could it affect overall energy balance? Using the results of chapter 8, we calculated that a surfeit of about 5 percent of daily energy intake could occur in the non-abdominal obese compared to the abdominal obese, assuming equal energy intakes, degrees and intensities of physical activity in both groups. An energy retention of 5 percent of total daily energy intake could certainly contribute to weight gain. It should be realized, however, that a positive energy balance induces changes in energy expenditure that affect body weight gain. This implicates that a simple summation in time of the calculated daily energy surfeits divided by the energy equivalent of weight gain (29) is only useful for estimating the maximally possible weight gain. If the lowered RMR in the non-abdominal women is constitutional, then, it might be predicted that in particular these women will be most resistent to weight reduction.

The type of body fat distribution did not affect DIT, suggesting that a (pre)pubertal history of obesity, associated with the type of body fat distribution, is not an important determinant of dietary thermogenesis.

In addition to investigations on the intra- and inter-individual variation in RMR and DIT, studies were carried out to assess the impact of various non-nutritional and nutritional factors on these components of energy expenditure.

In chapter 5 it was shown that prior exercise did not have a significant 10 hours after-effect on RMR. In this study energy expenditure was not continuously measured after exercise. It is possible that the exercise induced a significant after-effect on RMR that lasted only a relatively short time. The results of this study suggest that exercise would not induce a greater energy loss than the loss that could be inferred from calculation of the crossproduct of the applied workload and workefficiency. From this study we may conclude that exercise would probably not be useful as an adjuvant for weight control in subjects occasionally engaged in recreational physical activity. Surprisingly, however, the study described in chapter 11 showed a significant 10-hours after-effect of exercise on RMR. The difference in results between these two studies is probably related to the difference in exercise duration and intensity. The exercise performed in the study described in chapter 11 was of the unduly heavy type. After a maximum work capacity test, subjects had to cycle for another 80 minutes at varying percentages of their maximum workload. The combination of prolonged, alternating intense and less intense exercise proved to be a particular effective stimulus of resting metabolic rate. Such heavy rates of exercise are obviously not frequently encountered under normal conditions. Thus, the results of chapter 11 do not invalidate the conclusion on the efficacy of occasional recreational acitivity for weight control purposes.

Another aspect which was studied related to diurnal variation in RMR. Garrow, an authority on studies of energy balance and obesity in humans, points out that diurnal variation in RMR exists and that it could seriously affect the calculation of DIT (29). At close inspection it seems that Garrow bases his opinion on a single reference, the classical paper by Aschoff and Pohl, on rhytmic variations in energy metabolism (30). This paper gives a figure on oxygen uptake of a female subject measured in resting conditions over a whole day in thermoneutral conditions. It can not be concluded from this paper whether this subject was measured under strict fasting conditions or periodically fed. It is premature to conclude from this observation that diurnal variation in RMR exists. The results of studies presented in chapter 3

and in chapter 4 clearly show that time of the day does not significantly impact on RMR and DIT. The results of these studies are not at all spectacular, but they have important methodological consequences for the measurement of RMR and DIT, as explained in chapter 3.

AD 4

The effects on DIT of palatability, ethanol ingestion and short-term carbohydrate overfeeding were investigated to test various hypotheses on the mechanisms of postprandial energy expenditure.
Meals with relatively high palatability were expected to induce a relatively large pre-absorptive, ie, cephalic, insulin reponse, and, consequently, a higher post-absorptive insulin response. This was expected to cause enhanced storage of carbohydrates and, possibly, activation of the sympathetic nervous system. Both processes could lead to increased DIT. This hypothesis was not supported by the findings of the study presented in chapter 10. No difference in DIT occurred between meals of different palatability. Power calculations revealed that the design used in the study was sufficiently sensitive to detect a within-person change in DIT in response to treatment of around 25 percent. It seems, therefore, that humans, in short-term, do not protect themselves against the undesirable effects on energy balance of an occasional overindulgence at dinner. The absence of a significant effect of palatability on DIT was explained by assuming that highly unpalatable meals evoke a stress reaction, that increases facultative thermogenesis. Evidence for this notion comes from the study on the impact of psychological stress on DIT. In the palatability study two meal types were compared, a palatable versus a highly unpalatable meal. The results of this study do not preclude the possibility that the thermic response to food differs between palatable meals and ordinary meals.
The impact of ethanol on DIT was assessed in a study described in chapter 9. Ethanol ingested in a moderate dosis induced a significant thermic effect. According to the literature (32) the impact of ethanol on energy expenditure observed in this thesis is similar to glucose- or fat-induced thermogenesis. We did not compare the impact of iso-energetic loads of the three individual macronutrients on RMR to that of ethanol. Ethanol ingestion immediately prior to meal ingestion (aperitif) did not potentiate DIT in comparison with an equal amount of calories given in a mixed meal form. Again, the ethanol was not compared for its effects on DIT with each individual macronutrient. It was

expected that ethanol ingestion would increase nutrient storage and thereby DIT, not by an effect on insulin response, but, simply, because ethanol would act as an energy source in cellular metabolism, substituting for fat, glucose or proteins. Evidence for this mechanism came from the observed increase in DIT after ethanol ingestion in the second and third hour of the postprandial period. Overall, however, ethanol ingestion did not potentiate DIT.
This thesis does not offer an explanation for the paradoxical finding in observational studies that ethanol is usually added on top of the diet, but that its use is not associated with increased levels of adiposity (33-37). Three comments should be made. First, equality of body mass index (weight (kg)/height^2 (m)) between drinkers and abstainers of ethanol does not mean equality of metabolically 'active' mass or, as a corollary, equality of body fat stores (38). Secondly, ethanol use might covary with other lifestyle factors affecting energy expenditure, ie, smoking, coffee consumption, sleeping behavior (39) or physical activity. Ideally, observational studies should adjust the relationship between ethanol intake and body weight for these potential confounders. Thirdly, chronic ethanol use could induce thermogenic processes, for example, by effects on the autonomic nervous system (40). Finally, inferring about the chronic effects of a stimulus using results of acute effects of that stimulus should always be done cautiously.
In a last study described in chapter 11, the impact was assessed on RMR and DIT of unduly heavy previous exercise and short-term carbohydrate overfeeding. It was expected that carbohydrate overfeeding would result in an increase of DIT. This was indeed found; carbohydrate overfeeding increased DIT significantly. The effect of carbohydrate overfeeding on DIT was increased by prior exercise. No effect of carbohydrate overfeeding on RMR was observed. Unduly prior heavy exercise significantly affected RMR and DIT. Surprisingly, the carbohydrate overfeeding reduced the impact of prior exercise on RMR. These findings suggest that part of the after-effect of exercise on energy expenditure is related to replenishing the body's glycogen stores. Such a process would be relatively diminished after previous carbohydrate loading. The study described in Chapter 11 provides evidence for the existence of facultative, ie, adaptive thermogenesis. The increased DIT reduced the energy surfeit caused by the carbohydrate overfeeding. The response after exercise does not seem to indicate an adaptive response with respect to restoring energy balance. Both stimulus and response tend to decrease the body's energy stores. This indicates that after heavy exercise, restoring the original steady state in the body's glycogen stores is of greater importance in

comparison to balancing short-term fluctuations in the body's energy stores. Although this study gives evidence for the existence of the facultative component of DIT, it is difficult to assess from this study the contribution to overall DIT of neuronally-mediated stimulation of facultative thermogenic processes and, for example, of an increased turnover between the mobilisation and storage forms of a nutrient.
General textbook knowledge states that ingested carbohydrates are oxydized or converted to fat (41). In the carbohydrate overfeeding study it was shown that, even after carbohydrate overfeeding, net transformation of glucose into fat was of negligible importance for the non-oxidative storage of carbohydrates. This confirms previous findings (42,43) and supports Björntörp's and Sjöström's speculations and quantitative considerations with regard to carbohydrate storage in men (44). Only after massive overfeeding with carbohydrates de novo lipogenesis becomes a quantitatively important pathway for storing ingested carbohydrates (45). In such conditions daily energy expenditure increases markedly, largely, due to the energy expended in converting carbohydrates to fat (45).

Finally, does the work described in this thesis contribute to the solving of some of the major problems of the study of human energy exchange?

This thesis shows that individual differences in resting energy expenditure exist, even after accounting statistically for differences in age, body weight and body composition. Whether these inter-individual differences are associated with differences in the degree or intensity of habitual physical activity among our subjects can not be inferred from the studies described in this thesis. Secondly, after accounting for differences age, in body weight and body composition, this thesis shows that simple personal characteristics, ie, sex and type of body fat distribution in obesity, are still related to inter-individual differences in resting energy expenditure.
Short-term manipulation of the plane of nutrition, such as carbohydrate overfeeding, was shown to induce some form of a protective response in DIT against energy overnutrition. Whether this response would be of significance in total daily energy expenditure, and, whether a chronic dietary manipulation would induce such a response, can not be concluded from the results of this thesis. These questions remain unanswered.
The work described in this thesis shows that obese women with a relative predominance of body fat on buttocks and hips (non-abdominal type of fat

distribution) are characterized by a relatively reduced RMR. Our studies do not allow to draw a definite conclusion on the importance of this finding for the pathogenesis of non-abdominal obesity. They do suggest that studying energy expenditure in human obesity in relation to body fat distribution may prove to be valuable for understanding the nature of human obesity.
This thesis also shows that the origin of DIT is not exclusively determined by the postprandial processing of ingested nutients. Some simple non-nutritional factors, in particular psychological stress, sex of a subject and prior exercise, can significantly affect the postprandial rise in energy expenditure. To account for the impact of these factors, a revised 'model' of the origin of DIT is presented in Figure 1.

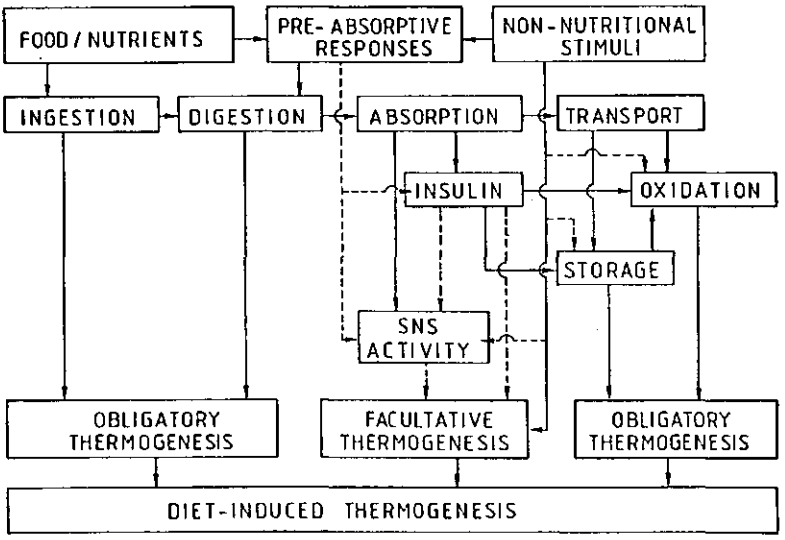

Figure 1. Revised 'model' of origin of diet-induced thermogenesis, incorporating the impact of non-nutritional factors. Thick lines indicate established contributions, interrupted lines indicate contributions still in doubt (SNS = sympathetic nervous system).

Non-nutritional factors were shown to affect dietary thermogenesis. The precise mechanism of these effects could not be elucidated from the work described in this thesis. Tentatively, I state that the effects of these factors on DIT could have been mediated through pre-absorptive influences on the post-absorptive insulin response, the rate of digestion and absorption of nutrients, in particular of carbohydrates, the routing of ingested nutrients over storage and immediate oxidation, the turnover between storage and mobilisation forms of nutrients and, finally, through a direct or indirect, ie, through stimulation of the activity of the sympathetic nervous system, stimulatory effect on the rate of ion pumping or substrate cycli. More research is needed to assess the contribution of these various processes to overall DIT.

CONCLUSIONS

This thesis shows that intra-individual variation in diet-induced thermogenesis is relatively large. This finding is important in the design of studies that aime to compare diet-induced thermogenesis between different groups or to quantify within-person responses of diet-induced thermogenesis to a thermogenic stimulus. Diet-induced thermogenesis showed a large inter-individual variation, but no evidence was obtained that, in particular, the obese would have a blunted thermogenic response to food. Men had a significantly higher diet-induced thermogenesis than women did. It is hypothetized that this is related to a difference between the sexes in the postabsorptive insulin response, probably mediated by a difference in carbohydrate digestion and absorption rate. Men were found to have lower resting metabolic rates per unit weight of fat-free mass when compared to women. This could indicate a higher effiency of fasting energy utilization in men. Alternatively, this finding could be related to a relative hypertrophy in men of components of the fat-free mass with relatively low metabolic activity. Obese women with a non-abdominal type of body fat distribution were found to have reduced resting metabolic rates compared to young non-obese men, young non-obese women, age-matched non-obese women and in comparison with obese women with an abdominal type of body fat distribution. It is hypothetized that the reduced resting metabolic rates in the non-abdominal obese women are causally related to their obesity. To test this hypothesis it is necessary to measure energy expenditure in groups of obese women, subdivided according to

type of body fat distribution, before, during and after weight loss, and, to compare their data to those of non-obese controls.

The within-person responses of resting metabolic rate and diet-induced thermogenesis to various stimuli were assessed. Resting metabolic rate was not systematically affected by psychological stress, time of the day or by moderate to heavy previous exercise bouts. Unduly heavy previous exercise bouts induced a significant rise in resting metabolic rate. Moderate ingestion of ethanol had a significant effect on resting metabolic rate. However, this effect did not indicate a reduced efficiency of energy utilization after ethanol ingestion. Diet-induced thermogenesis was significantly elevated by psychological stress, previous unduly heavy exercise bouts and by short-term carbohydrate overfeeding. Palatability of food did not affect diet-induced thermogenesis. Moderate prior ethanol ingestion did not potentiate subsequent diet-induced thermogenesis. It is concluded that these results support a role for non-nutritional factors in the origin of diet-induced thermogenesis.

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Summary

This thesis contains studies in humans on individual differences in resting metabolic rate (RMR) and diet-induced thermogenesis (DIT), and on the impact of various nutritional and non-nutritional factors on RMR and DIT.
RMR and DIT account for between 70-85 percent of total daily energy expenditure in the average individual. RMR is the energy expenditure of a postabsorptive subject in resting conditions. DIT is the increase in energy expenditure, relative to RMR, in a resting individual after food ingestion. Interest for studying RMR and, in particular, DIT arose at the suggestion that obese persons would have a relatively lowered DIT when compared to lean persons. A lowered DIT was considered to be caused by an increased 'efficiency' of energy metabolism, contributing to the pathogenesis of obesity. Opinions on this issue are, however, divergent.
From a different point of view interest in RMR and DIT has also been expressed. It has been suggested that individuals could reduce energy use through RMR or DIT, for example, during energy undernutrition or during increased energy requirements. Such a reduction would result in a more 'efficient' energy metabolism, resulting in decreased energy requirements. Little is known about the nature and magnitude of individual differences in healthy adult subjects of RMR and DIT and on the impact of nutritional and non-nutritional factors on RMR and DIT. The objective of the studies described in this thesis is to provide new knowledge on these matters.
Chapter 1 gives an introduction to the field of study and states the questions that were investigated in this thesis.
In Chapter 2 a review is given of aspects of the study of human energy exchange and their relation to problems of energy balance. The components and determinants of energy expenditure are discussed. The control of food (energy) intake is reviewed as well as the control of energy balance in relation to energy under- and overnutrition. 'Models' of RMR, DIT, the control of food intake and energy balance are presented.
Chapter 3 contains methodological studies on the measurement in humans of RMR, DIT and fuel utilization with a ventilated hood system. It was found that RMR measurements showed good reproducibility. DIT measurements were less reproducible. Strict control over the antecedent diet seemed not to reduce the within-person variability of DIT. The importance of the relatively low reproducibility for the study of DIT is discussed. No systematic effect of the phase of the menstrual cycle on RMR and DIT was observed. DIT was positively

associated with the energy content of the testmeal. For mixed meals with an energy content between about 1.5-2.5 MJ DIT can be assessed with sufficient accuracy within three hours.

Chapter 4 reports on the impact of time of the day on the measurement of RMR and DIT. No significant diurnal variation in RMR and DIT was observed.

Chapter 5 contains a study to assess whether prior moderate to heavy exercise bouts affect RMR in fasting subjects. No significant 10-hours after-effect of exercise on RMR was found.

Chapter 6 describes a study to measure the impact of psychological stress on RMR and DIT. Psychological stress did not systematically affect RMR. Surprisingly, psychological stress increased DIT.

Chapter 7 reports on the nature and magnitude of inter-individual variation in RMR and DIT in lean and obese subjects. No significant difference in DIT was found between lean and obese female subjects. DIT was on average 24 percent higher in men compared to women. Men had a relatively reduced RMR compared to women per unit weight of fat-free mass. Obese women with a non-abdominal type of body fat distribution had significantly lower RMR's, adjusted for body weight, body composition and age, compared to non-obese men and women, but also, in comparison to obese women with an abdominal type of body fat distribution.

Chapter 8 reports in detail on the impact of body fat distribution on resting energy expenditure in obese women. Three groups of obese women were studied. A group with a gluteal-femoral type of body fat distribution, a group with an intermediate type of body fat distribution and a group with a typical abdominal type of body fat distribution. A control group of age-matched non-obese women was also studied. RMR's adjusted for body weight and body composition were similar in the non-abdominal (gluteal-femoral/intermediate type of body fat distribution) obese women. Adjusted RMR's in the non-obese and the abdominal obese women were not statistically different. Adjusted RMR's of the latter groups were on average 15 percent higher compared to the RMR's of the non-abdominal obese women. The non-abdominal obese reported a relatively earlier onset obesity. It is concluded that the relatively reduced RMR's in the non-abdominal obese are probably of pathogenetic significance.

The thermic effect of ethanol (alcohol) and the impact of ethanol on DIT are described in Chapter 9. Ethanol induced a significant thermic effect. No effect of concentration on the thermic effect of ethanol was found. Ingestion of ethanol did not potentiate DIT when compared to an iso-energetic mixed meal.

The impact of the palatability of food on DIT was evaluated in a study described in Chapter 10. Differences in palatability among otherwise identical mixed meals or sucrose solutions did not produce systematic differences in DIT.

Chapter 11 describes a study on the influences of prior unduly heavy exercise bouts and short-term carbohydrate overfeeding on RMR and DIT. Prior exercise increased RMR and DIT significantly. Carbohydrate overfeeding did not affect RMR, but raised DIT significantly. The impact of exercise on RMR was attenuated by carbohydrate overfeeding.

Chapter 12 contains a general discussion. The results of the various studies are discussed, among other things in relation to the problems of energy balance and to a 'model' of DIT.

Samenvatting

Dit proefschrift bevat studies bij de mens naar individuele verschillen in ruststofwisseling en thermogeen effect van voeding (Engels, diet-induced thermogenesis (DIT)) alsmede naar de invloed van verschillende voedings- en andersoortige factoren op ruststofwisseling en DIT.
Ruststofwisseling en DIT dragen bij de gemiddelde mens voor 70-85 procent bij aan de totale dagelijkse energie-afgifte. De ruststofwisseling is de energie-afgifte van nuchtere personen in rust. DIT is de stijging van de energie-afgifte bij een persoon na voedselinneming ten opzichte van het nuchtere rustniveau.
De interesse voor onderzoek naar de ruststofwisseling en, met name, DIT nam toe na de suggestie, dat vetzuchtige personen een verlaagde DIT zouden hebben in vergelijking met mensen met een normaal vetpercentage in het lichaam. Een verlaagde DIT zou een gevolg zijn van een 'efficiëntere' energiewisseling. Een efficientere energiewisseling zou bijdragen aan het ontstaan van vetzucht. De meningen over deze zaak lopen echter bepaald uiteen.
Van een andere invalshoek is er ook interesse voor ruststofwisseling en DIT. Zo is gesuggereerd dat mensen hun energie-afgifte in ruststofwisseling of DIT zouden kunnen verlagen, bijvoorbeeld, bij ondervoeding of een langdurig verhoogde energiebehoefte. Een dergelijke verlaging zou resulteren in een verlaagde energiebehoefte.
Over de aard en grootte van individuele verschillen in ruststofwisseling en DIT bij gezonde volwassenen alsmede over de invloed van voedings- en andersoortige factoren op ruststofwisseling en DIT is nog weinig bekend. Het doel van de studies in dit proefschrift is over deze zaken nieuwe kennis te verkrijgen.
Hoofdstuk 1 schets het algemene kader waarbinnen het onderzoek valt en bevat een opsomming van de onderzoeksvragen.
In hoofdstuk 2 wordt een overzicht gegeven van aspecten van de studie van de energiewisseling bij de mens en hun relatie met problemen van de energiebalans. De componenten en determinanten van de energie-afgifte worden besproken. De regulatie van de voedsel(energie)inneming passeert de revue alsmede de controle van de energiebalans in relatie tot onder- en overvoeding. 'Modellen"' van de ruststofwisseling, DIT, de controle van de voedsel(energie-)inneming en de energiebalans worden gepresenteerd.
Hoofdstuk 3 bevat studies, die ingaan op methodologische aspecten van metingen bij de mens van de ruststofwisseling, DIT en substraatgebruik met een

'ventilated hood' systeem. Metingen van de ruststofwisseling waren goed reproduceerbaar. Metingen van DIT vertoonden een slechtere reproduceerbaarheid. Strikte controle op de voedselopname leek geen noemenswaardig effect te hebben op de binnen-persoonsvariatie van DIT. Het belang van de relatief slechte reproduceerbaarheid van DIT metingen voor onderzoek naar DIT wordt uitgediept. De fase van de menstruele cyclus had geen significant effect op ruststofwisseling en DIT. DIT vertoonde een positieve samenhang met de energie-inhoud van de testvoeding. Bij gebruik van een gemengde testvoeding met een energie-inhoud van ongeveer 1.5-2.5 MJ kan DIT bij een meetduur van drie uur voldoende nauwkeurig gemeten worden.
Hoofdstuk 4 rapporteert over de invloed van het tijdstip van de dag op de ruststofwisseling en DIT. Tussen ochtendmetingen en middagmetingen van ruststofwisseling en DIT werd geen systematisch verschil gevonden.
Hoofdstuk 5 bevat een studie naar het effect van voorafgaande inspannende lichamelijke aktiviteit op ruststofwisseling. Inspannende lichamelijke aktiviteit had na 10 uur geen aantoonbaar effect op de ruststofwisseling.
In hoofdstuk 6 wordt een studie beschreven naar de invloed van psychologische stress op ruststofwisseling en DIT. Psychologische stress had geen invloed op de ruststofwisseling. Verassend genoeg verhoogde psychologische stress de DIT.
Hoofdstuk 7 bevat een studie naar de aard en grootte van individuele verschillen in ruststofwisseling en DIT in vetzuchtige en niet-vetzuchtige personen. DIT verschilde niet significant tussen vetzuchtige en niet-vetzuchtige personen. DIT was gemiddeld 24 procent hoger in mannen in vergelijking met vrouwen. Mannen hadden in vergelijking met vrouwen gemiddeld een lagere ruststofwisseling per kilo vetvrije massa. Vetzuchtige vrouwen met een niet-abdominale lichaamsvetverdeling hadden gemiddeld een lagere ruststofwisseling, ook na correctie voor verschillen in lichaamsgewicht, lichaamssamenstelling en leeftijd, in vergelijking met niet-vetzuchtige personen, maar ook in vergelijking met vetzuchtige vrouwen met een abdominale lichaamsvetverdeling.
Hoofdstuk 8 rapporteert in detail over de invloed van de lichaamsvetverdeling bij vrouwen met vetzucht op ruststofwisseling en DIT. Drie groepen vrouwen werden bestudeerd. Een groep met een gluteaal-femoraal type vetverdeling, een groep met een intermediair type vetverdeling en een groep met een typisch abdominale vetverdeling. Een controle groep van niet-obese vrouwen, overeenkomend in leeftijd met de vetzuchtige groep, werd ook onderzocht. De ruststofwisseling, gecorrigeerd voor verschillen in lichaamsgewicht en lichaamssamenstelling, was overeenkomstig bij de vrouwen met een

niet-abdominale vetverdeling (gluteaal-femoraal/intermediair type vetverdeling). De ruststofwisseling verschilde niet significant tussen niet-vetzuchtige vrouwen en vrouwen met abdominale vetzucht. Gemiddeld lag de ruststofwisseling bij de niet-abdominale vetzuchtige groep 15 procent lager in vergelijking met de andere groepen. De vetzucht bij de niet-abdominale vrouwen was relatief op jongere leeftijd ontstaan. De conclusie wordt getrokken dat de lagere ruststofwisseling van de vrouwen met een niet-abdominale lichaamsvetverdeling van waarschijnlijk pathogenetisch belang is.
Het thermogeen effect van ethanol (alcohol) en de invloed van ethanol op de DIT worden beschreven in hoofdstuk 9. Ethanol had een significant thermogeen effect. Het thermogeen effect werd niet beïnvloed door verschillen in concentratie. Ethanol verhoogde de DIT niet in vergelijking met een iso-energetische gemengde testvoeding.
De invloed van aangenaamheid van voedsel op de DIT werd nagegaan in een studie, die in hoofdstuk 10 staat beschreven. Verschillen in aangenaamheid tussen verder identieke testmaaltijden of identieke suikeroplossingen leidden niet tot significante verschillen in DIT.
Hoofdstuk 11 beschrijft een studie naar de invloed op de ruststofwisseling en DIT van voorafgaande zeer zware lichamelijke inspannende aktiviteit en van kortdurende overvoeding met koolhydraten. Zeer inspannende lichamelijke aktiviteit had in deze studie nog na 10 uur een systematisch verhogend effect op ruststofwisseling en DIT. Overvoeding met koolhydraten verhoogde de DIT, maar had geen duidelijk invloed op de ruststofwisseling. Het effect van de aktiviteit op de ruststofwisseling was geringer wanneer overvoeding met koolhydraten plaatsvond.
Hoofdstuk 12 bevat een algemene discussie. De resultaten van de verschillende studies worden besproken, onder andere in relatie met problemen van de energiebalans en in relatie tot een 'model' voor DIT.

Curriculum vitae

Jan Adriaan Weststrate werd geboren op 23 januari 1959 in Krabbendijke. In 1977 behaalde hij het gymnasium-B diploma aan de Chistelijke Scholengemeenschap Walcheren te Middelburg. In datzelfde jaar begon hij zijn studie aan de toenmalige Landbouwhogeschool te Wageningen. In het studiejaar 1982/1983 werd een stageperiode doorgebracht bij het Dunn Clinical Nutrition Centre te Cambridge in Engeland. In september 1984 behaalde hij aan de Landbouwuniversiteit Wageningen het diploma van landbouwkundig ingenieur in de afstudeerrichting "Humane Voeding" met als hoofdvakken, Humane Voeding en Toxicologie, en als bijvakken, Wiskundige Statistiek en Methoden en Technieken van Sociaal-Wetenschappelijk Onderzoek. Vanaf januari 1985 tot september 1988 was hij verbonden als wetenschappelijk assistent aan de Vakgroep Humane Voeding van de Landbouwuniversiteit. Vanaf medio 1985 verrichtte hij onderzoek naar de energiewisseling en lichaamssamenstelling bij de mens. Het onderzoek naar de energiewisseling bij de mens resulteerde in een proefschrift. Het onderzoek naar de lichaamssamenstelling leidde tot 9 publicaties, waarvan 6 in internationale vaktijdschriften. Vanaf september 1988 is hij werkzaam als voedingskundige bij Unilever Research Laboratorium in Vlaardingen.