ENERGY REQUIREMENTS OF LEAN AND OVERWEIGHT WOMEN, ASSESSED BY INDIRECT CALORIMETRY

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J.O. DE BOER

ENERGY REQUIREMENTS OF LEAN AND OVERWEIGHT WOMEN, ASSESSED BY INDIRECT CALORIMETRY

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN

DOCTOR IN DE LANDBOUWWETENSCHAPPEN,

OP GEZAG VAN DE RECTOR MAGNIFICUS,

DR. C.C. OOSTERLEE,

IN HET OPENBAAR TE VERDEDIGEN

OP VRIJDAG 13 SEPTEMBER 1985

DES NAMIDDAGS TE VIER UUR IN DE AULA

VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

Aan mijn Ouders

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STELLINGEN

1. De energiebehoefte van gezonde volwassen vrouwen is sterk positief gerelateerd aan het lichaamsgewicht.
dit proefschrift

2. Het toenemen in lichaamsgewicht, nadat gestopt is met het volgen van een afslankdieet, valt niet toe te schrijven aan een verhoogde verteerbaarheid of beschikbaarheid van de voedselenergie.
dit proefschrift.

3. Het is een hachelijke zaak conclusies te verbinden aan voedselconsumptie gegevens, die met behulp van op enquêtes gebaseerde methoden zijn verkregen, ten aanzien van de energiebehoefte en de efficientie van het energiemetabolisme van personen met overgewicht.
Lansky, D. & Brownell, K.D., Am. J. Clin. Nutr. 35:727-732, 1982
Warnold, I., Carlgren, G. & Krotkiewsky, M., Am. J. Clin. Nutr. 31: 750-763,1978
dit proefschrift

4. De term "energie-opname" geeft niet aan of daarmee de bruto, de verteerbare dan wel de beschikbare energie-opname wordt bedoeld, en is als zodanig onbruikbaar in fysiologisch onderzoek.

5. In advertenties van en artikelen over afslankmethoden en afslankmiddelen dient naast het aantal kilo's dat men kan afvallen, eveneens het aantal kilo's te worden aangegeven, dat men na het stoppen van de kuur alleen al door maag- en darmvulling weer aankomt.

6. De stimulering van het sympatisch zenuwstelsel door overvoeding dient nader onderzocht te worden, aangezien deze stimulering een verklaring kan zijn voor de verhoogde incidentie van hypertensie en van hart- en vaatziekten bij mensen met overgewicht.
Young, J.B. & Landsberg, L., J. Chron. Dis. 35: 879-885, 1982

7. Omdat gevonden is dat dikke jonge volwassen mannen in hun vrije tijd minder lichamelijk actief zijn dan de niet dikke, kan worden verwacht dat verder gaande arbeidstijdverkorting negatieve invloed zal hebben op het resultaat van acties die tot doel hebben overgewicht bij mannen te bestrijden.
Baecke, J.A.H., Van Staveren, W.A. & Burema, J., Am. J. Clin. Nutr. 37: 278-286, 1983

8. De zinnen " Voor tamelijk dikke mensen (20-25% vetmassa voor mannen, 30-35% vetmassa voor vrouwen) is de ruststofwisseling circa 10% lager. Omdat dikke mensen in het algemeen ook minder actief zijn bij grote lichamelijke inspanning en magere mensen een grotere activiteit vertonen, kan de totale energiebehoefte voor deze categorieën ook 10% lager resp. hoger worden berekend.", wekken ten onrechte de indruk dat mensen met overgewicht een lagere energiebehoefte hebben dan magere mensen.
   Nederlandse Voedingsmiddelentabel, 34ste druk, Voorlichtingsbureau voor de Voeding, Den Haag, 1983.

9. Gezien de toenemende automatisering van de verwerving, opslag en verwerking van gegevens in het voedingsonderzoek is het gewenst in de opleiding van voedingskundigen aandacht te schenken aan de informatica.

10. Het feit dat ratten een normaal lichaamsgewicht in stand houden op rattenvoer, maar overgewicht ontwikkelen op "cafetaria" voedsel, is een ondersteuning van de zegswijze: "Verandering van spijs doet eten".

Proefschrift van J.O. de Boer.
Energy requirements of lean and overweight women, assessed by indirect calorimetry.
Wageningen, 13 september 1985.

CONTENTS

VOORWOORD

De in dit proefschrift beschreven experimenten maken deel uit van een onderzoek naar het energiemetabolisme van de mens, dat sinds 1980 op de vakgroep Dierfysiologie, in nauwe samenwerking met de vakgroep Humane Voeding, van de Landbouwhogeschool te Wageningen wordt uitgevoerd. De Nederlandse Hartstichting levert aan dit onderzoek financiële ondersteuning.

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juni 1985

Janna de Boer

SAMENVATTING

De prevalentie van overgewicht in de Westerse wereld en de verhoogde kans op sterfte en ziekte van mensen met overgewicht zijn de aanleiding tot vele onderzoekingen naar de balans tussen de energie-opname en het energieverbruik van mensen. Er zijn nog maar weinig gegevens over het energieverbruik gedurende 24 uur (24 uurs energieverbruik) en de energiebehoefte van mensen met een normaal gewicht en mensen met overgewicht. Om hierin wat meer inzicht te krijgen is in 1980 een onderzoek gestart om, met behulp van indirecte calorimeters, die geschikt zijn voor het verblijf van mensen van enkele dagen, dit energieverbruik te meten. Tijdens deze metingen kregen de deelnemers een proefvoeding verstrekt, zodat de energie-opname bekend was. De energiebehoefte kon dan berekend worden, gebruik makend van het gemeten 24 uurs energieverbruik en de energie-opname.

In dit proefschrift worden de experimenten beschreven, die de afgelopen drie jaar zijn uitgevoerd, aangaande de energiebehoefte van vrouwen zonder en met overgewicht.

In de inleiding (hoofdstuk 1) wordt een definitie gegeven van overgewicht. In dit hoofdstuk wordt ook ingegaan op de gezondheidsrisico's van mensen met overgewicht.

Hoofdstuk 2 behandelt de reproduceerbaarheid van het 24 uurs energieverbruik bij mensen. Hiertoe werd het energieverbruik van tien vrouwelijke deelnemers twee maal gedurende drie achtereenvolgende dagen in de calorimeter gemeten. De periode, die tussen beide meetperioden lag, varieerde van 2 tot 24 maanden. De omstandigheden waaronder gemeten werd, wat betreft energie-opname en lichamelijke activiteit in de calorimeter, werden constant gehouden tijdens de twee meetperioden. Eén deelneemster viel gedurende de periode tussen de meetperioden 13 kilo af, en haar gegevens zijn niet in de resultaten verwerkt. Uit het experiment kwam naar voren dat de variatie in 24 uurs energieverbruik binnen personen en tussen meetperioden klein is, mits de omstandigheden hetzelfde zijn en de deelnemers reeds goed bekend zijn met de calorimeter tijdens de eerste meetperiode (binnenpersoons variatiecoëfficiënt ca. 2%). Met deze resultaten kan worden berekend hoeveel 24 uurs metingen per periode en hoeveel personen nodig zijn om een verschil in 24 uurs energieverbruik met een bepaalde significantie te kunnen toetsen.

Hoofdstuk 3 gaat over de energiebehoefte van vrouwen zonder en met overgewicht.

Het 24 uurs energieverbruik van 29 vrouwen met een normaal gewicht en van 18 vrouwen met overgewicht werd gedurende 3 achtereenvolgende dagen gemeten, nadat ze reeds 5 dagen een proefvoeding hadden gegeten. De proefvoeding verschafte zoveel energie dat de energiebalans van beide groepen vrouwen praktisch nul was. Daarom kon het gemeten 24 uurs energieverbruik dienen als schatting van hun energiebehoefte.

Het bleek dat de energiebehoefte van de vrouwen met overgewicht in absolute zin hoger was dan die van de vrouwen zonder overgewicht. Echter als de energiebehoefte werd uitgedrukt per kilo vetvrije massa of per kilo lichaamsgewicht dan was deze gelijk aan of lager dan die van de vrouwen zonder overgewicht. Een multiple regressie analyse op deze gegevens wees uit dat het 24 uurs energieverbruik kon worden voorspeld uit het lichaamsgewicht en het lichaamsvetpercentage (multiple $r = 0.91$). De gevonden resultaten worden in dit hoofdstuk vergeleken met die van andere onderzoekers.

In hoofdstuk 4 wordt ingegaan op de validiteit van een methode om de gebruikelijke voedselconsumptie te meten. De methode is de 7-daagse weeg- en opschrijfmethode. Hiermee werd de voedselconsumptie in het normale leven gemeten van die deelneemsters, waarvan ook het 24 uurs energieverbruik in de calorimeter werd gemeten (hoofdstuk 3). De resultaten van de weeg- en opschrijfmethode werden gevalideerd tegen die van de indirecte calorimetrie. De weeg- en opschrijfmethode bleek een juiste schatting te geven van de gebruikelijke energie-opname van vrouwen met een normaal gewicht. Echter, de gebruikelijke energie-opname van de groep vrouwen met overgewicht werd met deze methode onderschat. Dit was hoofdzakelijk te wijten aan 4 deelneemsters, die erg lage energie-opnames rapporteerden in relatie tot hun energieverbruik. Er wordt dan ook aanbevolen om de gerapporteerde voedselconsumptie te controleren met behulp van een methode, die onafhankelijk is van rapportage.

In het experiment, beschreven in hoofdstuk 5, is getracht om de energiebehoefte van mensen te verhogen door ze onregelmatig te laten eten (1 dag te weinig en 1 dag te veel). Dit experiment werd uitgevoerd met twee groepen deelnemers, elk bestaande uit 7 vrouwen en 1 man. De ene groep consumeerde eerst een proefvoeding die elke dag dezelfde hoeveelheid energie leverde en daarna een proefvoeding die de ene dag 50% en de andere dag 150% van die hoeveelheid leverde. De andere groep volgde het omgekeerde schema. Het 24 uurs energieverbruik werd gemeten tijdens beide soorten proefvoeding gedurende 3 of 4 achtereenvolgende dagen. De onregelmatige voeding bleek, in tegenstelling tot de verwachting, de energiebehoefte niet te verhogen. Dit wil echter niet zeggen dat een onregelmatige voeding, die alterneert tussen een paar dagen te weinig en een paar dagen teveel, niet het verwachte effect te zien geeft.

Hoofdstuk 6 beschrijft het effect van een 8 weken durende lage energie-opname op de energiebehoefte van vrouwen met overgewicht. Aan dit experiment hebben 14 vrouwen deelgenomen. Echter de gegevens van 2 vrouwen zijn niet verwerkt in de resultaten, aangezien het twijfelachtig was of zij zich aan de voorschriften hadden gehouden. Het 24 uurs energieverbruik van deze deelneemsters werd vier keer gemeten: de eerste keer tijdens een proefvoeding, die de energiebehoefte nagenoeg dekte (100% voeding), gevolgd door een meting na 5 dagen op een vermageringsproefvoeding (4.2 MJ/d). Vervolgens volgden de deelneemsters 6 weken lang een voorgeschreven vermageringsdieet (4.2 MJ/d). Na deze periode werden zij weer 2 keer gemeten en wel de eerste keer na 5 dagen op de vermageringsproefvoeding en de tweede keer na 5 dagen op de normale proefvoeding (100% voeding). Het 24 uurs energieverbruik van de deelneemsters daalde in de eerste week op de 4.2 MJ proefvoeding met 9%. Na acht weken op een vermageringsvoeding was dit gedaald tot 15%. Nadat de deelneemsters weer een normale hoeveelheid hadden gegeten lag het 24 uurs energieverbruik 10% lager, dan vóór het vermageren. De gemiddelde energiebehoefte van de deelneemsters was na het afvallen gedaald met 1.2 MJ/d. Deze daling was groter dan was voorspeld op grond van de verandering in lichaamsgewicht en lichaamssamenstelling. Het is niet bekend of deze grotere daling een gevolg is van de langdurig lage energie-opname of deze tijdelijk is, of dat het misschien een indicatie is van een lage energiebehoefte als men een normaal gewicht heeft, hetgeen heeft geleid tot het ontwikkelen van overgewicht.

De algemene discussie volgt in hoofdstuk 7. Hierin worden enige suggesties gedaan in welke richting dit soort onderzoek zou kunnen gaan. Eveneens worden enkele aanwijzingen gegeven met betrekking tot de behandeling van overgewicht.

Een appendix geeft een gedetailleerd overzicht van de bereiding en samenstelling van de proefvoedingen en van de calorimeters en de indirecte calorimetrie.

SUMMARY

The prevalence of overweight in the developed world and the increased mortality and morbidity risk of overweight people stimulate research into the imbalance between energy intake and energy expenditure. Little information is available about the 24 hour energy expenditure and energy requirement of lean and overweight people. This thesis describes experiments on 24 hour energy expenditure, measured in whole body indirect calorimeters, energy intake and energy requirements of lean and overweight female subjects.

The experiment described in chapter 2 was performed to gain an insight into the reproducibility of measurements of 24 hour energy expenditure in human subjects. Ten female subjects were measured twice over three successive 24 hour periods, under similar conditions in the calorimeter, with an interval of between 2 and 24 months. One woman lost 13 kg during the interval; her data were excluded from analysis. The data from this experiment indicate that the variability in 24 hour energy expenditure within subjects, between periods of measurements, is small under similar conditions and after sufficient adaptation to the calorimeter (within subject coefficient of variation ca 2%). The results are discussed with regard to the length of the trial and the number of subjects required to test for a difference in 24 hour energy expenditure.

Chapter 3 deals with the energy requirement of lean and overweight women. The 24 hour energy expenditure of 29 lean and 18 overweight women, after 5 days on an experimental diet, was measured over 3 successive days by indirect calorimetry. The observed mean 24 hour energy expenditure was considered to be an estimate of the energy requirement as energy balance was close to zero for both groups of women. The energy requirement of the overweight subjects was higher than that of the lean subjects. However, the energy requirements of the overweight subjects, relative to fat free mass and body weight, were respectively similar to and lower than those of the lean subjects. A multiple regression analysis was performed to predict 24 hour energy expenditure (energy requirement) from variables related to the 24 hour energy expenditure. The 24 hour energy expenditure of the adult female subjects was best predicted by body weight and body fat percentage. The data on energy requirements of lean and overweight subjects are compared with data of other investigators.

Chapter 4 deals with the comparison of metabolizable energy intake, during normal life, with the observed mean 24 hour energy expenditure in the calorimeter. The metabolizable energy intake was measured using a 7 day weighing record method. This method was assumed to provide an estimate of the *usual* energy intake required to maintain body weight (energy requirement). The 24 hour energy expenditure measurement was also considered to give an estimation of the energy requirement. The latter estimate was considered to be the most accurate. The estimated metabolizable energy intake was validated against the observed 24 hour energy expenditure for the groups of lean and overweight women. The 7 day weighing record method provided an accurate estimate of the *usual* energy intake of lean women. However, the use of this method to estimate *usual* energy intake of overweight women remains questionable.

Chapter 5 describes the effect of alternating daily energy intake on energy metabolism. Theoretically one might expect that alternating the energy intake would increase 24 hour energy expenditure in comparison with a constant daily energy intake. This would result in a zero energy balance at a higher level of energy intake. The experiment was performed with two groups of subjects, each consisting of 7 females and one male. One group consumed first a diet providing a constant daily energy intake, and then a diet with an alternating daily energy intake (one day low, one day high). The other group followed the reverse protocol. Twenty-four hour energy expenditure was measured on both diets and energy balance was calculated. The result, that alternating daily energy intake does not affect energy balance, is discussed with regard to the expected theoretical effect. It is suggested that alternating energy intake might affect energy balance, if it is alternated over periods longer than one day.

Chapter 6 deals with the effect of an eight week low energy intake on the energy requirements and energy metabolism of overweight women. The experiment initially involved 14 women, but because of questionable adherence to the experimental protocol by two subjects, the data of only twelve subjects are presented. Twenty-four hour energy expenditure of these subjects was measured four times. The first measurement was made when they were consuming an experimental diet that provided approximately the energy required to maintain body weight (100% diet); this was followed by a measurement after 5 days on a weight-reducing experimental diet (4.2 MJ/d diet). The subjects then consumed a prescribed diet of 4.2 MJ/d during 6 weeks. After this period they were measured while still on the 4.2 MJ/d experimental diet, and then again after 5 days of refeeding on the 100% diet. The 24 hour energy expenditure of the overweight women decreased by 9% within the first week on the 4.2 MJ/d experimental diet.

After 8 weeks on a low energy intake the 24 hour energy expenditure on the 4.2 MJ/d experimental diet had declined by 15%. After one week of refeeding with the 100% diet, 24 hour energy expenditure was still 10% lower than the initial value. Energy requirement of the subjects was calculated before and after weight reduction, using data on the energy balance and metabolizable energy intake on the 100% and 4.2 MJ/d diets. Energy requirement of the subjects after weight reduction had decreased by 1.2 MJ/d, which was more than would be predicted from the change in body weight and body fat percentage by the equation presented in chapter 3. It remains to be established whether this lower energy requirement than predicted (adaptation) is a consequence of the long term low energy intake or whether it is a temporary phenomenon or an indication of a low energy requirement that may have caused overweight.

The general discussion follows in chapter 7. In this chapter suggestions for future research on energy expenditure and energy requirement are presented. The chapter also considers some implications of the results of the experiments presented in this thesis for the dietary management of overweight.

An appendix describes in more detail the experimental diet and the calorimetric measurements.

1. INTRODUCTION

In 1980 a study on energy metabolism of human subjects was started at the Department of Animal Physiology, in cooperation with the Department of Human Nutrition, at the Agricultural University of Wageningen. The energy expenditure of humans was measured using whole body indirect calorimeters, which had originally been used for energy metabolism experiments in husbandry animals. These calorimeters were refurnished for use by men and women. The aim of the first experiment was to assess the feasibility of using the calorimeters for measurements on human subjects and to examine the energy requirements of humans. The results of this experiment were published by Van Es et al. (1984).

This thesis describes the experiments in this study of human energy metabolism, with emphasis on the energy metabolism of overweight subjects. These experiments were focussed on the energy requirements of lean and overweight women, the effect of an alternating daily energy intake on energy metabolism and the effect of a long term low energy intake on the energy metabolism of overweight women. This work was financially supported by a grant of the Netherlands Heart Foundation.

OVERWEIGHT AND HEALTH

Definition of overweight

Overweight is defined as an excess of body weight, which may be either fat mass or fat free mass or both. It is expressed by a weight for height index (Body Mass Index: BMI=Weight (kg)/Height^2 (m^2)). The term obesity refers to an excess of body fat mass. Body fat may be assessed from, among others, body density measurements, skinfold measurements, body potassium measurements etc. The terms overweight and obesity are often used in the same context, but they do not necessarily express the same thing. However, overweight individuals who are not engaged in heavy muscular work or sports will generally also be obese.

Garrow (1981) proposes a cut-off point of BMI $\geq$ 25 for obesity or overweight. The same is suggested in an abstract by James (1985) on the working definition of what is meant by overweight. Hautvast and Deurenberg (1985) recommend, especially in young adults, a primary intervention to reduce overweight at a BMI between 25 and 30. The National Health Council of the Netherlands (Advies, 1985) advises accepting a moderate degree (BMI between 25 and 30) of overweight, provided there are no health complications. However, when a BMI of 30 is observed, intervention is advised regardless of the presence of health problems.

In this thesis a cut-off point of 25 kg/m² is used. Using this value, prevalence of overweight in the Netherlands amounts to about 25 - 30% in adults aged 18-65 (Kok et al.,1981) and to about 24% for young adult men and 14% for young adult women aged 19-31 (Baecke et al.,1983a). The prevalence of obesity (BMI $\geq$ 30) is at present about 3% in Dutch adults (Kok et al.,1981; Baecke et al., 1983a).

Health risks of overweight

The mortality risk of overweight adults increases slightly above a BMI of 25, with an acceleration when BMI's larger than 30 are reached (Dyer et al., 1975; Keys, 1980). A positive relationship between overweight and several risk factors for cardiovascular disease has also been observed (Berchtold et al., 1981; Larsson et al., 1981). Larsson et al. (1981) reported that in moderately overweight men the morbidity risk for hypertension, diabetes mellitus, kidneystones and gall bladder disease was increased. In middle-aged women weight gain was positively correlated with the incidence of angina pectoris and hypertension (Noppa, 1980). Weight gain was also significantly correlated with systolic and diastolic bloodpressure, fasting bloodglucose, serum cholesterol, serum triglycerides and uric acid. However, the weight change explained only a small percentage of the change in these risk factors.

Recently attention has been focussed on the distribution of the excess body fat in the body. When the excess body fat is concentrated mainly in the abdominal region (which may be indicated by a high ratio of waist to hip or waist to thigh circumferences) significant associations with diabetes mellitus, hypertension and gall bladder disease in women aged 40-59 and with menstrual abnormalities in women aged 20-30 were observed (Hartz et al., 1984). Relatively more fat around the waist (as compared to the hips) was associated with higher disease prevalence even among women with comparable total body fat. It was suggested that abdominal obesity is a "malignant" subgroup of obesity which should be treated even when present only to a limited extent (Björntorp, 1985).

The high prevalence of overweight and the health risks associated with it, are reasons to try to prevent and treat overweight. Furthermore maintenance of a reduced body weight after weight loss seems difficult, as illustrated by a report of Drenick and Johnson (1978) on a long term follow-up of previously extremely overweight subjects: Fifty percent of the subjects returned to their original weight within two or three years; ten years later only seven of the initial 121 patients had remained at the reduced weight. Dwyer and Berman (1978) showed that about 60% gained weight after weight loss, in a two year follow-up study on members of a commercial slimming club. These findings all raise the questions: "Why are many people overweight?" and "Why do they remain overweight?". One of the possibilities is that overweight people have a lower energy requirement or use dietary or body energy more efficiently than lean people. A lower energy requirement may result from a lower physical activity in overweight people. This possibility is, however, not supported by the findings of Baecke et al. (1983b), Lincoln (1972), Maxfield and Konishi (1966) and McCarthy (1966). Nevertheless, the results of food consumption studies suggest that overweight people have a lower energy requirement or a higher efficiency of energy utilization than lean people. In those studies it was found that overweight people eat as much, or even less than lean people (Beaudoin and Mayer, 1953; Thomson et al., 1961; Kromhout, 1983); this is also true when the results are corrected for body weight change (Baecke et al., 1983b). However, the findings seem to depend on the dietary assessment method used to determine energy intake. Beaudoin and Mayer (1953) found that the one- and three-day food record gave lower energy intake values in overweight women than in lean women, but that the dietary history method gave the opposite results.

Another way to assess energy requirement of people may be by measuring their energy expenditure and using this to calculate their energy requirement. For this purpose whole body direct and indirect calorimeters can be used to estimate the energy expenditure of lean and overweight subjects (Irsigler et al., 1979, Ravussin et al., 1982, Blaza and Garrow, 1983). In our research program whole body indirect calorimeters are used to assess energy requirements and energy expenditure of lean and overweight subjects. The difference between our experiments and those by other investigators is that our study attempts as far as possible to simulate a normal life within the experimental setting. This is done by the introduction of a standard daily physical activity schedule, which includes five 15 minute bicycling sessions, by using an experimental diet that resembles a normal Dutch diet and by allowing the subjects to smoke, drink coffee and perform some spontaneous activities during the calorimetry period.

OUTLINE OF THIS THESIS

As mentioned above the whole body calorimeters were used to study energy requirements and energy metabolism of lean and overweight people. The experiments presented here, mainly involved women, because women are likely to show a greater response than men, in experiments of this type. The experiments all consisted of a period of a few days at home, while consuming the experimental diet, followed by a period of 2-4 successive days in the calorimeter.

Since little is known about the reproducibility of such measurements in human subjects, ten subjects were measured twice under similar conditions, with an interval of 2 - 24 months. The results of this reproducibility study are presented in chapter 2.

Chapter 3 describes the experiment on the energy requirements of lean and overweight women. Since the results of this experiment indicated the opposite of the results of the food consumption studies mentioned above, the results were compared to estimates of energy intake in normal life, in the same women, obtained using a 7 day weighing record method. Chapter 4 deals with this comparison.

In the context of prevention and treatment of overweight the effect of a daily alternating energy intake on energy requirement was examined. Theoretically, one might expect that alternating energy intake elevates energy expenditure, compared to that with a similar constant daily energy intake. This would mean that energy balance might be achieved at a higher level of energy intake. Chapter 5 describes this experiment.

The commonly observed weight gain after successful weight loss in overweight people was the reason for studying the change in energy requirement and energy metabolism of overweight subjects after long-term low energy intake. The results of this experiment are presented in chapter 6.

A general discussion of the experiments described in this thesis is given in chapter 7.

The experimental diet and the calorimetry measurements and procedures are described in detail in the appendix.

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2. REPRODUCIBILITY OF 24 HOUR ENERGY EXPENDITURE MEASUREMENTS USING INDIRECT CALORIMETRY

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ABSTRACT

1. Ten female subjects completed two experimental periods (period I and period II). Each of the periods consisted of consumption of a weight maintenance diet for 6 or 8 days, faeces and urine collection during the last 4 days, and occupation of a whole body indirect calorimeter during the last 3 days. Three 24 hour energy expenditure measurements were performed during the stay in the calorimeter. The energy intake was the same in both periods for all subjects, except one. The time interval between the two periods varied from 2 to 24 months. Reproducibility of the energy intake and 24 hour energy expenditure measurement was assessed at group and individual level. One subject lost 13 kg in the interval between experimental periods; her data were excluded from analysis of reproducibility between the periods of measurement.

2. Food, urine and faeces were analysed for energy. There was no significant difference in mean digestibility and metabolizability between periods. The within-subject coefficient of variation of metabolizability of the experimental diet between periods was 1.7%.

3. Mean 24 hour energy expenditure (MJ) over 3 days did not differ between period I (8.78 SD 0.63) and period II (8.73 SD 0.66). The within-subject coefficient of variation in mean 24 hour energy expenditure over 3 successive days between periods was 3.1%, but decreased, after deletion of data on subjects who were less adapted to the calorimeter, to 1.9%.

4. The results are discussed with regard to length of trial and the number of subjects required to test for a difference in energy metabolism.

INTRODUCTION

To investigate the effects of thermogenic stimuli -such as physical activity, cold or heat exposure, nutrient intake, smoking, drugs, etc.- on 24 hour energy expenditure, measurements are often performed on the same subject with and without the thermogenic stimulus (Dauncey, 1980; Blaza & Garrow, 1983; Dauncey & Bingham, 1983; Hofstetter et al., 1983; Dallosso & James, 1984; Van Es et al., 1984). For planning these experiments the size of the sample (number of subjects) must be estimated. For this estimation the investigator must decide upon:

a. the size of the true effect (d) of the stimulus on 24 hour energy expenditure, that is regarded as important
b. the desired probability (1-β) of obtaining a significant result if the true difference is d
c. the significance level α of the test, which may be either one- or two-tailed (Snedecor & Cochran, 1967).

In paired samples an assumption must be made of the standard deviation of the true difference in 24 hour energy expenditure. In this case knowledge of the within-subject variation or reproducibility of a single 24 hour energy expenditure measurement may be helpful.

So far, few data are available on the reproducibility of 24 hour energy expenditure (24hEE) measurements at group and individual levels. Dallosso et al. (1982) and Garby et al. (1984) found in male subjects with fixed physical activity within-subject coefficients of variation (CV_w) of a single 24hEE measurement of 1.5% and 2.2%, respectively, with an intervening period of one week. From two 24hEE measurements in the control period in studies of Webb & Abrams (1983) and Webb & Annis (1983) CV_w's of 3.3% and 6% were calculated, the time interval in the first study being about 14 days. Garrow & Webster (1985) stated that Blaza (1980) had found a mean difference between duplicate measurements of 2% in six obese women.

All of these estimates of CV_w in 24hEE measurements were derived from measurements with direct calorimeters, except for the figure of 1.5% which originates from indirect calorimetry (Dallosso et al., 1982). The CV_w includes errors of measurement, besides sources of variance like biological variability and differences in behaviour. Since techniques of direct and indirect calorimetry dif-

fer, figures derived from direct calorimetry may not be appropiate for use in indirect calorimetry. Furthermore most of the CV_w's originate from duplicate measurements with a time interval of two weeks or less. In the studies on thermogenic stimuli, however, the time interval between measurements varied from a few days to one month, and in studies on the effect of long-term over-feeding and under-feeding (for instance slimming) on energy metabolism, time intervals of 4 weeks and more are not uncommon (Norgan & Durnin, 1980; Webb & Abrams, 1983; Webb & Annis, 1983; Bessard et al., 1983).
The aim of this article is to present data on the reproducibility, at group level and individual level, of energy intake and 24hEE measured by indirect calorimetry. The data concern 10 female subjects who were measured twice, each time during three consecutive days, with the same energy intake and the same standardized physical activity pattern, and with a time interval of between 2 and 24 months.

MATERIALS AND METHODS

Subjects

The subjects were ten apparently healthy women. Each completed two experimental periods (period I and period II) of which the design is given below. Characteristics of the subjects are given in table 1. Three subjects were smokers; these were allowed to continue smoking during the experimental periods.
Subjects 1 to 6 had already spent one or four days in the calorimeter prior to experimental period I. It may, therefore, be assumed that these subjects were familiar with, and well adapted to, the calorimeter. Subjects 7 to 10 were fam-

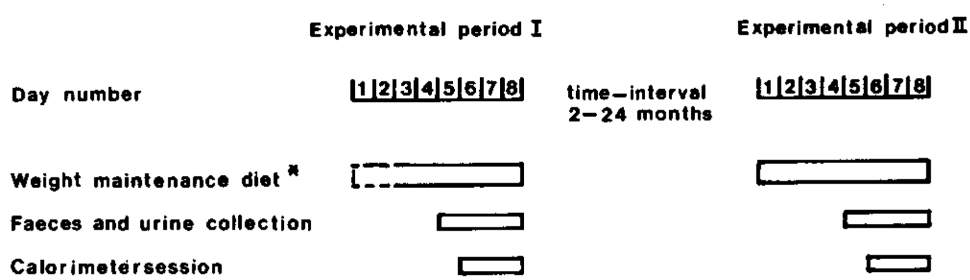

Figure 1. Timetable of the subjects.
* duration of experimental diets was 8 days for subjects 4 to 10, and 6 days for subjects 1 to 3 in experimental period I

iliarized by clear and careful explanation of the procedures and the calorimeter one to two months before and the evening before the measurements started.

Experimental design

Figure 1 summarizes the experimental timetable. Each subject was submitted to two experimental periods, period I and period II. The subjects followed the same protocol in both experimental periods except for subjects 1 to 3 whose experimental period I lasted 6 days (fig. 1). Each experimental period consisted of the consumption of an experimental diet (for 6 or 8 days), which was designed to meet the individual energy requirement of the subject. Faeces and urine were collected during the last 4 days and the subjects occupied the calorimeter during the last three days of each experimental period. Three 24hEE measurements were made during each calorimetry session.

Some of the data originate from two different studies, which had both been approved by the Ethical Committee of the department of Human Nutrition, Agricultural University, Wageningen.

Diets

The diets were designed with the use of the Dutch food composition table (1981). The contributions of energy from protein, fat and carbohydrate to the metabolizable energy intake of the experimental diet were 14%, 40% and 46% respectively, simulating the composition of an average Dutch diet.

Food items, food preparation, storage and sampling procedures have already been described by Van Es et al. (1984). The food was weighed out to the nearest 0.1 g. The subjects had to eat and drink everything provided.

The diet during experimental period I was identical to that in period II, except for subject 1, who consumed 1.2 MJ/d less in experimental period II.

Gross energy intake was calculated by multiplying the weight of the provided foods with their energy content. Subtraction of energy losses in faeces and urine from gross energy intake yielded the metabolizable energy intake.

Collection of faeces and urine

Faeces and urine were collected during the last 4 days of each experimental period. Faeces were collected, refrigerated and subsequently pooled, weighed, freeze-dried and sampled. Urine collection started and ended after voiding in the morning. Urine was collected in 1-litre plastic bottles containing mercury-iodide as a preservative. It was then stored in the refrigerator, pooled, weighed and sampled.

Measurements of energy expenditure

Apparatus: Two whole body indirect calorimeters were used. The calorimeters and gas exchange measurements have already been described by Van Es et al. (1984). Three consecutive 24 hour gas exchange measurements were made during each experimental period. Each measurement started at 7.30.

The 24 hour composite samples of the air entering and leaving the calorimeters were analysed volumetrically for oxygen (O_2)- and carbon dioxide (CO_2) concentration using a Sonden apparatus.

Brouwer's equation (1965) was used to calculate 24hEE from O_2 consumption, CO_2 production and urinary nitrogen (N_u):

24hEE (kJ) = 16.18 x O_2 consumption (l) + 5.02 x CO_2 production (l)- 5.99 x N_u (g).

Before calculation of 24hEE, O_2 consumed and CO_2 produced by burning cigarettes was subtracted from O_2 consumption and CO_2 production. The correction term for protein oxidation in the equation (-5.99xN_u) lowered 24hEE by an average of 0.7%. This term was used only in the calculation of the mean 24hEE over 3 days. In single 24hEE data this correction term was neglected, thus resulting in about 0.7% higher 24hEE values.

Procedure in the calorimeter

The two calorimeters were used simultaneously. Subjects could see each other through a large window in the connecting wall between the calorimeters, and could talk to each other by intercom. Subjects wore their own clothes and adjusted their clothing to their own comfort. The ambient temperature was set at 21-22^{o}C during the daytime, and at 19-20^{o}C at night, but was adjusted when subjects felt uncomfortable. Relative humidity was kept between 60-80%.

Subjects entered the chamber in the evening 8-9 h before the gas exchange measurement started. They were awoken at 7.45 and followed a standard daily activity schedule. This schedule included 5 times 15 min bicycling on a hometrainer at a speed of 24 km/h, without load: at 8.45, 12.15, 13.30, 17.30 and 22.30. Subjects prepared for bed at 22.45 and were in bed from 23.15 until 7.45. The rest of the day was filled with sedentary activities and some spontaneous activities, like preparing coffee, tea or meals, washing dishes etc. The schedule was meant to simulate a light housekeeping- or sedentary working-day.

Analyses of food, excreta and cigarettes

Energy content of food items, urine, faeces and cigarettes was determined using a static bombcalorimeter. Nitrogen content of urine was determined by the Kjel-

TABLE 1

Characteristics of 10 female subjects at the time of experimental periods I and II. Individual differences, between period I and II, in body weight (BW)[1] and body fat percentage (BF)[2] are indicated by ΔBW and ΔBF.

subject	age[3] year	height m	BW I kg	BW II kg	ΔBW kg	BF I %	BF II %	ΔBF %	interval months
1	22	1.77	63.0	62.6	0.4	22.5	23.4	-0.9	13
2	23	1.73	71.1	69.9	1.2	30.1	-	-	2
3	23	1.66	68.7	71.5	-2.8	32.7	36.4	-3.7	12
4	28	1.73	54.1	55.0	-0.9	20.8	19.0	1.8	7
5[4]	36	1.63	59.0	60.8	-1.8	24.3	-	-	5
6[4]	24	1.68	67.3	65.0	2.3	35.4	31.8	3.6	7
7	25	1.63	54.4	55.4	-1.0	25.5	26.7	-1.2	10
8	25	1.62	63.5	64.9	-1.4	28.5	32.5	-4.0	4
9[4]	29	1.76	60.4	58.4	2.0	24.2	21.1	3.1	24
10	31	1.66	113.2	100.6	12.6	40.9	36.3	4.6	11
Mean[5]	27	1.69	62.4	62.6	-0.2	27.1	27.3	-0.2	10
sd	5	0.06	6.0	5.9	1.8	5.4	6.5	3.1	6

[1]mean body weight during 3 day calorimetry session
[2]body fat % derived from underwaterweighing
[3]age at time of experimental period I
[4]smokers
[5]data of subject 10 excluded from mean BW I, BW II, ΔBW, BF I, BF II and ΔBF, because of large body weight change

dahl method (International Organization for Standardization, 1979) using mercury-oxide as a catalyst.

Analyses of results

Reproducibility is defined as the degree of agreement between repeated measurements, using one technique on the same subject or on the same group of subjects at different times. It depends on the variance due to error, which includes variance due to errors in measurements, interaction terms, biological variability and behaviour. This variance due to error will be referred to as within-subject variance.

Reproducibility at the group level was tested using Student's t-test for paired samples. When the difference between mean values was not significant ($p > 0.05$), the measurement was considered to be reproducible at the group level.

Reproducibility at the individual level is expressed by the within-subject coefficient of variation (CV_w). A small CV_w means that the measurement is highly reproducible. The CV_W was estimated as follows: the mean of individual differences and the variance of these differences (SD^2_{dif}) were calculated. Within-subject variance may be derived from SD^2_{dif} ($SD^2_W = SD^2_{dif} x 0.5$). The CV_W (= $\frac{SD_W}{mean}$ x100%) was calculated for all the data presented here.

Three different CV_W's between periods were calculated for 24hEE:

(1) CV_{W3} was calculated from the individual differences in the mean of three subsequent 24hEE measurements (day 6+7+8; fig. 1) between the two periods. In this calculation 24hEE was not corrected for protein oxidation.

(2) CV_{W2} was calculated from the individual differences in the mean of two subsequent 24hEE measurements (day 6+7; fig 1) between the two periods.

(3) CV_{W1} was calculated from the individual differences in the 24hEE for one day (day 6; fig. 1) between the two periods.

Analysis of variance was performed to test for an effect of the sequence of days spent in the calorimeter, and of the period of measurement on 24hEE. The CV_W within periods was calculated from these computations. Calculations were performed using SPSS (Nie et al., 1975)

RESULTS

Body weight and body composition

Table 1 shows the change in body weight and body composition of the individual subjects between the experimental periods. Subject 10 lost 13 kg in the 11 month interval between the experimental periods. Her data were excluded from analyses on reproducibility *between* periods. Mean body weight and body composition did not differ between periods I and II. The within-subject coefficient of variation in body weight was 2.0%, and that in body composition was 8.0%.

Energy intake

Individual gross energy intake, digestibility and metabolizability in each experimental period are presented in table 2. Data on subject 1 were excluded from testing for differences in gross energy intake and metabolizable energy intake between periods, because for experimental reasons her gross energy intake was not the same. Her data were included, however, in analyses on digestibility and metabolizability of the diet. Mean differences in gross energy intake, digestibility, metabolizability and metabolizable energy intake between periods were not significantly different from zero. Reproducibility at the individual level, as indicated by CV_W of digestibility and metabolizability were

TABLE 2

Gross energy intake (GE), digestibility (DE%) and metabolizability (ME%) of 10 female subjects during experimental periods I and II. Differences between period I and II are indicated by ΔGE, ΔDE% and ΔME%.

subject	GE I MJ	GE II MJ	ΔGE MJ	DE% I %	DE% II %	ΔDE% %	ME% I %	ME% II %	ΔME% %
1[1]	11.29	9.72	1.57	92.7	95.0	-2.3	88.6	91.2	-2.6
2	10.28	9.99	0.29	93.5	93.4	0.1	89.7	89.4	0.3
3	10.29	10.24	0.05	92.8	92.0	0.8	88.4	88.0	0.4
4	9.64	9.48	0.16	93.0	92.2	0.8	88.9	88.4	0.5
5	10.70	10.60	0.10	96.9	91.8	5.1	92.6	87.9	4.7
6	10.28	10.41	-0.13	93.8	94.3	-0.5	89.2	90.5	-1.3
7	10.49	10.71	-0.22	94.6	93.7	0.9	90.8	89.8	1.0
8	10.31	10.37	-0.06	93.9	93.3	0.6	89.8	89.0	0.8
9	10.50	10.94	-0.44	95.2	92.1	3.1	90.9	88.3	2.6
10	11.19	11.39	-0.20	91.7	94.5	-2.8	86.8	90.1	-3.3
mean[2]	10.31[3]	10.34[3]	-0.03[3]	94.0	93.1	1.0	89.9	89.2	0.7
sd	0.31	0.45	0.23	1.4	1.1	2.1	1.3	1.2	2.1

[1]Gross energy intake lower in experimental period II for experimental reasons
[2]data of subject 10 *not* included, because of large weight change
[3]data of subject 1 excluded from mean values of GE and ΔGE

1.7% and 1.7%.

Energy expenditure

Data on 24hEE measurements are shown in table 3. There was no indication that the 24hEE within experimental periods differed systematically between days. The within-subject coefficient of variation of 24hEE measurements in experimental period I was 2.6% and that in period II 1.8% (data of subject 10 included). Mean 24hEE (corrected for protein oxidation) over 3 days (MJ) in period I was 8.78 (SD 0.63) and in period II 8.73 (SD 0.66). Analysis of variance showed that the variance due to experimental period did not reach significance. The CV_{W^3} was 3.1% (SD_{W^3} = 270 kJ). The larger CV_W in 24hEE in period I (2.6%), compared to that in period II (1.8%), suggests that the subjects in experimental period I may have been insufficiently adapted to the calorimeter. Data of subjects 7 to 9 were therefore excluded from analysis (as already mentioned, these subjects were less familiar with the calorimeter). After exclusion of their data, the CV_{W^3} diminished to 1.9% (SD_{W^3} = 164 kJ; table 4). Table 4 presents values of the CV_{W^2} and CV_{W^1} of 24hEE, based upon data of all subjects (except nr 10) and

TABLE 3

Individual 24 hour energy expenditure (24hEE) data (MJ) and individual differences (Δ) in 24hEE (MJ) between periods (Δ_1 = 24hEE day 6 period I - 24hEE day 6 period II ; Δ_2 = mean 24hEE days 6+7 period I - mean 24hEE days 6+7 period II ; Δ_3 = mean 24hEE days 6+7+8 period I - mean 24hEE days 6+7+8 period II)

		PERIOD I			PERIOD II			Δ		
subject	day	6	7	8	6	7	8	1	2	3
1		8.49	8.45	8.51	8.38	8.17	8.42	0.11	0.19	0.16
2		8.23	8.07	8.02	7.96	8.20	8.10	0.27	0.07	0.03
3		8.32	8.36	8.57	8.76	8.41	8.82	-0.44	-0.24	-0.24
4		8.31	7.85	7.99	8.23	7.98	8.18	0.08	-0.02	-0.08
5		9.14	8.79	9.13	8.56	9.05	8.80	0.58	0.17	0.22
6		9.56	9.37	9.28	9.59	9.78	9.98	-0.03	-0.22	-0.38
7		9.46	9.66	8.99	8.34	8.57	8.58	1.12	1.10	0.87
8		8.94	8.51	9.18	9.03	9.04	8.87	-0.09	-0.32	-0.10
9		9.66	10.11	-[1]	9.81	10.12	9.78	-0.15	-0.08	-[1]
10		14.04	13.93	13.36	12.66	12.42	12.57	1.38	1.44	1.23
							mean[2]	0.16	0.07	0.06
							SD^{3}_{dif}	0.46	0.43	0.38
							SD^{4}_{w}	0.32	0.30	0.27

[1] no data on 24hEE due to technical failure
[2] data of subject 10 not included, because of large weight change between periods.
[3] standard deviation of the individual differences
[4] within-subject standard deviation

on the data from the well-adapted subjects.

DISCUSSION

Energy intake

Since digestibility and metabolizability of this experimental diet were fairly constant at the group level, the reproducibility of metabolizable energy intake is good, provided of course that gross energy intake remains constant.
At the individual level, variability in metabolizable energy intake may be due to the variation in gross energy intake (CV_W = 1.6%) as well as to variation in metabolizability (CV_W = 1.7%). Variation in gross energy intake, although exactly the same amount of food and the same food items were provided, may be ascribed to variation in energy density of the foods between experiments, and

to errors of measurement. Dairy products and sausages in particular were subject to (seasonal) variation in energy content between experiments. Taking into account the two above mentioned CV_w's, metabolizable energy intake may show a CV_w of $\sqrt{(1.6^2 + 1.7^2)}$= 2.3% (95% confidence interval 1.5% to 4.4%). By preparing experimental diets in a large batch for a whole series the CV_w in energy intake may be diminished, for the difference in gross energy content between diets will have disappeared. It is concluded that this variation in energy intake is small, and is likely to have very little impact on 24hEE.

TABLE 4

Within-subject coefficients of variation between periods when 1, 2 or 3 subsequent 24 hour energy expenditure measurements per period are performed. Within-subject standard deviation is presented between parantheses(kJ)

	n = 9[1]	n = 6[2]
within-subject coefficient of variation 1	3.7% (323)	2.8% (238)
within-subject coefficient of variation 2	3.4% (301)	1.6% (133)
within-subject coefficient of variation 3	3.1% (270)	1.9% (164)

[1]data on subject 10 excluded
[2]excluding data from subjects less well adapted to the calorimeter

Energy expenditure

Our results show that mean 24hEE over 3 days with a standardized daily activity pattern is highly reproducible for groups, provided no significant change in body weight or in body composition has occurred. The fact that the CV_w decreased after adaptation to the calorimeter indicates that reproducibility at the individual level improves after adaptation of the subjects to the calorimeter. One day's adaptation some weeks before the start of the experiment may be sufficient.

The observed within-period CV_w's in 24hEE (2.6% and 1.8%) are well within the range of values found by other investigators (Blaza, 1980; Dallosso et al., 1982; Webb & Abrams, 1983; Webb & Annis, 1983; Garby et al., 1984). These CV_w's are, however, not entirely comparable to those of the other investigators, because they originate from consecutive measurements. They may therefore be more strongly influenced by autocorrelation in 24hEE than the CV_w's of the others. The most comparable CV_w's are those presented in table 4. It may be assumed that these 24hEE measurements within subjects were independent, as the interval between measurements was rather long. The value of CV_{w1} = 3.7% (n=9; 95% confidence interval 2.5% - 7.1%) was obtained using similar methods to those of the other investigators, except that the interval between measurements was much longer. This CV_{w1} is higher than the 1.5%, 2.0% and 2.2% mentioned by others (Dallosso et al., 1982; Blaza, 1980; Garby et al., 1984). The CV_{w1} of the well adapted subjects (2.8%; 95% confidence interval 1.7% - 6.9%) is not significantly different from two of the earlier mentioned estimates: 2.0% and 2.2%. It should be noted that in our experiment sources of variation were probably present that were absent or limited in the experiments of other investigators. First, physical activity, body weight, body composition and living circumstances may have changed, thus influencing energy metabolism. Body weight showed a small CV_w; body fat percentage, however, showed a relatively large CV_w. This large CV_w may be due more to errors in the body density measurement than to true changes in body fat percentage. Secondly, metabolizable energy intake on the experimental diet showed a between-period CV_w of 2.3%, and this may have had a slight effect on 24hEE. Thirdly, spontaneous activities and behaviour of the subjects in the calorimeter may have differed between days and periods, although a standard activity pattern was prescribed. Another source of variation may have been the time of 24hEE measurement with regard to the menstrual cycle. Bisdee & James (1983) reported that sleeping metabolic rate increased, from the preovulatory to premenstrual stage of the cycle, by 6%. Webb (1985) mentions that the effect of the stage of the ovulatory cycle is strong in women in their 20's (7 to 15% increase in 24hEE after ovulation), but that this effect is reduced or absent in women in their 40's. The calorimetry sessions in our experiment were planned between menstruation periods, but since in some subjects the length of the menstrual cycle was unpredictable, this planning failed in some instances. Garby et al. (1984) and Dallosso et al. (1982) did not have this source of variation, because they used men. Webb & Abrams (1983) and Webb & Annis (1983), however, also used female subjects; this may be one of the reasons for the higher CV_w in 24hEE in their experiments.

Table 4 shows that the CV_w in 24hEE between periods decreases as the number of subsequent measurements is increased. The CV_{w2}, however, is smaller than the CV_{w3} in the 6 well adapted subjects. This seems likely to be an artefact, but the CV_w values given in table 4 were used to calculate the sample size required to test a change in 24hEE induced by, e.g., a thermogenic stimulus. The chosen levels of significance (α) are 0.05 and 0.01 (one-tailed) and the chosen probability (1-β) 80% and 90%. The expected change in 24hEE was selected as 3%, 5%

or 10%. The sample sizes required, when 1, 2 or 3 consecutive measurements are performed, are given in table 5.

TABLE 5
Number of female subjects required to test a change in 24 hour energy expenditure (24hEE) within subjects of 3% (260 kJ), 5% (430 kJ) and 10% (860 kJ), with significance levels of α = 0.05 or α = 0.01 and a probability (1-β) of 80% and 90%, when 1, 2 or 3 consecutive 24hEE measurements are performed per subject. The within-subject coefficients of variation used are 2.8% (238 kJ) for 1 day, 1.6% (133 kJ) for 2 day, and 1.9% (164 kJ) for 3 day measurements. (graphs by Owen (1962) were used).

		EXPECTED CHANGE IN 24hEE					
		3%		5%		10%	
number of 24hEE measurements	β	0.2	0.1	0.2	0.1	0.2	0.1
α = 0.05 (one-tailed)							
1		8	9	4	5	3	3
2		4	5	3	3	3	3
3		5	6	3	4	3	3
α = 0.01 (one-tailed)							
1		12	14	6	7	4	4
2		6	7	4	4	3	3
3		7	9	5	5	3	4

It is clear from this table that no beneficial effect is to be expected from increasing the number of measurements per subject, when the effect to be tested amounts to 10% or more of the 24hEE. However, in calorimetric studies intended to detect small differences in 24hEE, i.e. 5% or less, with a high level of significance and probability, performing several consecutive measurements per subject may be advantageous. The figures in the table show no benefits to be gained by performing three rather than two measurements per subject.
In conclusion, considering the within-subject coefficients of variation in 24hEE, the reproducibility of 24hEE is high, even over a long period of time. This is true provided, that body weight and body composition have not changed significantly during the interval, that energy intake and physical activity during the measurements are the same and, last but not least, that the subjects are already well adapted to the calorimeter. Part of the within-subject coefficient of variation is due to errors in measurement, suggesting that energy metabolism is a fairly constant phenomenon under comparable circumstances. Because the reproduc-

ibility of the 24hEE measurements in female subjects is high, one measurement on relatively few subjects may suffice to test an expected change of 10% or more in 24hEE. To test smaller changes in 24hEE (5% or less) it may become profitable to increase the number of consecutive 24hEE measurements, rather than increasing the number of subjects.

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3. ENERGY REQUIREMENTS AND ENERGY EXPENDITURE OF LEAN AND OVERWEIGHT WOMEN, MEASURED BY INDIRECT CALORIMETRY

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ABSTRACT

This energy balance study was designed to estimate the energy requirements of lean and overweight women. Twenty-nine lean and eighteen overweight women consumed a diet providing enough energy to maintain body weight (energy requirement level) for 8 days. The last 80 hours of the 8 day experiment were spent in a whole body indirect calorimeter. Three 24 hour energy expenditure measurements were made on each of the subjects. A standard physical activity schedule was imposed on the subjects during their stay in the calorimeter. This schedule simulated a light activity work- or light housekeeping dav.

The mean body weight of the lean subjects was 59 ± 1 kg (mean ± SEM), and that of the overweight subjects 94 ± 5 kg. Fat free mass (FFM) was higher in the overweight subjects (52 ± 2 kg) than in the lean subjects (45 ± 1 kg; $p < 0.001$). Actual metabolizable energy intake was measured by analyzing food, urine and faeces for energy. Mean energy intake was 8.88 ± 0.13 MJ in the lean subjects and 10.12 ± 0.29 MJ in the overweight subjects. Mean 24 hour energy expenditure (24hEE) of the lean subjects (8.58 ± 0.13 MJ) was lower than the mean energy expenditure of the overweight subjects (10.70 ± 0.45 MJ); $p < 0.001$). Energy balance, calculated as energy intake minus mean 24 hour energy expenditure, was close to zero in both lean and overweight subjects. Twenty-four hour energy expenditure was, therefore, used to estimate energy requirement.

Twenty-four hour energy expenditure was positively correlated with body weight

(r = 0.91), FFM (r = 0.78) and urinary creatinine excretion (r = 0.79). Multiple regression analysis showed body weight to be the best single predictor of 24 hEE. However, body fat % did contribute 2% to the total explanation of the variance in 24hEE. Energy expenditure while sleeping in bed, similar to resting metabolic rate, was best predicted by a combination of body weight, 24 hour creatinine excretion and smoking during the preceding daytime.
The observed EE values in the lean and overweight subjects are discussed with reference to data from other studies. It is concluded that, in consideration of counselling and treatment of overweight, our data may be useful for prediction of the energy requirements of women between 20-50, in normal life.

INTRODUCTION

Many people in affluent societies are overweight. Overweight may be defined as an excess of body weight in relation to height. It is normally expressed by the Body Mass Index (BMI; weight(kg)/height^2(m)). Garrow (1) proposes as a cut off point, for overweight as well as for obesity, which is an excess of body fat, a BMI of 25 kg/m^2. Prevalence rates of overweight in the Netherlands (2,3) are approximately 25-30% for males and females (18-65 yr) and 24% for males and 14% for females in a young adult population (19-31 yr). The health risk associated with overweight (4), the tendency of overweight persons to remain overweight and the weight gain after weight reduction (5,6,7) stimulate the search for underlying causes for the imbalance between energy intake and energy expenditure.
Spitzer and Rodin (8) reviewed studies on the eating behaviour of normal weight and overweight individuals. They state that "....research evidence from laboratory studies suggests that overweight subjects do not eat more than normal weight subjects...", but also that "...it appears that there is somewhat more support for a relationship between body weight and amount ordered and/or consumed in naturalistic studies than in laboratory studies". They suggest that these conflicting findings may be due partly to the overweight population under study; i.e. in most studies overweight subjects were in a weight-maintaining and not in a dynamic weight-gaining phase. A food consumption study using a 2 d food record (9) revealed a negative correlation between body fat percentage and energy intake in a young adult population even after adjusting for changes in body weight. These findings suggest that the overweight may have a lower energy requirement and/or use their food energy more efficiently than lean people, provided their physical

activity is not lower.

However, the results of studies on energy expenditure, measured by direct or indirect calorimetry in overweight and lean individuals, tend to lead to the opposite conclusion: that overweight individuals have higher energy requirements than lean individuals. Several investigators have found higher resting metabolic rates (RMR) in overweight subjects (10,11,12), suggesting higher energy needs. Resting metabolic rate measurements do not, however, include thermogenesis due to food intake, physical activity, smoking, caffeine consumption, stress etc. Since RMR may account for 60-75% of the total daily energy expenditure, relying only upon RMR may obscure a reduced thermogenesis in overweight individuals. Recently several studies on 24 hour energy expenditure (24hEE) in whole body calorimeters, with special reference to overweight and obese individuals have been published (13-17). These show that the 24hEE of overweight individuals is higher than that of lean individuals. In these experiments, however, physical activity was limited to sedentary activities and no strenuous exercise was allowed. In some experiments, subjects were not close to energy balance, possibly resulting in lower or higher 24hEE than would have been found, had energy intake been adjusted to energy requirement level.

To estimate the energy requirements of lean and overweight women in a weight maintaining phase we performed an energy balance study making use of two whole body indirect calorimeters. We attempted to simulate in the calorimeter a day occupied mainly by sedentary activities and some bicycling, resembling a normal sedentary work-, or a light housekeeping day. Energy intake was held constant at or near energy requirement level for 5 days before the calorimetry session. Metabolizable energy intake was assessed by analyses for energy in food, faeces and urine. Energy expenditure was measured over three 24 hour periods. Energy balance was assessed by subtracting mean energy expenditure over 3 days from metabolizable energy intake.

MATERIALS AND METHODS

Subjects and anthropometry

Twenty-nine lean and 18 overweight women completed the 8 day experiment. Women with a BMI $\leq$ 25 were classified as lean and women with a BMI > 25 were classified as overweight. All women were apparently healthy and used no drugs, apart from contraceptives, during the experiment. Smoking was permitted during the experiment,

TABLE 1
Characteristics of 29 lean and 18 overweight female subjects.

	lean		overweight		
	mean	SEM	mean	SEM	p value
Age yr	28.1	1.3	33.5	1.8	< 0.05
Body weight[1] kg	58.8	1.0	93.7	5.0	< 0.001
Height m	1.68	0.01	1.67	0.01	ns
Body Mass Index kg/m²	20.7	0.4	33.5	1.6	< 0.001
Body fat 1[2] %	23.6	0.9	42.2	2.2	< 0.001
Body fat 2[2] %	26.1***	0.8	38.6**	0.9	< 0.001
Fat free mass[2] kg	44.7	0.6	51.9	1.6	< 0.001
Tobacco g/d	1.6	0.7	3.2	1.2	ns

[1] Mean body weight during stay in calorimeter

[2] Body fat 1 assessed by underwater weighing; body fat 2 assessed by skinfold measurements. Fat free mass calculated using body fat 1

** different from body fat 1 overweight women $p < 0.05$
*** different from body fat 1 lean women $p < 0.001$

but the number of cigarettes was individually adjusted to a minimum during the calorimetry session. Table 1 presents some of the characteristics of the subjects. Body volume was assessed by underwater weighing, corrected for lung volume by He dilution. Siri's equation (18) was used to calculate body fat % from body density. The underwater weighing of one overweight subject failed.
Skinfold thicknesses were measured at 4 sites (biceps, triceps, subscapula and suprailiaca) using a Holtain caliper. In twelve of the overweight subjects, however, skinfolds at subscapula and/or suprailiaca could not be accurately measured. The equations of Durnin and Womersley (19) were used to calculate body fat % from the skinfold data.

Experimental protocol

To become accustomed to the calorimeter and the procedures, 38 subjects spent 30 hours in the calorimeter (adaptation day), one to two months before the actual 8 day experiment. At that time a 24hEE measurement was made. Four other subjects had already taken part in an earlier experiment. The remaining 5 subjects were familiarized with the calorimeter by showing and explaining the equipment and procedures.
The experimental protocol is presented in figure 1. The 8 day experiment consisted, for each subject, of consumption of an experimental diet close to the

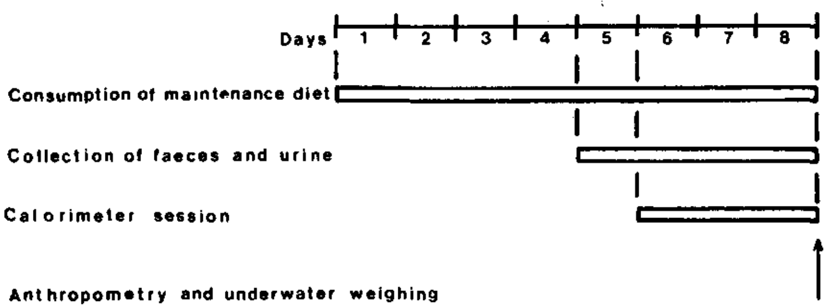

Figure 1. Experimental protocol of the 8 day experiment.

energy requirement level, and a stay, for the last 80 hours, in the calorimeter. Faeces and urine were collected during the last 4 days of the experiment. Anthropometry and under water weighing were performed immediately after the calorimetry session in all but 4 subjects. These four were measured three to four weeks before the experiment.

The study was approved by the Ethical Committee of the Department of Human Nutrition of the Agricultural University in Wageningen.

Diet

Energy requirement of each subject was estimated from the observed 24hEE during the adaptation day, from the food consumption measured by 7 d weighing record, and an enquiry on daily physical activity both during normal life. The experimental diet was then designed to meet this estimated energy requirement.

The experimental diet consisted of a limited number of foods commonly eaten in the Netherlands. The food composition was the same as that described by Van Es et al. (20). Tapwater, mineral water and tea were provided ad libitum. Coffee consumption depended upon the subjects normal consumption, up to a maximum of 10 g/d (powdered coffee), and was held constant throughout the experiment.

Metabolizable energy content of the experimental diet was calculated making use of Dutch food composition tables (21,22) and values from analysis of the energy content of foods not included in those tables. The relative contributions of protein, fat and carbohydrate to total metabolizable energy intake were maintained at 14%, 40% and 46% respectively, simulating the current Dutch eating pattern. Food preparation, storage and sampling procedures were as described by Van Es et al. (20). Food was weighed out to the nearest 0.1 g. All the food for one day was provided in the morning, and the subjects were free to choose how much food was eaten for breakfast, lunch or dinner. The actual metabolizable energy intake (ME) was determined from analyses of the energy in the food, faeces and urine.

Collection of faeces and urine

Faeces and urine were collected over the last 4 days of the experiment. Faeces produced were refrigerated, and afterwards pooled, weighed and freeze-dried. Urine collection started after voiding in the morning and ended 4 days later after collection of the morning urine. Urine was collected in 1 litre plastic bottles containing mercury-iodide as a preservative. Urine was stored in the refrigerator, pooled, weighed and sampled.

Indirect calorimetry

Procedure in the calorimeter. Two calorimeters were used simultaneously. Subjects could see each other through a large window in the connecting wall between the calorimeters, and could talk to each other by intercom. Subjects wore their own clothes and adjusted their clothing to their own comfort. The ambient temperature was set at 21-22°C at daytime and at 19-20°C at night, but was adjusted when subjects felt uncomfortable. Relative humidity was between 60 and 80%. The calorimeters are described in greater detail by Van Es et al. (20).
Subjects entered the calorimeter in the evening, 8-9 hours before the gas exchange measurements started. They were awoken at 7.45 and followed a standard daily activity schedule. This schedule included five 15 minute bicycling sessions on a hometrainer at a speed of 24 km/h without load, at 8.45, 12.15, 13.30, 17.30 and 22.30 (see also fig. 2). Subjects prepared for bed at 22.45 and were in bed before 23.15. The remainder of the time was occupied with sedentary activities and some spontaneous activities, e.g. preparing coffee, tea or meals, washing dishes etc. This activity pattern was intended to simulate light housekeeping or sedentary work.
The subjects weighed themselves each morning and evening. At this time they also measured their body temperature using rectal thermometry.
Gas exchange measurements. Gas exchange was measured between 7.30 am and 7.30 am on the next day. Oxygen (O_2) and carbon dioxide (CO_2) concentrations in the in- and outgoing air were measured by two different systems. With the first system, 24 h composite air samples were collected and analysed volumetrically for O_2 and CO_2 using a Sonden apparatus. This yielded for each subject, the mean O_2 consumption and CO_2 production over 24 h. To obtain O_2 consumption and CO_2 production over shorter periods another system (no. 2) was used. This consisted of a

paramagnetic O_2 analyser (Servomex 540A) and an infra-red CO_2 analyser (TPA 311). Ingoing air samples were analysed once every 6 h and air samples leaving the calorimeter were analysed and recorded every 20 min. for a period of 10 min. The response time of the second system was approximately 3 min.
Brouwer's equation (23) was used to calculate the mean 24 h energy expenditure over 3 days from O_2 consumption, CO_2 production and urinary nitrogen excretion. The protein correction factor in this formula was neglected in calculation of energy expenditure per day and during periods shorter than 24 h. Before calculation of energy expenditure O_2 consumed and CO_2 produced by burning cigarettes were subtracted.
Energy expenditure (EE) values were obtained every 15 min., day and night. For analysis the average for each 60 min. was used. Daytime (7.30-23.30) and nighttime (23.30-7.30) values were also considered. Values were also partitioned, somewhat artificially, into EE during three distinct physical activities:

1. EE due to lying in bed (sleeping EE), as EE during the night (23.30-7.30) multiplied by 3.
2. EE due to sedentary activities (sedentary EE), defined as the difference between the energy expended during sedentary activities and the sleeping EE.
3. EE due to bicycling (bicycling EE) defined as the difference between the energy expended in bicycling and the sum of sedentary EE and sleeping EE.

The overall calibration of the calorimeters was checked by infusing pure N_2 or CO_2, or by burning alcohol for 24 h. The measurements by the calorimeter agreed with those predicted from the N_2 and CO_2 infusion for 101% and 99.5 ± 0.7%, respectively, and with those predicted from burning alcohol for 99.5 ± 1.0% (O_2) and 99.7 ± 1.2% (CO_2).

Analyses

Energy contents of food, urine, faeces and cigarettes were determined using a static bombcalorimeter. Nitrogen content in food and excreta was determined by the Kjeldahl method using mercury-oxide as a catalyst (24). Analysis of urine for creatinine was performed by the method described in Klinisches Labor (25).
Statistical analysis: Differences between and within groups were tested using the t-tests for unpaired and paired samples, respectively. Analysis of variance was used to test for a difference in EE between the days spent in the calorimeter. Coefficients of variation were calculated from estimates of within- and between-subject variances resulting from the analysis of variance. The best prediction

equations for 24hEE and sleeping EE were computed by multiple regression analysis using the backward deletion method.

Computations were performed with SPSS (26). Statistical significance was reached when $p < 0.05$. Values in the text are presented as mean ± SEM, unless stated otherwise.

RESULTS

Body weight and body composition

Table 1 shows data on body weight and body composition of the subjects. The range of body weights in lean subjects was 47.0-69.9 kg and that in the overweight subjects 71.5-147.4 kg. Body fat % calculated from body density differed from body fat % calculated from skinfold thickness in lean as well as in overweight women. Body fat % derived from body density was considered to be more accurate and was used in the calculations and analyses presented below.

Fat free mass (FFM) in the lean subjects (44.7 ± 0.6) was lower ($p < 0.001$) than in the overweight subjects (51.9 ± 1.6). The excess body weight of the overweight subjects compared with the lean subjects consisted of 21% FFM and 79% fat mass. These values are close to the values mentioned by Webster et al.(27). They calculated that excess weight consisted of 22-30% FFM and 70-78% fat mass.

Creatinine excretion in the urine was measured as an index of muscle mass. Creatinine excretion in the lean subjects was 1.25 ± 0.02 g/d and in the overweight subjects 1.49 ± 0.05 g/d ($p < 0.001$). This difference cannot be explained by the 16 g higher meat intake of the overweight women but may be largely attributable to muscle mass. Creatinine excretion was positively correlated with body weight ($r = 0.76$) and FFM ($r = 0.81$).

Energy intake

Gross energy intake was calculated by multiplying the weight of foods provided by the energy contents of the foods, determined by bomb calorimetry. Actual ME intake was calculated by subtracting the energy lost in faeces and urine from the gross energy intake. Table 2 gives values of gross energy intake, digestibility, metabolizability and ME intake. Neither digestibility nor metabolizability differed significantly between lean and overweight subjects. Powdered coffee consumption during the experiment was higher in the overweight (7.9 ± 0.5 g/d) than in the lean subjects (5.8 ± 0.5 g/d; $p < 0.01$).

TABLE 2
Mean gross energy intake (GE), digestibility, metabolizability, metabolizable energy intake (ME) actual and calculated, in 29 lean and 18 overweight female subjects.

	lean		overweight		
	mean	SEM	mean	SEM	p value
GE MJ	9.90	0.14	11.34	0.32	<0.001
digestibility % of GE	93.6	0.2	93.3	0.2	ns
metabolizability % of GE	89.7	0.2	89.3	0.3	ns
ME actual MJ	8.88	0.13	10.12	0.29	<0.001
ME calculated MJ	8.67*	0.12	9.97*	0.29	<0.001

* different from actual ME p<0.005

Respiratory quotient

The respiratory quotient of the lean subjects (0.85 ± 0.01; mean ± sd) was significantly ($p = 0.045$) different from that of the overweight subjects (0.84 ± 0.01). Based on the nutrient composition of the diet a respiratory quotient of 0.85 was to be expected (28).

Energy expenditure

Three 24hEE measurements were obtained for each subject. Each 24hEE was partitioned into EE during daytime (7.30-23.30) and nighttime (23.30-7.30) (see pp 40) The within-subject coefficient of variation (CV_W)of 24hEE was similar in the lean (2.2%) and overweight subjects (2.1%). The CV_W in EE during day and night was 2.6% and 3.8%, respectively, in the lean subjects and 2.2% and 3.8%, respectively, in the overweight subjects. This increase in CV_W may be due to: (a) differences in metabolism and/or behaviour, which have a greater effect on EE over shorter periods; and (b) to an increased contribution of errors in measurement. Analysis of variance revealed no systematic difference between days in 24hEE or EE during day- or nighttime. EE values were therefore averaged over the 3 days, and mean values were used in further analyses (table 3). Mean 24hEE over 3 days was 2.12 MJ lower in lean than in overweight subjects. But 24hEE expressed per kg body weight was lower in the overweight than in the lean subjects. When 24hEE was expressed in relation to FFM the 24hEE of the overweight subjects did not differ from that of the lean subjects (table 3).

TABLE 3

Twenty-four energy expenditure (24hEE), also expressed as a function of body weight (BW) and fat free mass (FFM), and energy balance of 29 lean and 18 overweight female subjects

		lean		overweight		
		mean	SEM	mean	SEM	p value
24hEE	MJ	8.58	0.13	10.70	0.45	<0.001
$24hEE/_{BW}$	MJ/kg	147	3	115	2	<0.001
$24hEE/_{FFM}$	MJ/kg	192	3	203	6	ns
Energy balance	MJ	0.30	0.08	-0.58	0.29	<0.01

Fig. 2 illustrates the EE pattern over 24 h. Data on sleeping EE, sedentary EE and bicycling EE are given in table 4. The EE values of the lean subjects were significantly lower than those of the overweight subjects. Sleeping EE contributed 70% to the total 24hEE, sedentary EE 25% and 24%, and bicycling EE 5% and 6% in lean and overweight subjects respectively. Energy expenditures over 24 h and during the various activities were correlated with body weight and body composition parameters as shown in table 5.

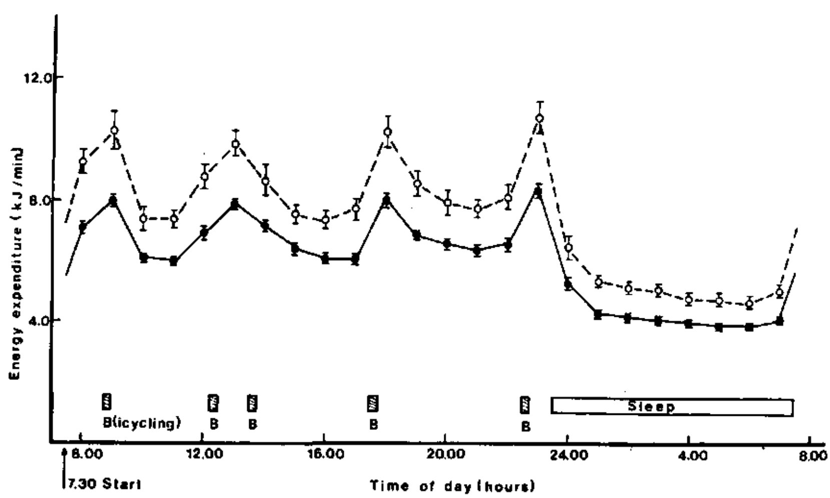

Figure 2. Energy pattern over 24 hours (mean of 3 days). Mean energy expenditure every 60 minutes of 29 lean women (dotted circles) and 18 overweight women (open circles. SEM represented by vertical bars.

TABLE 4
Energy expenditure (EE) during various activities[1] (kJ/min.) of 29 lean and 18 overweight female subjects in the calorimeter.

	lean		overweight		p value
	mean	SEM	mean	SEM	
EE sleeping	4.2	0.1	5.2	0.2	<0.001
EE sedentary activities	2.3	0.1	2.7	0.1	=0.01
EE bicycling	5.6	0.3	9.2	0.9	<0.001

[1] see for details pp. 40

Energy balance
Energy balance of the lean subjects was slightly positive: 0.30 ± 0.08 MJ/d. That in overweight subjects was not different from zero (see table 3). Even on a 1.24 MJ/d higher ME intake the overweight subjects showed a 0.88 MJ lower energy balance than the lean subjects. Mean weight change over 3 days in the calorimeter was not significantly different from zero in either group of subjects.

Between-subject variation in energy expenditure
Coefficient of variation due to between-subject variation in 24hEE was 17.7% for the total group of 47 subjects. In lean subjects the between-subject CV was 8.4%, and in overweight subjects this was 17.9%. Body weight explained 82% (n = 47) and FFM 61% (n = 46) of the variance in 24hEE. Prediction equations were calculated for 24hEE and sleeping EE, by introducing variables correlated significantly with these EE's.

TABLE 5
Pearson correlation coefficients between energy expenditure (EE)[1] data and body weight and body composition parameters.

	24h EE	EE sleeping	EE sedentary activities	EE bicycling
Body Weight	0.91	0.87	0.66	0.75
Fat free mass	0.78	0.76	0.54	0.58
Creatinine	0.79	0.80	0.59	0.49

[1]See for details p.40

24hEE and sleeping EE were best predicted by the following equations:

24hEE (kJ) = 4446 + 69 x body weight (kg); r^a = 0.906 (n = 47)

24hEE (kJ) = 4452 + 88 x body weight (kg) - 46 x body fat%; r^a = 0.904 (n = 46)

sleeping EE (kJ/min) = 1.50 + 0.02 x body weight (kg) + 1.27 x creatinine (g/d) + 0.37 x smoking[b]; r^a = 0.897 (n = 46)

a Multiple r

b no smoking = 0; smoking = 1

Age, height, body temperature and coffee consumption did not contribute significantly to the prediction of 24hEE or sleeping EE.

DISCUSSION

Energy intake

The observed digestibility and metabolizability of the mixed diet agree with values found by other investigators (16,20,29,30). No difference in digestibility or metabolizability was observed between lean and overweight subjects. This suggests that, in general, maintenance of overweight cannot be explained by higher digestibility or metabolizability of gross energy.

Actual and calculated ME intake differed by, on average, 2% (table 2). This mean difference is not very large, but the individual difference between actual and calculated ME intake varied between -6% and 3%.

Energy expenditure and energy requirement

There is no evidence that 24hEE decreased during the three day stay in the calorimeter, as was found in an earlier study (20). The within-subject CV's in 24hEE, derived from measurements on successive days, are similar to those reported by Garby et al. (31) and to those found in RMR by James et al. (10), but are higher than those found by Dallosso et al. (32). These investigators estimated the within-subject CV from duplicate measurements with a time-interval of several days to one week. The small CV_w may be ascribed to the adaptation day, one to two months before the actual experiment, to a preliminary period of 5 days for adaptation to the diet, and to the standardization of physical activity in the calorimeter. A reduction of the number of days spent in the calorimeter may thus only slightly reduce precision. Where partitioning of 24hEE into shorter time periods (day, night) or among activities is a main object of study, this reduction may be inadvisable, since differences in metabolism or behaviour and errors in measurement become relatively larger and may obscure the effects under study. Twenty-four hour energy expenditure has been shown to increase during over-feeding (positive energy balance) (16,20,33) and decrease during under-feeding (negative energy balance) (20,33,34). Use of 24hEE to estimate energy requirement introduces an error into the estimated energy requirement, if energy balance is positive or negative. With respect to the groups of women, 24hEE would overestimate actual energy requirement by only 0.7%, in the lean subjects. The actual energy requirements of the overweight subjects may be considered to be estimated by 24hEE without error, since the energy balance was not significantly different from zero. For individual subjects, however, the error in estimation of energy requirement from 24hEE may be larger (in this experiment -3% to +3%).

The observed higher energy requirement of overweight women during their stay in the calorimeter agrees with findings on 24hEE in overweight and lean subjects by other investigators (13-15). For comparison, data on 24hEE, EE during night or sleep, and RMR in women, estimated by other investigators, are presented in table 6. Since studies differ in type of calorimetry, protocol, diet composition, energy intake level, physical activity etc., direct comparison of the presented data is difficult. It may be seen from this table that the 24hEE of our women is higher than those found by others, but that EE during the night, which may be considered to be similar to RMR (17,35), is within the range found by others. EE dropped during the early morning hours (3.30-6.30) to 3.9 ± 0.1 kJ/min in our lean and to 4.8 ± 0.2 kJ/min in our overweight subjects (fig. 2). The higher 24hEE in our study may have been caused by spontaneous activities as well as bicycling and other energy expenditure-increasing factors (smoking, caffeine consumption, environmental temperature, social contact) during the daytime.

The simulation of a normal sedentary work- or light housekeeping day by introduction of some physical activity, the consumption of a mixed diet close to energy requirement, and the permission to smoke and to drink coffee may enable us to extrapolate these data to life outside the calorimeter. This is supported by the enquiry on physical activity in everyday life of the lean and overweight subjects which did not show a higher or lower average physical activity than in the calorimeter. The energy requirement found for the lean subjects is similar to the recommended daily energy intake of an adult woman of 60 kg engaged in light activity (21). The higher energy expenditure during sleeping and the higher energy expenditure during physical activities, in overweight subjects (table 4) may explain why they require more energy to maintain their body weight than lean women. However,

TABLE 6

Data on body weight, body composition, 24h energy expenditure (24hEE)[1], EE during sleep or night, resting metabolic rate (RMR)[2] and metabolizable energy intake (ME) of lean and overweight women as found by several investigators.

LEAN WOMEN		n	age years	body weight kg	fat free mass kg	24hEE MJ/d	EE during sleep kJ/min	RMR kJ/min	ME MJ/d
Bessard et al.	(35)	6	24	55.1	41.8	7.30	3.6	3.6	7.37
Blaza and Garrow	(15)	5	23	58.4	49.1	5.33	-	4.2	3.32
Dauncey	(36)	9	32	57.1	44.8	7.72(28°C)[3] 8.26(22°C)	- -	3.9 4.3	10.46 10.46
Irsigler et al.	(13)	10	22	59.6	-	6.80	-	-	7.16
Ravussin et al.	(14)	5	22	54.6	42.0	7.49	-	3.5	10.42
Schutz et al.	(17)	8	24	55.0	41.4	-	3.7	3.7	7.20
Webb and Annis	(16)	3	51	55.6	41.0	7.11	-	-	7.35
Felig et al.	(12)	10	24	62.8	43.4	-	-	3.8	-
Hoffmans et al.	(11)	13	20-30	58.6	46.4	-	-	4.1	-
Jung et al.	(37)	7	50	49.5	-	-	-	3.3	-
Kaplan and Leveille	(38)	4	19-31	57.7	39.0	-	-	5.2	-
				weighted mean		7.25	3.7	3.9	
				range		5.3-8.3		3.3-4.3	
Our study		29	28	58.8	44.7	8.58	4.2	-	8.88
OVERWEIGHT WOMEN									
Bessard et al.	(35)	6	27	84.8	51.6	9.24	4.8	4.8	8.96
Blaza and Garrow	(15)	5	35	96.3	53.4	8.30	-	5.3	3.28
Irsigler et al.	(13)	4	24	83.2	-	8.38	-	-	6.51
Ravussin et al.	(14)	11	29	83.4	50.8	9.06	-	4.6	10.42
Schutz et al.	(17)	20	30	88.5	54.3	-	4.9	4.9	9.22
Webb and Abrams	(34)	4	34	76.4	47.0	7.92	-	-	9.09
Webb and Annis	(16)	3	45	80.0	44.5	7.68	-	-	7.97
Doré et al.	(39)	19	31	104.5	-	-	-	6.1	-
Felig et al.	(12)	10	36	103.6	54.1	-	-	4.8	-
Halliday et al.	(40)	22	33	90.2	49.4	-	-	5.3	-
Hoffmans et al.	(11)	15	20-30	71.1	47.1	-	-	4.5	-
Jung et al.	(37)	6	47	84.1	-	-	-	4.3	-
Kaplan and Leveille	(38)	4	19-31	94.2	52.1	-	-	7.6	-
				weighted mean		8.63	4.9	5.2	
				range		7.7-9.2		4.3-7.6	
Our study		18	34	93.7	51.9	10.70	5.2	-	10.12

[1] *For comparison of data used: 1 Watt=0.06 kJ*
[2] For comparison of data used:1 ltr O_2=21 kJ
[3] Environmental temperature

the overweight subjects had a lower energy requirement per kg body weight than lean subjects, and the two groups had an equal energy requirement per kg FFM, which agrees with the findings of Ravussin et al. (14).

Prediction of 24hEE

82% of the variance in 24hEE of our subjects could be explained by body weight alone. Other investigators (14,41) have found that FFM explained a similar proportion of the variance. In our study FFM explained 61% of the variation in 24hEE and, in combination with fat mass 81%. The contribution of fat mass is not surprising in view of the extra energy needed to carry and move this weight during physical activities. The lower correlation between 24hEE and FFM in our study may be due to several factors. In the two studies cited (14,41) male subjects were included, resulting in a wider range in FFM, and the physical activity of the subjects was restricted to sedentary activities.

Since RMR is responsible for 60-75% of total 24hEE in individuals with a normal physical activity, sleeping EE was also considered. Body weight, urinary creatinine excretion (as an index of muscle mass) and smoking, contributed significantly to the prediction of sleeping EE. Doré et al. (39) report a regression equation based on RMR data from 140 women aged between 16 and 68. Body weight and body potassium (as index of lean mass) were positively correlated, and age negatively, with RMR. The smaller age range in our experiment (20-47) may explain why age did not appear in our equation. The observed effect of smoking on sleeping EE is supported by the findings of Dallosso and James (42). Hofstetter et al. (43), however, observed a decrease in 24hEE during a non-smoking day, but found no effect on RMR.

The regression equations given may be used for prediction of energy requirements (or 24hEE) of individual women (20-45 yr), when inside the calorimeter. For women engaged in light work, these equations may be used with care to predict energy requirement outside the calorimeter. The 99% confidence interval of the predicted energy requirement for a lean woman (60 kg) is 6.6-10.5 MJ/day and that for an overweight woman of 80 kg 8.0-11.9 MJ/day. The error in prediction for individual women may thus be quite large: ± 20%. It may still be useful, however, in counselling and treatment of overweight individuals. The data on predicted 24hEE of an overweight woman suggest that in general weight may be reduced by adherence to weight-reduction diets of 4.2 MJ/day, provided that no metabolic adaptation occurs during prolonged low energy intake.

In conclusion, energy requirements of overweight women in a weight-maintaining phase are higher than those of lean women. However, when expressed in relation to body weight or FFM, energy requirements of overweight women are lower and similar, respectively. The prediction equations for 24hEE give an indication of the energy requirements of women between 20-45 years and body weight is a useful parameter for this prediction.

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4. ENERGY AND NUTRIENT INTAKE OF LEAN AND OVERWEIGHT WOMEN, ASSESSED BY THE 7 DAY WEIGHING RECORD METHOD, VALIDATED BY 24 HOUR ENERGY EXPENDITURE MEASUREMENTS IN A WHOLE BODY INDIRECT CALORIMETER

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ABSTRACT

Usual metabolizable energy and nutrient intake of 27 lean and 18 overweight women were measured using the 7 day weighing record method. Twenty-four hour energy expenditure of these women was measured over three successive days in whole body indirect calorimeters. Energy intake and 24 hour energy expenditure were assumed to be estimates of the energy requirements of the women. The 7 day weighing record method was validated by comparing the energy intake with the 24 hour energy expenditure.
Energy intake of lean and overweight women was 8.61 ± 1.74 MJ/d (mean ± sd) and 8.78 ± 2.15 MJ/d, respectively. Protein contributed 15%, fat 37%, carbohydrate 45% and alcohol 3% of the total metabolizable energy intake, when data of the two groups were pooled.
Energy intake of the lean women, as estimated by the 7 day weighing record method, was similar to the 24 hour energy expenditure. The overweight women, however, reported a 1.9 ± 2.9 MJ/d lower energy intake than their 24 hour energy expenditure. The energy intake of the individual lean women was positively correlated with their 24 hour energy expenditure ($r = 0.58$; $p < 0.005$). No relationship was found in the overweight women. However, after deletion of the data of four excessively under-reporting overweight women, a positive relationship was found in this group too ($r = 0.79$; $p < 0.001$).
It is concluded that the 7 day weighing record method gives valid estimates of

the *usual* energy intake of lean women. The use of this method for estimation of *usual* energy intake in overweight women, however, remains questionable.

INTRODUCTION

In an earlier experiment on energy requirements of lean and overweight women, making use of whole body calorimeters (1), an estimate of the *usual* metabolizable energy intake over an extended time in everyday life was required. This estimate was used, together with other estimates of energy requirements, to design a diet to be used during the calorimetry experiment. In this experiment, 24 hour energy expenditure of lean and overweight women was measured over three successive days in whole body indirect calorimeters. In the calorimeter a standardized physical activity schedule was imposed on the subjects, to simulate the activity of individuals engaged in sedentary occupations with light activity during leisure time. The energy balance of the women in the calorimeter was close to zero, and it may therefore, be assumed, that the observed 24 hour energy expenditure was a good measure of energy requirement (1).
In this calorimetry experiment the 7 day weighing record method was used to measure metabolizable energy intake in everyday life, as an indication of energy requirement. This dietary assessment method has been referred to as ' .. a "gold standard" against which other methods are assessed. '(2). Block (3) mentions, in his review of validations of dietary assessment methods, that '.... further attempts to perfect and validate the methods are desirable ...'. Few studies, however, have validated this record method (with or without weighing) for estimating the *usual* food consumption (4, 5, 6).
In this report the validity of the 7 day weighing record method for measuring *usual* energy intake is discussed with reference to the observed 24 hour energy expenditure in the calorimeter.

MATERIALS AND METHODS

The female subjects for the calorimetry experiment were selected from volunteers on the basis of their body mass index (BMI: Weight (kg)/Height^2 (m^2)). Subjects with a body mass index of more than 25 kg/m^2 were classified as overweight. The sample is thus not representative of the Dutch female population.
Twenty-seven lean and eighteen overweight subjects completed both the 7 day

weighing record and the calorimetry experiment. The lean women were younger (28.5 ± 7.0 yr; mean ± sd) than the overweight women (33.5 ± 7.6 yr; $p < 0.05$). Mean body weight of lean women was 58.2 ± 5.3 kg, and that of the overweight women was 93.7 ± 21.2 kg; the mean BMI's in the two groups were 20.7 ± 1.9 and 33.5 ± 6.9, respectively.

Each subject was carefully taught, at the department, how to weigh and record her food intake over 7 consecutive days. All food items and drinks were weighed by the woman herself using modern electronic scales incorporating a zeroing button, and having a large digital readout (Sartorius GMBH, Göttingen, West Germany, type 1203 MP; weighing range 0-4000 gr:accuracy 1 g). Everything eaten or drunk had to be weighed and recorded: leftovers were also weighed. When not able to weigh food or drinks, the subjects were asked to estimate and record the weights or volumes in household measures. This situation occurred during meals at homes of friends or family or at restaurants, and while shopping.

Record forms for 7 days were supplied, together with an example of a food record and written instructions about how to describe food items in detail, how to record the ingredients of composite dishes, etc. The subjects were instructed to adhere to their usual food intake and refrain from dieting. The subjects started to record their food intake over 7 consecutive days at a time convenient to them, so that recording during holidays etc. was avoided. It was emphasized that the subjects should not hesitate to call the department if anything was unclear during the recording period. After completion of the record the forms were sent or brought to the department. They were reviewed by the dietician. If incomplete or questionable, the subjects were called and asked for additional information by the dietician.

Each food item on the record was coded according to a uniform food encoding system (7). Metabolizable energy and nutrient intake were then calculated using a Dutch computerized food composition table (7). In what follows,"energy intake" will be used to indicate metabolizable energy intake.

Within three months of completion of the record 24 hour energy expenditure was measured in a whole body indirect calorimeter during 3 consecutive days. The physical activity in the calorimeter was standardized. The activity pattern consisted of 8 hours lying in bed, 5 times 15 minutes bicycling on a hometrainer, and sedentary activities (including some spontaneous activities like preparing meals, coffee or tea, washing dishes, etc.). This activity pattern was chosen to simulate the physical activity of individuals engaged in sedentary occupations or light house-keeping. Details of indirect calorimetry and the study on energy requirements of lean and overweight women are given in de Boer et al (1).

The 24 hour energy expenditure may be considered to be an estimate of the energy requirement, provided the energy balance is zero or close to zero. Twenty-four hour energy expenditure is affected by over-feeding and under-feeding: it increases by about 15% (8,9) of the extra energy intake, or decreases by about 5-10% (8,9) of the deficit in energy intake. These values refer to short-term over- and under-feeding, i.e. for between one day and one week. In the case of over- and under-feeding a correction should be made to estimate energy requirement from energy expenditure. In the calorimetry experiment (1) the energy balances of the groups of lean and overweight subjects were close to zero, so the observed 24 hour energy expenditure (mean of three measurements) may be considered to be an estimate of the energy requirement in the calorimeter.
The measurements of energy intake and energy expenditure were performed over a period of 2 years, and were not confined to a specific season.

TABLE 1
Energy and nutrient intake of 27 lean and 18 overweight women. (assessed by 7 day weighing record method)

	lean women		overweight women		pooled (n=45)	
	mean	sd	mean	sd	mean	sd
Energy (MJ)	8.61	1.74	8.78	2.15	8.68	1.89
Protein (% energy)	14.1	3.0	15.6	3.2	14.7	3.1
Fat (% energy)	36.6	4.2	38.0	5.7	37.2	4.8
Carbohydrate (% energy)	45.8	4.8	42.8	7.9	44.6	6.3
Alcohol (% energy)	3.5	3.3	3.6	3.9	3.5	3.5
Cholesterol (mg per 4.2 MJ)	145	47	178*	62	158	55
Dietary fiber (g per 4.2 MJ)	13.5	3.8	10.9*	2.7	12.4	3.5

* different from lean women: $p < 0.05$

Student's t-test for unpaired samples was used to test differences in food consumption between lean and overweight subjects. The t-test for paired samples was used to compare energy intake with 24 hour energy expenditure in lean and overweight subjects. Analysis of variance was performed on the daily energy and nutrient intake, to distinguish between the variation due to subjects and that due to days of the week. The within-subject between-days standard deviation was calculated from the residual mean square, after analysis of variance with subjects as the main effect. This residual mean square included the variation due to day of the week. The within-subject between-days (to be referred to as within-subject standard deviation and the between-subject standard deviation was divided by the mean energy or nutrient intake of the respective group of subjects, yielding the within-subject and between-subject coefficients of variation. Statistics were computed with SPSS (10).

RESULTS

Table 1 gives the mean energy- and nutrient intake of the lean and overweight subjects and the pooled data. No significant difference was observed between energy or nutrient intake of the two groups, except for cholesterol and dietary fiber intake. Cholesterol intake was higher, and dietary fiber intake lower, in the overweight subjects.

TABLE 2

Relative contributions of the different variance components to the total variance in energy and nutrient intake[1] of 27 lean and 18 overweight women.

	between subject	day of the week	residual
lean women	%	%	%
Energy	26.1	1.3	72.6
Protein (% energy)	47.6	1.6	50.8
Fat (% energy)	21.4	4.2	74.4
Carbohydrate (% energy)	28.5	5.3	66.2
overweight women			
Energy	49.9	0.0	50.1
Protein (% energy)	36.1	0.0	63.9
Fat (% energy)	35.5	2.6	61.9
Carbohydrate (% energy)	51.2	1.2	47.6

[1] assessed by 7 day weighing record method

In table 2 the relative contribution of the different sources of variance to the total variance are presented. It is apparent that the major sources of variance are the between-subject and residual variance. Variance due to day of the week was significant ($p < 0.05$) only for fat and carbohydrate intake in lean women. Fat intake was higher and carbohydrate intake was lower, on Friday, Saturday and Sunday. Since the effect due to day of the week was negligible in energy and protein intake in both groups, and in fat and carbohydrate intake in the overweight group, the variance due to day of the week was included in the resid-

ual variance. This resulted in the within-subject variance, as described in the methods section.
Table 3 presents the between-subject and within-subject coefficients of variation. There is no indication that the within-subject coefficients of variation differ between the two groups of subjects.

TABLE 3

Between-subject and within-subject coefficients of variation in energy and nutrient intake[1] of 27 lean and 18 overweight women

	between subject	within-subject
lean women	%	%
Energy	17.1	28.7
Protein (% energy)	20.0	21.0
Fat (% energy)	9.0	17.3
Carbohydrate (% energy)	9.2	14.7
overweight women		
Energy	22.9	22.6
Protein (% energy)	18.2	24.0
Fat (% energy)	13.8	18.7
Carbohydrate (% energy)	17.2	16.8

[1]assessed by 7 day weighing record method

Mean energy intake was similar to 24 hour energy expenditure in the lean women, but was 1.9 ± 2.9 MJ/d (mean ± sd) lower in the overweight women (table 4). For the pooled data, energy intake was 0.8 ± 2.3 MJ/d lower than 24 hour energy expenditure ($p < 0.05$). The Pearson correlation coefficients between energy intake and 24 hour energy expenditure are also given in table 4. Figure 1 shows the individual data on energy intake and 24 hour energy expenditure of the lean and overweight subjects. A positive relationship was found between energy intake and 24 hour energy expenditure in lean subjects ($r = 0.58$), but not in overweight subjects.

TABLE 4

Energy intake (MJ), 24 hour energy expenditure (24hEE, MJ) and Pearson correlation between energy intake and energy expenditure of 27 lean and 18 overweight women.

	Energy intake[1]		24hEE		Pearson r	significance of r
	mean	SEM	mean	SEM		
lean women	8.61	0.33	8.62	0.14	0.58	0.002
overweight women	8.78*	0.51	10.70	0.45	-0.01	n.s.
pooled	8.68*	0.28	9.45	0.25	0.16	n.s.

[1] assessed by 7 day weighing record method

* significantly different from expenditure: $p < 0.05$

DISCUSSION

The mean energy- and nutrient intakes of the lean and overweight subjects are similar to those found in other samples of adult Dutch females (11,12). The reported energy intake of both groups is in accordance with the recommended daily energy intake for an adult woman between 20 and 35, with a body weight of 60 kg, who is engaged in light work (13). Considering the extra 30 kg body weight of the overweight subjects, the observed energy intake in this group is lower than would be recommended, to maintain that weight.
The observed within-subject coefficients of variation in energy intake are within the range of those calculated from data of Van Staveren et al. (11) and those reported by Beaton et al. (14). However, the between-subject coefficient of variation in energy intake in our subjects appears to be lower than those (24% and 26%, respectively) in previous reports (11,14). This may be caused by the fact that the subjects of our study were not randomly selected.
Many studies on food consumption of lean and overweight people have found lower or similar energy intakes in overweight people compared with those of lean people (12,15-21). Our overweight subjects reported an energy intake that was similar to that of the lean subjects. This finding, as well as that of the other investigators, is in conflict with physiological reasoning and with observations on 24 hour energy expenditure of lean and overweight subjects, measured in whole body

calorimeters (22,23,24). The 24 hour energy expenditure of overweight subjects in those studies was higher than that of the lean subjects (the physical activity was similar), as might be expected, since the resting metabolic rate of overweight subjects is higher (25,26), and movement and carrying of the extra body weight costs extra energy. This discrepancy may be explained by a lower physical activity of the overweight people in everyday life.

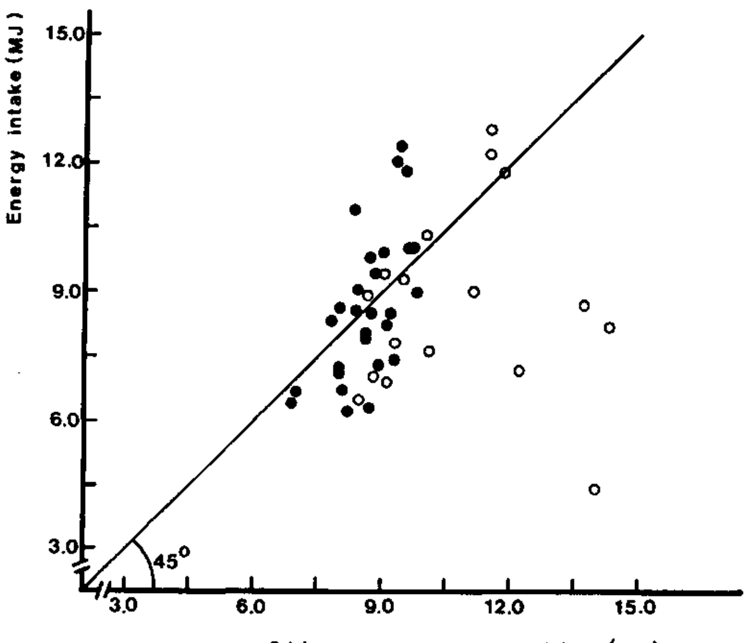

Figure 1. Energy intake, assessed by the 7 day weighing record method, and 24 hour energy expenditure, assessed by indirect calorimetry of 27 lean women (dotted circles) and 18 overweight women (open circles).

However, several investigators have reported that physical activity of overweight people is no lower than that of lean people (12,20,21). The current average physical activity of women in the developed countries is fairly light. This is supported also by the observed mean energy intake of the lean women, which is similar to the recommended energy intake for a woman engaged in light work (13). A daily physical activity that is lower than this light work is unlikely in overweight women between 20 and 45, who are engaged in their normal occupation and who are not limited in physical activity by extremely high body weight. A lower activity of overweight people cannot, therefore, explain the discrepancy. Another reason for the discrepancy may be that the methods used to estimate *usual* energy intake are subject to systematic error, and are therefore giving inaccurate data. The validity of the dietary assessment method (the "7 day weighing record") may be tested with the observed 24 hour energy expenditure in the calorimeter. Although it may be questioned whether the physical activity in the calorimeter resembles the normal daily activity, a questionnaire about physical activity over the 7 days when recording the food intake gave us no reason to doubt that, for our groups of subjects, the physical activity in the calorimeter reflected normal daily activity.

The data on energy intake and 24 hour energy expenditure indicate that the 7 day record method gives a valid estimate of the *usual* energy intake of the lean subjects. However, the former does not apply to the group of overweight subjects. The estimate of their *usual* energy intake was significantly lower than their observed 24 hour energy expenditure. It has been reported that in elderly subjects (4) and pregnant women (6) the 7 day record provides valid results. However, Warnold et al. (27) mention that the overweight patients in their study, when on a reducing diet, did not record their true energy intake during two 7 day records. This validation was based on measurements of 24 hour urinary nitrogen excretion, which gives an insight into protein consumption. Other investigators have questioned the value of food records for estimating energy intake in overweight patients (15,28). Recently, Van Staveren et al.(29) observed that the female subjects in the lowest quintile of energy intake, assessed by a 24 hour recall repeated monthly 14 times, were on average heavier and included relatively more overweight subjects than those in the other quintiles. Comparison of the nitrogen intake data with urinary nitrogen excretion data suggested that the subjects in the lowest quintile of energy intake had underreported their energy intake, and that those in the highest quintile had overreported their energy intake.

The fact that reported energy intake increased significantly between repeated dietary histories in massively obese individuals (30) may indicate that more 7 day records or more than 7 day records, may be needed to estimate accurately the *usual* energy intake in overweight subjects. This last statement suggests that the within-subject coefficient of variation in overweight women might be high. The within-subject coefficient of variation in overweight women in our experiment was no higher than that of lean women. It is possible however that the within-subject between-weeks coefficient of variation in overweight women is higher than the within-subject between-days coefficient of variation.

If it is assumed that the physical activity inside the calorimeter does reflect daily physical activity of the *individuals* outside the calorimeter, the validity of the 7 day weighing record, as an assessment of *usual* energy intake of individuals in everyday life, may be tested. A positive relationship was found between the individual data on energy intake and 24 hour energy expenditure in the lean subjects. The regression coefficient expressing the relation between energy intake and 24 hour energy expenditure was 1.35 ± 0.39 (mean ± sd) and was not significantly different from one. This suggests that the method may be valid for estimation of *usual* energy intake of individual lean women. In the overweight women, however, no such relationship was found. This was due mainly to four overweight women, who reported an energy intake 37-69% lower than their 24 hour energy expenditure. For these subjects the bicycling exercise in the calorimeter may have increased their physical activity above their normal levels. However. subtraction of the energy expenditure due to bicycling reduced the discrepancy between recorded energy intake and 24 hour energy expenditure only to 34-66%. The other overweight women did show a positive relationship between energy intake and 24 hour energy expenditure ($r = 0.79$; $p < 0.001$). Pooling of the data of those overweight subjects with those of the lean subjects yielded a positive relation between energy intake and 24 hour energy expenditure ($r = 0.64$; $p < 0.001$; $b = 1.10 \pm 0.21$). The "flat slope syndrome" (4), which was present before deletion of the data of the excessively under-reporting overweight subjects, had thus disappeared.

The positive relationship between reported energy intake and 24 hour energy expenditure suggests that, with use of the 7 day weighing record, individuals may be classified into subgroups of small and large eaters. The reported energy intakes so obtained, should be compared, however, with independent indicators of the energy intake. One such indicator may be the 24 hour urinary nitrogen excretion (5,6,27). For women who are in a weight maintaining phase, the 24 hour energy expenditure may be predicted from regression equations based upon body weight or upon body weight and body fat percentage, as given by de Boer et al. (1).

It is concluded that the 7 day weighing record method gives valid results on *usual* energy intake for a group of lean women. However, the validity of the method for use in overweight women remains questionable. Critical evaluation of reported intakes with regard to body weight, body weight change and physical activity is required before any conclusions can be drawn about *usual* energy and nutrient intake, and the requirements of groups, or, in particular, individuals.

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5. EFFECT OF ALTERNATING DAILY ENERGY INTAKE ON ENERGY METABOLISM, USING INDIRECT CALORIMETRY

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ABSTRACT

1. Sixteen subjects consumed a mixed diet for 14 days. The diet provided a constant energy level (C regime; 100%) for 8 days and alternated between 50% and 150% of the C level (L/H regime) for 6 days. Eight subjects (group I) were subjected first to the C regime and then to the L/H regime. The other eight subjects (group II) followed the reverse scheme. Two indirect calorimetry sessions were performed, one for three days, while subjects were on the C regime and one for four days while on L/H regime. Analyses of food, faeces and urine for energy permitted estimation of metabolizable energy intake. Energy balances over the last 4 days of the C and L/H regimes were assessed from metabolizable energy (ME) intake and mean 24 hour energy expenditure.

2. Neither digestibility nor metabolizability, nor metabolizable energy intake differed significantly between the two dietary regimes.

3. Twenty-four hour energy expenditure (24hEE) decreased by 5% on the L/H regime in group I, compared to that on the C regime. In group II, however, 24hEE, when on the L/H regime, was 3% higher than that on the C regime. The opposite reactions by the two groups may be partly explained by insufficient adaptation of the subjects to the calorimeter during their first session. After pooling of the data of both groups, no difference was observed in 24hEE between the two

dietary regimes. The observed decrease during the 50% days and increase during the 150% days in 24hEE, while on L/H regime, relative to the 100% days, amounted to 10% of the deficit in ME intake and 6% of the extra ME intake, respectively.

4. Energy balance was not affected by the L/H regime.

5. The data of this study do not support the view that alternation of daily energy intake elevates 24hEE and changes energy balance.

INTRODUCTION

Several investigators have reported the effects of over-feeding and under-feeding with a mixed diet on basal metabolic rate (BMR), 24-h-oxygen consumption and 24-h-energy expenditure (24hEE). Apfelbaum et al. (1971) found an increase of 11% in 24-h-oxygen consumption after over-feeding their subjects for 15 days. In other studies 24hEE increased by 10% after 1 day over-feeding (Dauncey, 1980), by 6% after 7 to 8 days over-feeding (Van Es et al., 1984) and by 10% after 30 days over-feeding (Webb & Abrams, 1983). When expressed as a percentage of the extra energy intake, the increase in 24hEE in these three studies was calculated to be 16%, 14% and 20% respectively. A reduced 24hEE was observed during under-feeding. Reported decreases in 24hEE were 6% after 1 day under-feeding (Dauncey, 1980), 2% after 7 to 8 days under-feeding (Van Es et al., 1984) and 12% after 6 weeks of under-feeding (Webb & Annis, 1983). These decreases, expressed as a percentage of the deficit in energy intake, amounted to 9%, 5% and 30% respectively. Long-term under-feeding results in loss of body weight and loss of metabolically active tissue. These losses, and a possible metabolic adaptation to long-term low energy intake, may be the cause of the greater reduction in 24hEE in the study of Webb & Annis (1983). Another large decrease in energy expenditure after long-term under-feeding was reported by Keys et al. (1950), who found a 32% reduction in BMR after 12 weeks under-feeding.

The increase in 24hEE, expressed as a percentage of the extra energy intake due to short-term over-feeding (1 to 8 days) with a mixed diet appears to be greater (14-16%) than the decrease in 24hEE expressed as a percentage of the energy deficit, due to short-term under-feeding (5-9%). This is to be expected on theoretical grounds. In over-feeding the extra energy in the form of protein, fat and carbohydrate must be absorbed, transported and stored. This is coupled with an increase in 24hEE depending upon the form in which these nutrients are stored. Storage of dietary carbohydrate as adipose fat may dissipate 28% of the energy of the ingested carbohydrate, but only 3-5% is dissipated by storage as glycogen (Flatt, 1978). During under-feeding, however, less nutrients are to be absorbed, transported and stored, so 24hEE is reduced. Considering the theoretical values given by Flatt (1978) the reduction in 24hEE due to short-term under-feeding might, for a mixed diet, amount to about 7% of the deficit in energy intake. The larger increase in 24hEE during over-feeding (compared with the decrease in 24hEE in under-feeding) is thus attributable largely to the conversion of dietary carbohydrate to adipose fat.

We hypothesize, therefore, that an increased energy expenditure, relative to that during periods of constant energy intake, may result when short periods of over-feeding are alternated with short periods of under-feeding. Zero energy balance may then be reached at a higher level of energy intake. In everyday life daily energy intake is not constant but fluctuates, as shown by coefficients of variation of daily energy intake of about 30% (Beaton et al., 1979; Todd et al., 1983). Calorimetric studies, however, are often performed with diets providing a constant daily energy intake. If short-term fluctuation in energy intake affects energy balance, as hypothesized, this might be helpful in treatment and prevention of overweight.

The study reported here was designed to test the above-mentioned hypothesis. The subjects consumed a diet, which either provided a constant amount of energy per day or alternated between 50% and 150% of the constant level. Twenty-four-h-energy expenditure was measured as heat production, during the two diet periods, in whole-body indirect calorimeters.

MATERIALS AND METHODS

Subjects

Sixteen apparently healthy volunteers (fourteen women and two men) participated in the study. Physical characteristics of the subjects are given in table 1. Three subjects smoked, and were allowed to smoke during the experiment.

Experimental protocol

A summary of the experimental timetable is given in figure 1. Two dietary regimes were applied to each subject (1) a C (constant) regime, providing the subject daily with the same amount of energy (requirement level=100%) and (2) a L/H (Low/High) regime, which alternated daily between 50% and 150% of the requirement level. The

TABLE 1
Physical characteristics of subjects (M= male, F= female)

	Subject no.	Sex	Age (years)	Height (m)	Weight[1] (kg)	Body fat[2] (%)
GROUP I						
	1	F	28	1.68	61.6	31.7
	2	F	47	1.64	70.0	40.1
	3	F[3]	29	1.76	60.4	24.2
	4	F[3]	43	1.62	53.5	28.2
	5	F	20	1.63	64.2	31.2
	6	F	22	1.83	72.0	24.8
	7	F	25	1.63	54.3	25.5
	8	M	23	1.84	69.0	12.9
		Mean	30	1.70	63.1	27.3
		SD	10	0.09	7.0	7.8
GROUP II						
	9	F	22	1.77	63.0	22.5
	10	F[3]	63	1.65	68.7	41.0
	11	M	26	1.81	74.0	16.2
	12	F	23	1.75	84.0	33.7
	13	F	22	1.60	45.9	23.0
	14	F	23	1.73	71.3	30.1
	15	F	23	1.66	68.8	32.7
	16	F	36	1.56	63.2	39.9
		Mean	30	1.69	67.4	29.9
		SD	14	0.09	10.9	8.7

[1] Mean body weight during stay in calorimeter
[2] Body fat percentage assessed by underwaterweighing; expressed as a percentage of body weight.
[3] Smokers

subjects were divided into two groups. For group I the experiment consisted of an initial period of 8 days on C regime followed by a further 6 days on L/H regime. These subjects occupied the calorimeter for days 6-8 and 11-14. Group II followed the dietary regimes in the reverse order. These subjects started with the C regime for 2 days, followed by the L/H regime for 6 days and then by the C regime for another 6 days. Subjects of group II occupied the calorimeter for days 5-8 and 12-14. The study received ethical approval from the Ethical Committee of the Department of Human Nutrition.

Diets

Prior to the study the energy requirement of each subject was estimated by a 3 day weighing record and an enquiry on daily physical activity. The experimental diets

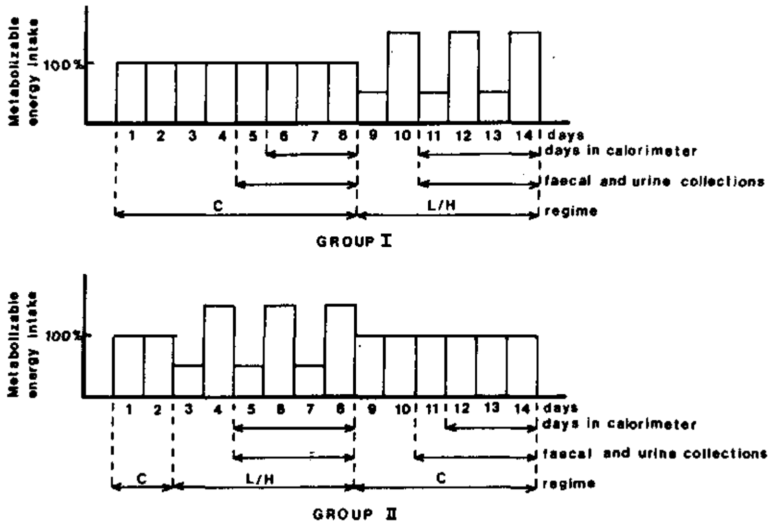


Fig. 1. Experimental protocol.

were then designed to meet this energy requirement. The diets consisted of a limited number of foods commonly eaten in the Netherlands. The food composition was the same as described by Van Es et al. (1984). Tapwater, mineral water and tea were provided ad libitum. Three subjects preferred to drink daily 1 to 2 glasses of alcoholic beverages. Coffee consumption depended upon the subjects' normal consumption, up to a maximum of 10 g/day (powdered coffee), and was held constant throughout the experiment.

Metabolizable energy (ME) content of the C and L/H diets was calculated making use of the Dutch Food Composition Table (1981), so that the mean ME intake on C and L/H regimes would be identical. The relative contributions of protein, fat and carbohydrate to total ME intake were maintained at 14%, 40% and 46%, respectively, simulating the current Dutch eating pattern. Alcohol contributed 2% to the ME intake of the three above mentioned subjects.

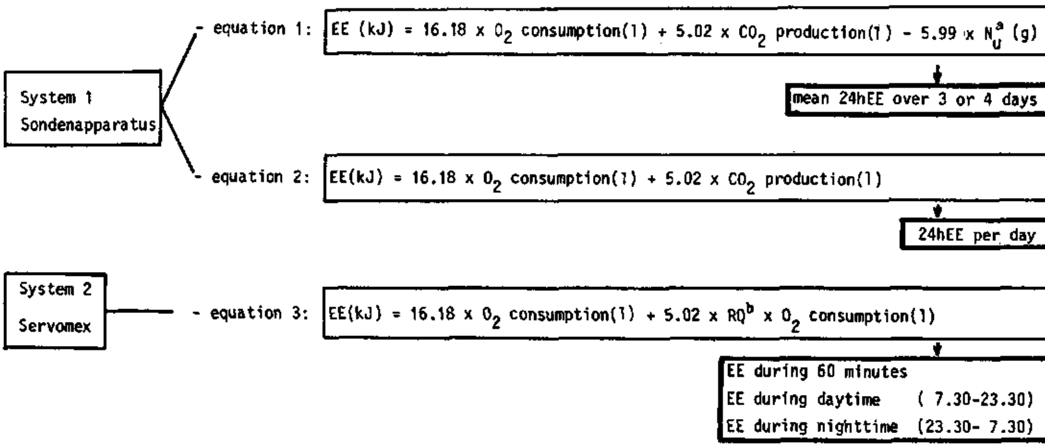
Food preparation, storage and sampling procedures were as described by Van Es et al. (1984). Food was weighed out to the nearest 0.1 g. All the food for one day was provided in the morning and the subjects were free to choose how much food and which food items to eat for breakfast, lunch and dinner. The subjects had to eat and drink everything provided. The actual ME intake was determined from analyses of energy in the food, faeces and urine.

Collection of faeces and urine

Faeces and urine were collected over the last 4 days of each dietary regime (fig. 1). Faeces produced were refrigerated, and afterwards pooled, weighed and freeze-dried. Urine collection started after voiding in the morning and ended 4 days later after collection of the morning urine. Urine was collected in 1 litre plastic bottles containing mercury-iodide as a preservative. Urine was stored in the refrigerator, pooled, weighed and sampled.

Indirect calorimetry

Procedure in the calorimeter: Subjects had been familiarized with the calorimeter by showing and explaining the equipment and the procedure.
Two calorimeters were used simultaneously. Subjects could see each other through a large window in the connecting wall between the calorimeters, and could talk to each other by intercom. Subjects wore their own clothes and adjusted their clothes to their own comfort. The ambient temperature was set at 21-22°C at daytime and at 19-20°C at night, but was adjusted when subjects felt uncomfortable. Relative humidity was between 60% and 80%. The calorimeters are described in greater detail by Van Es et al. (1984). The subjects entered the calorimeter in the evening, 8-9 hours before the gas exchange measurements started, so that they could adapt to the calorimeter. They were awoken at 7.45 and followed a standard daily activity schedule.This schedule included five 15 minutes bicycling sessions on a hometrainer at a speed of 24 km/h without load, at 8.45, 12.15, 13.30, 17.30 and 22.30 . The subjects prepared for bed at 22.45 and were in bed around 23.15. They weighed themselves each morning and evening. The remainder of the time was occupied with sedentary activities and some spontaneous activities, e.g. preparing coffee,tea or meals, washing dishes, etc. This activity pattern was intended to simulate a day of sedentary work or light housekeeping.
Gas exchange measurements: Gas exchange was measured between 7.30 and 7.30 the next day. Figure 2 gives a summary of the systems and equations used for measurement and calculation of energy expenditure. Oxygen (O_2) and carbon dioxide (CO_2) concentrations in the in- and outgoing air of the calorimeter were measured by two different systems. With system 1, 24 hour composite air samples were collected and analysed volumetrically for O_2 and CO_2 using a Sondenapparatus. This yielded for each subject, the mean O_2 consumption and CO_2 production over 24 hour. To obtain O_2 consumption and CO_2 production over shorter periods system 2 was used. This system consisted of a paramagnetic oxygen analyser (Servomex; model OA 137). This system analysed ingoing airsamples once every 6 hours and samples leaving the calorimeter every 20 min over a period of 10 min. The response time of system 2



[a] N_u = nitrogen excretion in urine
[b] Respiratory quotient calculated from analyses on oxygen (O_2) and carbondioxide (CO_2) from system 1

Fig. 2. Summary of systems and equations used to calculate energy expenditure (EE).

was approximately 3 min. The mean O_2 concentration over 24 hours in outgoing air as measured by system 2, was on average 100.0% (SD 0.1%) of the O_2 concentration measured by system 1.

Energy expenditure was calculated from O_2 consumption, CO_2 production and urinary nitrogen, using the equation derived by Brouwer (1965) and simplified versions of this equation (see fig. 2). Before calculation of energy expenditure, the O_2 consumed and CO_2 produced by burning cigarettes was subtracted. Equation 1 was used only for the calculation of mean 24hEE over 3 or 4 days. The protein correction factor ($-5.99 \times N_{urine}$) accounted for only 0.8% of 24hEE. Energy expenditure values were calculated for each 24 hour (system 1, equation 2), but were also obtained every 15 min of the day and night (system 2, equation 3). For purposes of analysis, however, the mean for each 60 min was used. The values were also examined as daytime (7.30-23.30) and night-time (23.30-7.30) values. Daily 24hEE calculated from measurements by system 1, equation 2 was 0.8% (SD 0.7%) lower than the 24hEE obtained using system 2, equation 3.

The overall calibration of the calorimeters was checked by infusing pure nitrogen (N_2) or CO_2 or by burning alcohol for 24 hours. The measurements by the calorimeter agreed with those predicted from N_2 and CO_2 infusion for 101% and 99.5% (SD 0.7%), respectively, and with those predicted from burning alcohol for 99.5% (SD 1.0%: O_2) and 99.7% (SD 1.2%: CO_2).

Analysis of food, excreta and cigarettes

The energy contents of food, urine, faeces and cigarettes were determined using a static bomb calorimeter. Nitrogen content in foods and excreta was determined using the Kjeldahl method, with mercury-oxide as a catalyst (International Organization for Standardization, 1979).

Analysis of results

Differences between the C and L/H regimes, were tested using the t-test for paired samples. Analysis of variance was used to examine differences in energy expenditure (EE) during the 3 C (100%) days and to examine differences in EE between C (100%), L (50%) and H (150%) days. Within-subject coefficients of variation were calculated from estimates of within-subject variances resulting from the analysis of variance. The variance due to interactions was included in the within-subject variance. Computation of the data was performed using SPSS (Nie et al., 1975). Statistical significance was reached when $p < 0.05$.

RESULTS

Energy intake

Individual gross energy intake was calculated by multiplying the weight of foods provided by the energy content of the food, as determined by bomb calorimetry. Metabolizable energy intake was then obtained by subtracting faecal and urinary energy losses from gross energy intake. Table 2 shows the individual values of gross energy intake, faecal and urinary energy, and the observed metabolizable energy intake on both dietary regimes.

Gross energy intake differed between the two regimes by less than 1% ($p < 0.05$) in both groups. Neither digestibility nor metabolizability were affected by the type of diet. The observed ME intake on the L/H regime was not significantly different from that on the C regime. The ME calculated, using the Dutch Food Composition Table (1981), differed by up to 3% from ME observed in individuals, but did not differ significantly at the group level from the observed ME intake. Further calculations and analyses used the observed ME intake.

Energy expenditure

Total 24hEE values were collected over 3 days on the C regime and 4 days on the L/H regime and are presented in table 3. Only two measurements were made of the

TABLE 2

Individual gross energy intake (GE), energy losses in faeces (FE) and urine (UE), observed metabolizable energy intake (ME_{obs}) and ME_{cal} calculated using the Dutch Food Composition Table (1981) (MJ/d) on C regime and L/H regime[1].

Subject no.	C regime GE	FE	UE	ME_{obs}	ME_{cal}	L/H regime GE	FE	UE	ME_{obs}	ME_{cal}
GROUP I										
1	10.39	0.53	0.39	9.47	9.43	10.44	0.63	0.41	9.40	9.38
2	10.98	0.71	0.41	9.86	9.70	10.88	0.73	0.33	9.82	9.66
3	10.50	0.50	0.46	9.54	9.40	10.39	0.75	0.42	9.22	9.34
4	9.96	0.45	0.37	9.14	9.11	9.91	0.62	0.32	8.97	9.03
5	10.51	0.62	0.44	9.45	9.43	10.40	0.98	0.41	9.01	9.38
6	11.55	0.94	0.45	10.16	10.41	11.48	0.80	0.42	10.26	10.40
7	10.49	0.57	0.39	9.53	9.43	10.38	0.71	0.39	9.28	9.38
8	13.81	0.80	0.55	12.46	12.56	13.73	0.70	0.52	12.51	12.54
Mean	11.02	0.64	0.43	9.95	9.93	10.95**	0.74	0.40	9.81	9.89
SD	1.22	0.17	0.06	1.06	1.13	1.21	0.11	0.06	1.17	1.14
GROUP II										
9	11.29	0.83	0.46	10.00	10.10	11.14	0.63	0.36	10.15	10.01
10	9.52	0.84	0.41	8.27	8.19	9.44	0.90	0.48	8.06	8.26
11	12.15	0.79	0.46	10.90	10.69	12.07	0.82	0.49	10.76	10.68
12	11.30	0.82	0.46	10.02	10.09	11.17	0.80	0.43	9.94	10.04
13	8.82	0.67	0.35	7.80	7.82	8.82	0.44	0.34	8.04	7.77
14	10.28	0.67	0.39	9.22	9.00	10.25	0.83	0.40	9.02	8.99
15	10.29	0.75	0.44	9.10	9.00	10.28	0.63	0.40	9.25	8.99
16	9.32	0.65	0.37	8.30	8.20	9.34	0.63	0.36	8.35	8.16
Mean	10.37	0.75	0.42	9.20	9.14	10.31*	0.71	0.40	9.20	9.11
SD	1.14	0.08	0.04	1.06	1.06	1.10	0.15	0.06	1.02	1.04
Pooled Mean	10.70	0.70	0.42	9.58	9.54	10.63**	0.72	0.40	9.50	9.50
SD	1.19	0.14	0.05	1.09	1.13	1.16	0.13	0.06	1.11	1.13

[1] For details see p. 68 and 69
* different from GE under C regime: $p<0.05$ ** different from GE under C regime: $p<0.005$

24hEE of subject 3 on C regime, owing to a technical failure. The mean of those two values was used in further analyses. The data on subject 8 were deleted, since this subject had been extremely busy and stressed during his first calorimeter run. Twenty-four hour energy expenditure on 50% days in group I was 0.82 MJ (SD 0.22; $p < 0.001$) lower than on 100% days. No difference was observed in 24hEE between 100% and 150% days in group I. Group II, however, showed no difference in 24hEE between 50% and 100% days, but had an increase in 24hEE of 0.57 MJ (SD 0.24; $p < 0.001$) on 150% days compared to 100% days. The resulting mean values of 24hEE over 3 or

TABLE 3.
Individual 24-h-energy expenditure[1] (MJ/d) during constant (C) and during alternating (L/H) daily energy intake.

	C regime			L/H regime			
Energy intake	100%	100%	100%	50%	150%	50%	150%
Subject no.							
GROUP I							
1	9.60	9.39	9.43	8.94	9.33	8.54	9.34
2	9.56	9.02	9.31	8.67	9.04	8.50	9.07
3	9.66	10.11	-	8.92	9.59	9.18	9.83
4	8.65	8.40	8.55	8.25	8.47	7.86	8.57
5	10.25	10.09	9.79	8.72	9.66	8.83	9.82
6	10.72	10.56	10.01	9.43	10.87	9.40	10.66
7	9.46	9.67	8.99	8.42	9.60	8.77	9.38
8	11.67	11.69	10.84	9.94	10.64	8.91	10.30
Mean[2] (n=7)	9.70	9.61	9.42	8.76	9.51	8.73	9.52
SD	0.65	0.74	0.52	0.39	0.73	0.50	0.66
GROUP II							
9	8.49	8.45	8.51	8.47	9.16	7.80	8.83
10	8.60	8.29	8.48	8.74	8.93	8.07	9.03
11	11.20	11.35	11.31	10.91	12.06	10.90	11.84
12	10.14	10.08	10.33	10.62	11.18	10.45	11.22
13	6.61	6.73	6.88	6.34	6.83	6.60	7.65
14	8.23	8.07	8.02	8.35	8.75	8.15	8.89
15	8.32	8.36	8.58	8.06	8.56	8.01	8.66
16	8.24	8.45	8.34	8.32	8.80	8.46	8.79
Mean	8.73	8.72	8.81	8.73	9.28	8.56	9.36
SD	1.38	1.39	1.38	1.46	1.63	1.42	1.42
Weighted mean[3]	9.22	9.16	9.11	8.74	9.40	8.64	9.44
SD	1.15	1.16	1.05	1.03	1.23	0.99	1.07

[1] calculated with equation 2; figure 2
[2] data no. 8 deleted from analysis, see also text pp.73
[3] weighting factor 8/7 used for data of Group I

4 days are presented in table 4. Twenty-four-h-energy expenditure under the C regime was higher than under the L/H regime in group I, but the 24hEE of group II reacted in the opposite way. A possible explanation for this discrepancy is that subjects of both groups were not sufficiently adapted to the calorimeter during their first session. This may be illustrated by the decrease in 24hEE and EE during daytime (fig. 3) in group I on C regime and by higher within-subject coefficients

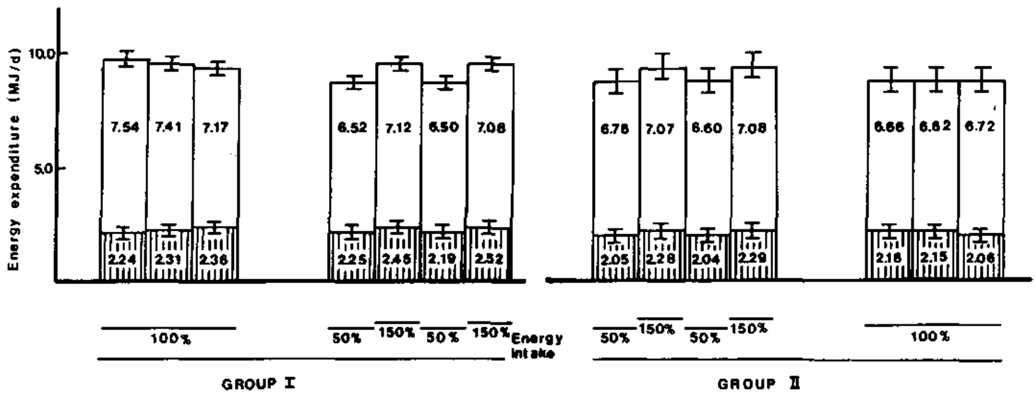


Fig. 3. Mean energy expenditure during daytime (7.30 - 23.30;) and nighttime (23.30 - 7.30;) and standard errors of the mean, represented by vertical bars, for the two groups on the calorimetry days.

of variation (cv) during the first calorimetry sessions than in the second sessions. Within-subject cv's were 2.8% (group I) and 2.6% (group II) during the first calorimetry session, but decreased to 2.4% and 1.3% respectively during the second session. Pooling of the 24hEE data of the two groups resulted in disappearance of the difference in 24hEE between the two dietary regimes, as shown in table 4. Fig. 4 shows the pattern of EE over 24 hours on 50%, 100% and 150% days for 15 subjects. Analysis of variance revealed an interaction between energy intake (50% 100%, 150%) and subject in 24hEE and EE during the daytime ($p < 0.001$), but the interaction disappeared during the night. Owing to this interaction the increase and decrease in 24hEE and in EE during daytime on 150% and 50%, respectively, relative to 100% days are not additive.

Net nutrient oxidation

Net oxidation of nutrients during C and L/H regimes was calculated using urinary nitrogen x 6.25 for protein oxidation, and values on O_2 consumption and CO_2 production (Brouwer, 1965) for fat- and carbohydrate oxidation. Fig. 5 shows the quantities of nutrients oxidized. Fat oxidation was higher ($p < 0.01$) on the C regime (109 g; SD 17) than on the L/H regime (91 g; SD 10) in group I. Group II showed the opposite effect, namely a higher fat oxidation on the L/H regime (94 g; SD 23) than on C regime (85 g; SD 25). There was no difference in net nutrient oxidation between the two dietary regimes when data on the two groups were pooled.

Respiratory quotient

Mean respiratory quotient (RQ) of all subjects was 0.85 (SD 0.02) on 100% days, 0.84 (SD 0.02) on 50% days and 0.86 (SD 0.02) on 150% days. Oxidation of the

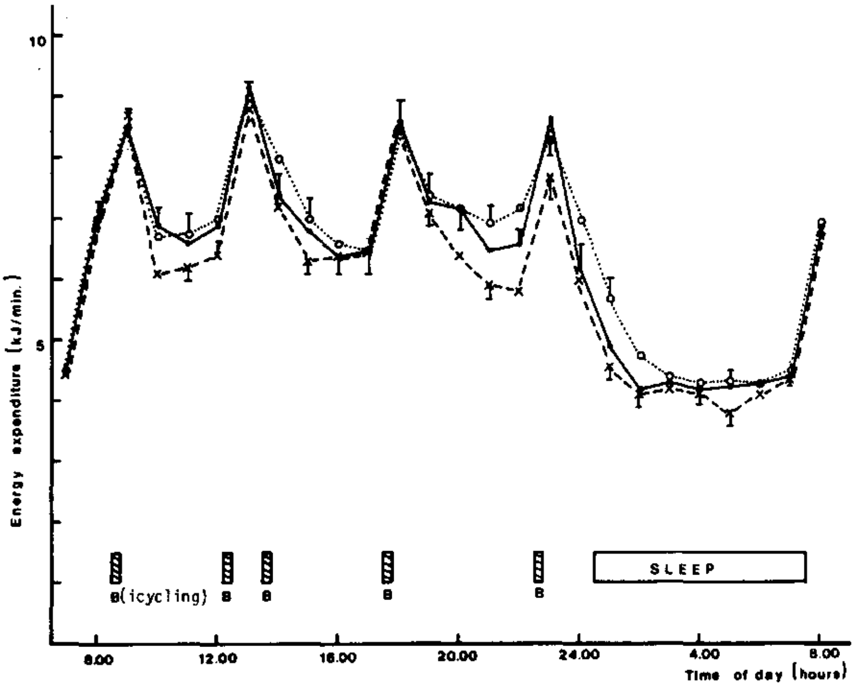


Fig. 4. Effect of under-feeding (50%) and over-feeding (150%) on pattern of 24 hour energy expenditure. Mean values (n=15) and standard errors of the mean, represented by vertical bars, of energy expenditure (kJ/min) in each 60 minute period on the three 100% days (dotted circles), the two 50% days (crossed bars) and the two 150% days (open circles) are shown.

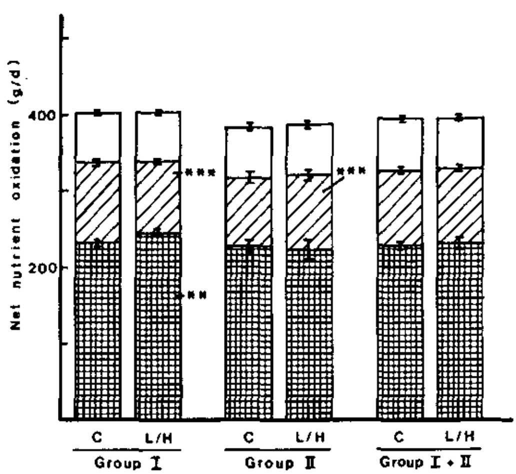


Fig. 5. Mean net nutrient oxidation (carbohydrate ▦ fat ▨ and protein ▭) of the two experimental groups and of the two groups combined on C and L/H regime.
** different from carbohydrate oxidation on C regime: $p < 0.05$
*** different from fat oxidation on C regime: $p < 0.01$

dietary mixture would have shown a food quotient of 0.85 (Flatt, 1978). The observed RQ values suggest that nutrients were oxidized in the body in the same proportions as they are present in the dietary mixture.

Energy balance

Energy balance, expressed as the difference between metabolizable energy intake and 24hEE, was affected by the use of the L/H-diet in group I, but was not affected in group II, as shown in table 4. Pooling of the data showed that energy balance was not changed by the L/H dietary regime.

DISCUSSION

Diet

Analysis of faeces and urine collected over the last four days of each dietary regime enabled us to account for differences in metabolizable energy intake between the two regimes. Faecal energy losses under the two dietary regimes were 6-7%, and urinary energy losses amounted to 3-4%. These values are within the range of 4-9% for faecal losses and 3-5% for urinary losses on mixed diets reported by other investigators (Norgan & Durnin, 1980; Göranzon et al., 1983; Webb & Abrams, 1983; Webb & Annis, 1983; Dallosso & James, 1984; Van Es et al., 1984). Neither digestibility nor metabolizability was affected by the type of dietary regime, suggesting that the use of C type diets, often used in experiments for

TABLE 4
Mean metabolizable energy intake (ME), 24-h-energy expenditure (24hEE) and energy balance (EB) on constant (C) and alternating (L/H) daily energy intake (MJ/d).

	C regime						L/H regime					
	ME		24hEE[1]		EB		ME		24hEE[2]		EB	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
GROUP I n=7	9.59	0.33	9.51	0.63	0.08	0.47	9.42	0.46	9.07***	0.54	0.35***	0.44
GROUP II n=8	9.20	1.06	8.69	1.37	0.51	0.73	9.20	1.02	8.92**	1.46	0.28	0.95
Weighted mean[3]	9.40	0.78	9.10	1.11	0.30	0.63	9.31	0.77	8.99	1.07	0.31	0.72

[1] Mean of 3 days at 100% diet
[2] Mean of 2 days at 50% and 2 days at 150%
[3] Weighting factor 8/7 used for data of group I
** different from C regime: $p<0.02$ *** different from C regime: $p<0.005$

reasons of convenience, does not influence metabolizable energy intake. Metabolizable energy intake in every day life can thus be simulated by C type diets.

Energy expenditure

The expected decrease and increase in 24hEE during the under-feeding (50%) and over-feeding (150%) days, respectively, relative to the days on requirement (100%) intake was not consistent in the 2 experimental groups. The observed decrease in 24hEE during under-feeding in group I was 9% (17% of the deficit in energy intake). No significant decrease was observed in group II. No increase in 24hEE on the over-feeding days was found in group I, but an increase of 6% (12% of the extra energy intake) was observed in group II. The sequence of regimes imposed on the subjects seemed to have an effect on the results. The observed interaction between energy intake and subjects may be due to insufficient adaptation of the subjects during their first calorimeter run, although they had entered the calorimeter 8 - 9 h before the measurements started. Presumably, the subjects felt less comfortable during the daytime hours, since the interaction is not present in the night-time. The within-subject coefficients of variation in 24hEE were higher during the first calorimetry session than during the second session. When all days (3 x 100%, 2 x 50%, 2 x 150%) were considered, the within-subject cv of groups I and II were similar: 2.6% and 2.4% respectively.
These cv's are slightly higher than reported within-subject cv's of 1.5% and 2.2% (Dallosso et al., 1982; Garby et al., 1984). Van Es et al., (1984) found a 1-3% higher 24hEE on the first day of the first calorimeter run and Garrow & Webster (1985) reported recently that anxiety or mental effort might elevate 24hEE by about 2%. The observed higher net fat oxidation within groups during their first calorimeter run (fig. 5) supports the assumption that the subjects were stressed. Stress results in a release of catecholamines, and in an increased supply of ACTH and adrenocorticoid hormones. This eventually results in an increased supply of free fatty acids to the liver, muscles etc., so these are readily available as substrates to meet the higher energy requirement during normal intake and under-feeding. During over-feeding less energy will be available for fat synthesis, resulting in a higher net fat oxidation. The above mentioned findings all emphasize that subjects should be made familiar with the calorimeter and/or that experiments should be set up in a cross-over design, if possible.

Energy balance

After pooling of the data of the two groups, energy balance was not affected by a daily alternating energy intake; this does not conform to our expectations. Kirchgessner &

Müller (1981) performed a similar experiment with 12 non-gravid, non-lactating sows, for 6 weeks: 3 weeks on C regime (100%) and 3 weeks on L/H regime (25%, 175%). They, too, found no difference in energy balance between the two regimes, although the diet they provided was high in carbohydrate and low in fat. They reported a more pronounced effect of the over-feeding day on EE during night-time, which agrees with findings of Dauncey (1980). Our own experiment shows a 8% increase in EE during the night of the over-feeding days, but this effect disappeared towards early morning (fig. 3) and during the daytime the EE was not significantly elevated. On the under-feeding days EE decreased by 6% by day, and 4% by night. In Dauncey's (1980) study, however, the effect of under-feeding was more pronounced in the daytime.

In our experiment mean 24hEE increased by 3% (6% of the extra energy intake) on the over-feeding days, and decreased by 5% (10% of the deficit in energy intake) on the under-feeding days. Kirchgessner & Müller (1981) concluded that the excess energy of their high carbohydrate feed on the over-feeding day was not stored as fat, as the increase in 24hEE was too low to account for fat storage. This also, seems to be the case in our own experiment.

If the extra energy retained on over-feeding days has the same composition as the diet (14% as protein, 40% as fat, 46% carbohydrate), as supported by the observed RQ of 0.86, 24hEE (as a percentage of the extra energy) should increase by 8%: 0.14 x 25%* = 3.5% protein storage, 0.4 x 4%* = 1.6% fat storage, plus 0.46 x 7%* = 3.2% glycogen storage. The observed value (6%) is of the same magnitude. Had the excess carbohydrate been stored as fat, the theoretical increase in 24hEE would have amounted to 17%. It may be concluded that no significant fat synthesis from carbohydrate occurred on the over-feeding days. The observed value of 6%, however, is lower than the value of 16% found by Dauncey (1980) after 1 day over-feeding. Her subjects, however, had consumed a normal diet 7 days prior to the measurements, so glycogen stores may already have been filled and fat synthesis may have occurred. Another possible explanation is, that some of our subjects were selected because of a tendency towards overweight. Some investigators have reported a lower dietary induced thermogenesis over 24 hour in obese persons (Zed & James, 1982; Steiniger et al., 1983; Schutz et al. 1984), although others have found no such difference (Blaza & Garrow, 1983; Webb & Annis, 1983).

The decrease of 5% in 24hEE (10% of the deficit in energy intake) on under-feeding days is fairly similar to the values found by Dauncey (1980).

*values derived from Flatt (1978).

Although energy balance seems to be unaffected by alternating daily energy intake longer periods of under-feeding (2-4 d) alternating with longer periods of over-feeding (2-4 d) may affect energy balance as hypothesized. In that case glycogen stores, and stores in gut, blood and other tissues, may be filled after one to two days over-feeding. Fat may then be synthesized, resulting in an increased energy dissipation in the over-feeding period. The decrease in EE during under-feeding, over two to four days, may remain constant, because the time is too short to evoke considerable weight loss or loss of metabolically active tissue.
It is thus of interest, with regard to prevention and treatment of overweight, to study the effect of the above-described eating pattern on the energy balance of overweight and lean subjects.

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6. ADAPTATION OF ENERGY METABOLISM OF OVERWEIGHT WOMEN TO AN EIGHT WEEK LOW ENERGY INTAKE, STUDIED WITH INDIRECT CALORIMETRY

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ABSTRACT

Energy requirement of 14 overweight women was assessed before and after weight reduction. Twenty-four hour energy expenditure was measured four times in a whole body calorimeter. The first measurement was performed before weight reduction, while consuming a weight maintenance diet (100% diet), this was followed by a second measurement after one week on a 4.2 MJ diet. After eight weeks on a weight reduction diet the subjects were measured again on the 4.2 MJ diet; this measurement was followed by a measurement after one week of refeeding with the 100% diet. Two subjects lost less weight than might have been expected from their observed energy expenditure in the calorimeter, so their data were excluded from the analyses. Mean body weight of the other 12 women was 93.3 ± 7.4 kg (mean ± sd) at the start and 83.4 ± 7.7 kg at the end. Loss of fat mass accounted for 84% of the total body weight loss. Twenty-four hour energy expenditure (24hEE) decreased from 10.52 ± 0.83 MJ on the 100% diet to 9.58 ± 0.75 MJ on the 4.2 MJ diet before weight reduction. After eight weeks of slimming 24hEE on the 4.2 MJ diet had decreased by 15% of the initial 24hEE (on the 100% diet) to 8.92 ± 0.65 MJ. After refeeding, the 24hEE increased to 9.45 ± 0.75 MJ. The energy requirement before weight reduction was calculated to be 10.62 ± 0.88 MJ/d, and that after weight reduction was 9.39 ± 0.79 MJ/d. The decrease in energy requirement (and 24hEE at the 100% diet) was more than that predicted from the change in body weight and body composition. It is suggested that an adaptation occurs, which may be metabolic. Whether this adaptation is temporary is not known.

INTRODUCTION

The often observed easy weight gain after slimming (1-3), may be caused simply by overeating. It may also be that during long term low energy intake energy metabolism adapts, and is not restored to normal during refeeding. It is known that energy expenditure decreases during both short- and long term under-feeding (4,5,6,7). In the latter case part of the decrease in energy expenditure may be explained by the loss of body weight and fat free mass. Neither the reaction of energy expenditure to refeeding after long-term low energy intake, nor the persistence of altered metabolism have been extensively studied in humans. Evidence from studies with rats suggests that the efficiency of energy retention is increased during refeeding after starvation (8). Other investigators report that less energy is needed to maintain body weight in previously obese rats to keep their body weight equal to that of the control animals (9).

The present study reports on the energy balance and 24 hour energy expenditure, measured by indirect calorimetry, of 14 overweight women, before, during, and after weight loss. The subjects were measured four times: first, before weight reduction, while consuming a weight maintenance diet, then after consuming a weight reduction diet for 7 days. The subjects were measured again after eight weeks on a weight reduction diet and then again after 1 week of refeeding.

MATERIALS AND METHODS

Subjects and anthropometry

Fourteen overweight women (body mass index greater than 25 weight (kg)/ height^2 (m^2)) participated. Thirteen women were apparently healthy and did not use drugs, apart from contraceptives, during the experiment. One woman suffered from rheumatism, but she used no drugs and was healthy enough to complete the experiment. Table 1 gives some characteristics of the women. Body weight, as presented in the table, was the mean body weight during the first calorimetry session before weight reduction. Body volume was assessed by underwater weighing and was corrected for lung volume by He dilution. Siri's equation (10) was used to calculate body fat percentage from body density. Weight of fat mass was calculated by multiplying body fat percentage by body weight on the morning of the underwater weighing.

TABLE 1

Characteristics of 14 overweight women before weight reduction

subject	age year	body weight[1] kg	height m	body mass index kg/m²	waist/thigh[2]
1	36	99.3	1.66	36.0	1.22
2	37	96.1	1.67	34.5	1.38
3	37	97.0	1.64	36.1	1.53
4	30	91.8	1.70	31.8	1.31
5	32	106.7	1.74	35.2	1.34
6	30	95.7	1.82	28.9	1.31
7	40	81.5	1.74	26.9	1.29
8	41	88.9	1.62	33.9	1.40
9	32	95.7	1.55	39.8	1.17
10	30	98.0	1.70	33.9	1.51
11	36	87.8	1.66	31.9	1.18
12	30	81.2	1.70	28.1	1.26
13	32	98.0	1.76	31.6	1.47
14	26	84.6	1.60	33.0	1.26

[1]mean body weight during stay in calorimeter on the 100% diet, before weight reduction
[2]waist circumference divided by thigh circumference (mean of both thighs)

Habitual smokers were permitted to smoke during the experiment, but were restricted to a maximum number of cigarettes per day while occupying the calorimeter.

Experimental protocol

The subjects were familiarized with the calorimeter and the procedures by spending 30 hours in the calorimeter (adaptation day) some months before the actual experiment. A measurement of their 24 hour energy expenditure (24hEE) was made during this 30 hour stay (data not presented here). This was used to estimate their energy requirement, which was required to design the experimental diet. At that time they were also asked to refrain from dieting and to maintain their body weight until the start of the experiment.

The 10 week experiment (figure 1) consisted of consumption the experimental diet during the initial two weeks. During the first week the diet provided energy near requirement level for each individual (the *100%* diet) and the second week a weight reduction diet was given (the *4.2 MJ* diet). During the subsequent 6 weeks the subjects were asked to follow a prescribed weight reduction diet of 4.2 MJ/d. This was followed by another two weeks on the experimental diet. The *4.2 MJ* diet was provided in the first week. In the second week the subjects were refed with the same *100%* diet as at the start of the experiment.

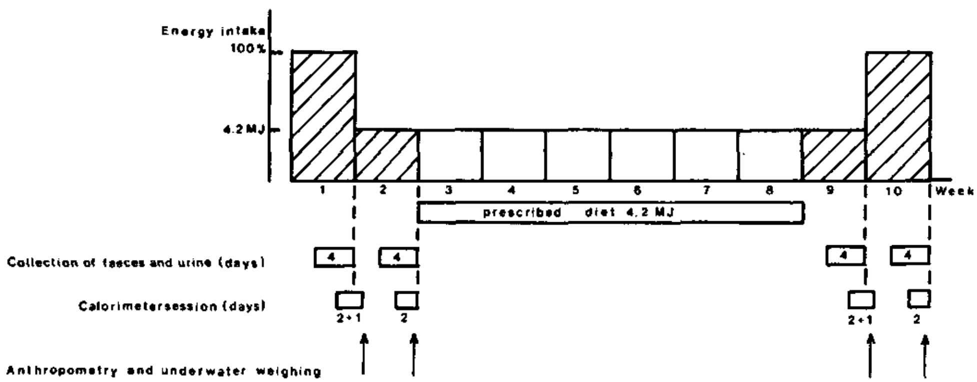

Figure 1. Summary of experimental protocol.
The shaded areas indicate the experimental diet periods.

Faeces and urine were collected during the last four days of each week on the experimental diets. The last two days (days 6 and 7) of each experimental diet week were spent in the calorimeter. The day (day 1) of the change of diet level was also spent inside the calorimeter. Under water weighing and anthropometry were performed immediately after each calorimetry session. Throughout the experiment the subjects weighed themselves weekly on a calibrated scale in the morning, after voiding, and without clothes.

Diets

Experimental diets (the 100% and 4.2 MJ diets):
Energy requirement of each subject before weight reduction was estimated using the 24hEE measurement made during the adaptation day, and from a food consumption measurement, made using a 7 day weighing record method, and an enquiry on daily physical activity, both during every day life. The *100%* diet was then designed to meet this energy requirement. The experimental diet consisted of a limited number of foods usually eaten in the Netherlands. The food composition was as described by Van Es et al. (5). Tapwater, mineral water and tea were provided *ad libitum*. Coffee consumption depended upon the subject's normal consumption, but was served to a maximum of 10 g powdered coffee per day and was held constant during the experimental diet weeks.
The *100%* and *4.2 MJ* diets consisted of the same fooditems. They were designed using Dutch food composition tables (11,12) and our own estimates of the energy of foods not included in those tables. It was decided that the relative contribution of protein (14%), fat (40%) and carbohydrate (46%) to total metabolizable energy (ME) intake of the *100%* and *4.2 MJ* diets would be the same, so that the results would be comparable to those of Van Es et al. (5), and that the diet would simulate the current normal Dutch eating pattern. Food preparation, storage and sampling procedures are described in Van Es et al. (5). Food was weighed out to the nearest 0.1 g. All food for one day was provided in the morning. Everything provided had to be eaten. The subjects were free to choose how much food was eaten for breakfast, lunch or dinner. Actual ME intake during the experimental diet weeks was determined by use of the analyses of energy of all food items, faeces and urine.

Prescribed diet: Between the two 2-week periods of experimental diet the subjects followed a prescribed diet, which was designed to provide 4.2 MJ/d. The composition of this prescribed diet was different from that of the *4.2 MJ* diet. Protein content of the prescribed diet represented 26% of the ME intake, to prevent substantial loss of fat free mass. Fat and carbohydrate contributed 24% and 50%, respectively.
The subjects were visited weekly by the dietician to check on adherence to the diet and to keep them motivated. The food intake of the previous week was then discussed in relation to the observed weight loss in that week.

Collection of faeces and urine.

Faeces and urine were collected over the last 4 days of each experimental diet week. The faeces were refrigerated and afterwards pooled, weighed and freeze-dried. Urine collection started after voiding in the morning and ended 4 days later after collection of the morning urine. Urine was collected in 1 litre plastic bottles containing mercury-iodide as a preservative. It was stored in the refrigerator, pooled, weighed and sampled.

Indirect calorimetry

Procedure in the calorimeter:.

Two calorimeters were used simultaneously. Subjects could see each other through a large window in the connecting wall of the calorimeters and could talk to each other by intercom. Subjects wore their own clothes and adjusted their clothing to their own comfort. The ambient temperature was set at 21-22° C during the daytime, and at 19-20° C at night, but was adjusted if the subjects felt uncomfortable. Relative humidity was kept between 60-80%. A more detailed description of the calorimeters is given by Van Es et al (5).

Subjects entered the respirationchamber in the evening 8-9 h before the gas exchange measurements started. They were awoken at 7.45 and followed a daily standard activity schedule. This schedule included five times 15 minutes bicycling sessions on a home trainer at a speed of 24 km/h without load at 8.45, 12.15, 13.30, 17.30 and 22.30 (see figure 2). Subjects prepared for bed at 22.45 and lay down from before 23.15 until 7.45. The remainder of the time was filled with sedentary activities and some spontaneous activities such as preparing coffee, tea and meals, washing dishes etc. The activity pattern was meant to simulate light housekeeping or sedentary work. The subjects weighed themselves each morning after voiding, and every evening, without clothes, in the calorimeter.

Gas exchange measurements:

Over each 24 hour of the occupation of the calorimeter gas exchange measurements were made between 7.30 and 7.30 the next morning.

Oxygen (O_2) and carbon dioxide (CO_2) concentrations in the in- and outgoing air were measured using two different systems. With the first system, 24 hour composite air samples were collected and analyzed volumetrically for

O_2 and CO_2 using a Sondenapparatus. Mean O_2 consumption and CO_2 production over each 24 h was thus obtained for each subject. To obtain O_2 consumption and CO_2 production over shorter periods another system (no. 2) was used. This system consisted of a paramagnetic O_2 analyser (Servomex 540A) and an infra-red CO_2 analyser (TPA 311). Ingoing air samples were analysed once every 3 h and air samples leaving the calorimeter were analysed and recorded every 20 min. over a period of 10 min. The response time of the second system was approximately 3 min.

Brouwer's equation (13) was used to calculate energy expenditure from O_2 consumption, CO_2 production and urinary nitrogen excretion. The protein correction factor in this formula was neglected in the calculations of energy expenditure during periods shorter than 24 h. Before calculation of energy expenditure O_2 consumed and CO_2 produced by burning cigarettes was subtracted.

Energy expenditure values were obtained over each 24 h, (system 1) but also over shorter periods of the day and night (system 2).
Values were partitioned somewhat artificially into energy expenditure (EE) values during three distinct physical activities:

- EE due to sleeping in bed as energy expenditure during the night (22.30 - 7.30) multiplied by three.
- EE due to sedentary activities defined as the difference between the energy expended during sedentary activities and the EE due to sleeping.
- EE due to bicycling defined as the difference between the energy expended in bicycling and the sum of the EE due to sleeping and sedentary activities.

The overall calibration of the calorimeters was checked by infusing pure nitrogen (N_2) or CO_2 or by burning alcohol during 24 hours. The measurements by the calorimeter were 101% and 99.5 ± 0.7% of those predicted from N_2 and CO_2 infusion , respectively, and 99.5 ± 1.0% (O_2) and 99.7 ± 1.2% (CO_2) of those predicted from burning alcohol.

Calculation of energy requirement and efficiency of energy utilization

The data on metabolizable energy intake and energy balance before and after weight reduction can be used to calculate energy requirement before and after weight reduction (5), as follows:

$$\frac{\text{ENERGY REQUIREMENT}}{W_1} = \frac{ME_1}{W_1} - \frac{\frac{RE_1}{W_1}}{k_m} \qquad (1)$$

$$k_m = \frac{\frac{RE_1}{W_1} - \frac{RE_2}{W_2}}{\frac{ME_1}{W_1} - \frac{ME_2}{W_2}} = \frac{\text{efficiency dietary energy}}{\text{efficiency body energy}} \qquad (2)$$

ME and RE stand for metabolizable energy intake and retained energy (energy balance) and W for a body weight parameter. W is substituted by body weight (kg), metabolic weight ($kg^{\frac{3}{4}}$) or fat free mass (kg), when energy requirement is to be expressed as a function of those parameters. If not, W equals 1. The subscript 1 refers to the ME, RE and W during the *100%* diet, and subscript 2 to those during the *4.2 MJ* diet, before or after weight reduction. The subscript m refers to "maintenance" of the body.

The ratio k_m is a way of expressing the efficiency of utilization of dietary energy for maintaining the body in relation to the efficiency of utilization of body energy for the same purpose (with negative RE's). For instance, if $k_m = 1$, a dietary deficit of 5.8 MJ is supplemented by 5.8 MJ of body energy to maintain the body. If $k_m = 0.6$ only 0.6 x 5.8 MJ of body energy is needed to replace the dietary energy deficit of 5.8 MJ. Clearly k_m may change according to the composition of the diet. The values used in this article are therefore applicable only to a mixed diet. Equation 2 is valid only with the assumption that the energy requirement remains unchanged on the *100%* and *4.2 MJ* diets. If energy requirements change due to the treatment the k values obtained no longer truly reflect the ratio of the efficiency of utilization of dietary energy to the efficiency of the utilization of body energy for maintaining the body.

Analyses.

Energy content of foods, faeces, urine and cigarettes was determined using a static bomb calorimeter. Nitrogen content of foods and excreta was determined with the Kjeldahl method, using mercury-oxide as a catalyst (14).

Analyses of results: The t-test for paired samples was used to test differences within subjects. Computations were performed with SPSS (15). Values in the text are presented as mean ± SD, unless stated otherwise.

TABLE 2.

Body weight and body fat of 12 overweight women on the 100% diet and the 4.2 MJ diet before and after weight reduction

	Before weight reduction				After weight reduction			
	100% diet		4.2 MJ diet		4.2 MJ diet		100% diet	
subject	body weight kg	body fat %	body weight kg	body fat %	body weight kg	body fat %	body weight kg	body fat %
1	99.3	47.6	96.1	46.4	88.3	41.7	89.3	41.0
2	96.1	43.6	93.2	40.6	85.7	40.2	87.8	40.9
3	97.0	50.2	94.7	50.0	85.5	47.0	86.9	46.4
4	91.8	45.4	88.9	44.8	80.2	39.0	81.5	38.9
5	106.7	51.5	104.6	51.5	95.3	48.6	95.8	47.4
6	95.7	36.6	92.8	37.6	82.4	31.2	83.5	32.0
7	81.5	33.4	78.7	33.5	68.2	25.7	68.9	24.8
8	88.9	43.6	86.2	43.1	76.4	38.8	77.5	38.1
9	95.7	51.4	93.6	51.4	88.2	47.5	89.3	46.2
10	98.0	41.2	95.0	40.2	87.5	37.7	87.6	34.9
11	87.8	41.4	85.2	41.9	80.4	38.9	80.4	37.6
12	81.2	39.1	79.3	37.6	72.0	33.5	72.4	33.1
mean	93.3	43.8	90.7	43.2	82.5	39.2	83.4	38.4
sd	7.4	5.8	7.4	5.8	7.6	6.8	7.7	6.6

RESULTS

Body weight and body composition

Table 2 gives the individual data on body weight and body fat, before and after weight reduction, at the time of the calorimetry sessions. Subjects 13 and 14 lost 2.1 kg and 4.8 kg body weight, respectively, during the experiment. Twenty-four hour energy expenditure measurements indicated that greater weight losses would have been reached by better adherence to the prescribed diet of 4.2 MJ/d. The data of these subjects were excluded from further analyses. Total body weight loss was calculated as the difference between the mean body weight (in the calorimeter on the *100%* diet) before and after weight reduction (week 1 minus week 10; fig. 1). The weight loss thus calculated may be assumed to be the actual weight loss, which is not affected by changes in the amount of food ingested and food digesta. This would not have been the case if the weight loss had been calculated as the difference

between the body weight on the *100%* diet before weight reduction and that on the *4.2 MJ* diet after weight reduction (week 1 minus week 9; fig.1). Mean body weight decreased by 9.9 ± 1.9 kg, and body fat by 5.4 ± 1.6% between the two weeks on the *100%* diet. The fat mass decreased by 1.3 ± 1.0 kg (50% of the weight lost) in the first week on the *4.2 MJ* diet. The decrease in fat mass between the first week (no 1) on the *100%* diet and the last week (no 9) on the *4.2 MJ* diet was 7.8 ± 1.5 kg (73% of the weight lost), and the decrease between the two weeks (no 1 and 10) on the *100%* diet was 8.3 ± 1.5 kg (84% of the total body weight loss).
The weekly rate of weight loss was highest in the first week on the *4.2 MJ* diet (2.6 ± 0.5 kg) and lowest in the first week (0.9 ± 0.8 kg) and the fifth week (0.9 ± 0.4 kg) of the prescribed weight reduction diet. The mean rate of weight loss during the 8 weeks of weight reduction was 1.4 kg per week.

Energy intake during experimental diets

The mean gross energy intake, digestibility and metabolizability of the *100%* diets and the *4.2 MJ* diets before weight reduction were not different from those after weight reduction, as table 3 shows. Metabolizability of the *4.2 MJ* diet was lower than that of the *100%* diet. This may be attributed to a relatively higher excretion of urinary nitrogen, as the subjects were in negative nitrogen balance on the *4.2 MJ* diet. Energy content in urine is strongly correlated with its nitrogen content (5), which explains the lower metabolizability of the *4.2 MJ* diet. Metabolizable energy intake of each subject is presented in table 4. The metabolizable energy intakes on the *100%* and *4.2 MJ* diets, respectively, were the same before and after weight reduction.

TABLE 3

Mean gross energy intake, digestibility and metabolizability of the 100% diet and the 4.2 MJ diet for 12 overweight women before and after weight loss.

		Before weight reduction				After weight reduction			
		100% diet		4.2 MJ diet		4.2 MJ diet		100% diet	
		mean	sd	mean	sd	mean	sd	mean	sd
Gross energy intake	MJ	11.20	0.94	4.79	0.09	4.81	0.07	11.23	0.99
Digestibility	%	93.3	1.8	94.2	1.9	92.8	3.1	93.8	1.6
Metabolizability	%	89.6	1.9	87.3**	2.3	87.4*	3.4	90.7	1.6

*different from metabolizability of the 100% diet after weight reduction; $p = 0.01$
** different from metabolizability of the 100% diet before weight reduction; $p = 0.001$

TABLE 4

Metabolizable energy intake (ME) and 24 hour energy expenditure (24hEE) of 12 overweight women on the 100% diet and the 4.2 MJ diet before and after weight reduction

	Before weight reduction					After weight reduction				
	100% diet		1st day on 4.2 MJ	4.2 MJ diet		4.2 MJ diet		1st day on 100% diet	100% diet	
subject	ME MJ	24hEE[1] MJ	24hEE MJ	ME MJ	24hEE[1] MJ	ME MJ	24hEE[1] MJ	24hEE MJ	ME MJ	24hEE[1] MJ
1	10.94	11.58	11.40	4.17	10.48	4.03	9.48	10.27	11.14	9.96
2	10.25	11.46	11.10	4.05	10.42	4.26	9.83	10.33	10.70	10.15
3	9.85	9.82	9.37	4.21	8.73	4.41	7.91	8.61	10.28	8.38
4	10.03	10.41	9.94	4.29	9.80	4.20	9.06	9.52	10.19	9.58
5	11.09	11.80	10.90	4.13	10.86	4.07	9.26	10.12	11.42	10.04
6	11.30	10.65	9.81	4.39	9.44	4.52	8.33	8.94	10.97	8.99
7	10.06	10.33	10.19	4.11	9.30	4.34	8.67	9.16	10.12	9.50
8	9.53	9.77	9.43	4.08	8.84	4.01	8.20	8.87	9.60	8.65
9	9.92	9.87	9.45	4.29	9.13	4.25	8.92	9.72	9.59	9.50
10	10.31	11.19	10.63	4.05	10.12	3.97	10.01	10.25	10.44	10.90
11	8.49	10.22	9.80	4.16	9.27	4.01	8.88	9.65	8.46	9.32
12	8.68	9.15	8.50	4.25	8.52	4.37	8.42	8.39	9.23	8.46
mean	10.04	10.52	10.04*[1]	4.18	9.58*[2]	4.20	8.92*[3]	9.49*[4]	10.18	9.45*[4]
sd	0.86	0.83	0.84	0.11	0.75	0.18	0.65	0.68	0.85	0.75

[1]*mean of two 24hEE measurements (days 6 and 7 of the experimental diet weeks)*
*[1] different from 24hEE on the 100% diet before weight reduction; $p<0.001$
*[2] different from 24hEE on the 100% diet and the 1st day on 4.2 MJ diet before weight reduction; $p<0.001$
*[3] different from 24hEE on the 100% diet and 4.2 MJ diet before weight reduction; $p<0.001$
*[4] different from 24hEE on the 100% diet before weight reduction and from 24hEE on the 4.2 MJ diet after weight reduction; $p<0.001$

Energy expenditure

The individual data on 24hEE during the four calorimetry sessions are presented in table 4. The mean 24hEE on the *100%* diet was 10.52 ± 0.83 MJ. On the first day of the *4.2 MJ* diet 24hEE decreased by 0.48 ± 0.23 MJ (8% of the energy deficit). By the 6th and 7th day of this *4.2 MJ* diet the 24hEE had declined further to 9.58 ± 0.75 MJ. This decrease represented 16% of the energy deficit. After 6 weeks on the prescribed weight reduction diet and 5 days on the *4.2 MJ* diet the 24hEE on this diet had declined to 85% of the initial 24hEE on the *100%* diet. On refeeding, 24hEE increased on the first day by 0.57 ± 0.26 MJ (10% of the extra energy intake). This increase, however, did not continue further in the 7 days of refeeding. The difference between the

24hEE on the *100%* diet before and after weight reduction was 1.1 ± 0.5 MJ. Data on energy expenditure during sleeping, sedentary activities and bicycling are presented in table 5. Energy expenditure during these activities changed in the same direction as total 24hEE on the various diets, before and after weight reduction.

Figure 2 gives the energy expenditure pattern per 30 minutes on the *100%* diet before and after weight reduction.

TABLE 5

Energy expenditure (mean ± sd) due to sleep, sedentary activities and bicycling of 12 overweight women before and after weight reduction on the 100% and the 4.2 MJ diet.

		Before weight reduction			After weight reduction		
		100% diet	1st day 4.2 MJ	4.2 MJ diet	4.2 MJ diet	1st day 100% diet	100% diet
sleep	(kJ/min)	5.0 ± 0.4	4.8 ± 0.4	4.6 ± 0.3	4.2^{++} ± 0.2	4.5 ± 0.3	4.5^{**} ± 0.3
sedentary activities	(kJ/min)	2.8 ± 0.4	2.6 ± 0.5	2.4 ± 0.4	2.3 ± 0.4	2.5 ± 0.5	2.4^{*} ± 0.4
bicycling	(kJ/min)	9.4 ± 3.2	9.5 ± 3.7	9.9 ± 2.8	8.7^{+} ± 2.5	8.5 ± 3.0	8.7 ± 2.5

*different from energy expenditure on the 100% diet before weight reduction; $p<0.005$
**different from energy expenditure on the 100% diet before weight reduction; $p<0.001$
+ different from energy expenditure on the 4.2 MJ diet before weight reduction; $p<0.05$
++ different from energy expenditure on the 4.2 MJ diet before weight reduction; $p<0.001$

Energy balance

Energy balance was negative (-0.48 ± 0.62 MJ) on the *100%* diet before weight reduction, but became positive (0.73 ± 0.85 MJ) after weight reduction. On the *4.2 MJ* diet, energy balance was -5.39 ± 0.80 MJ before and -4.71 ± 0.77 MJ after weight reduction.

Energy requirement and k_m

Table 6 presents the k_m and energy requirement before and after weight reduction. K_m increased after weight reduction, regardless of the body weight parameter used. Using these k_m values the decrease in energy requirement was calculated as 1.24 ± 0.55 MJ/d. Energy requirement as a function of body weight did not change, but when expressed in relation to fat free mass, energy requirement decreased from 204 ± 16 before weight reduction to 185 ± 12 kJ/kg after weight reduction ($p < 0.001$).

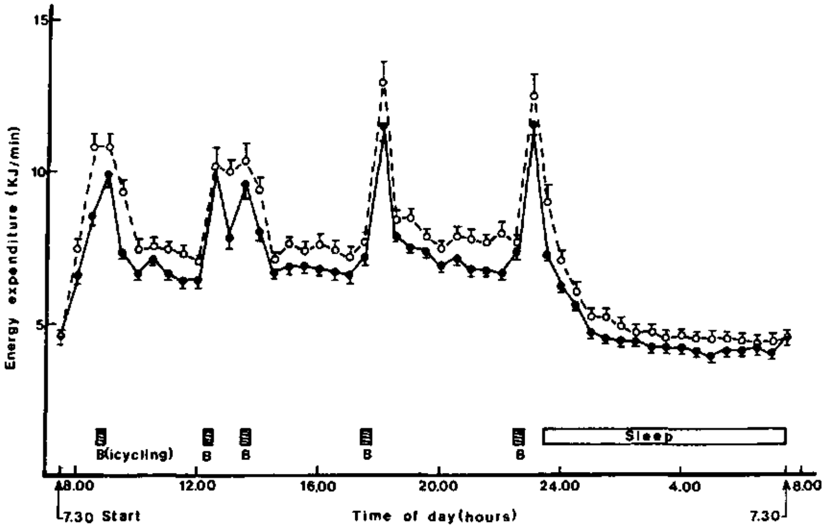

Figure 2. Energy expenditure pattern over 24 hours, on the 100% diet, of 12 overweight women before (open circles) and after (dotted circles) weight reduction. Mean of observations on two days (sem represented by vertical bars).

TABLE 6

Mean values of k_m (efficiency of dietary energy utilization/efficiency of body energy utilization) and energy requirement before and after weight reduction for 12 overweight women. k_m and energy requirement are expressed in absolute terms (W=1), per kg body weight (W=BW) and per kg fat free mass (W=FFM). For details see p. 89

	W=1		W=BW		W=FFM	
	mean	sd	mean	sd	mean	sd
k_m						
before weight reduction	0.84	0.03	0.88	0.03	0.87	0.03
after weight reduction	0.91**	0.04	0.93*	0.04	0.95**	0.03
ENERGY REQUIREMENT						
before weight reduction	10.62	0.88	114	7	204	16
after weight reduction	9.39**	0.79	113	11	185**	12

* different from k_m or energy requirement before weight reduction; $p<0.01$
** different from k_m or energy requirement before weight reduction; $p<0.001$

DISCUSSION

Body weight and body composition.

The observed rate of weight loss of 1.4 kg per week is in accordance with the "optimum rate of weight loss" as stated by Garrow (16). Calculation of the optimum rate was based on a weight loss of 2 kg per week for the first 4 weeks and 1 kg per week thereafter. These rates of weight loss correspond to an energy deficit of about 4.2 MJ per day (16). The energy deficit in this experiment during the 8 weeks on weight reduction diets was larger, but this did not result in a higher rate of weight loss. This may be attributed to a higher loss of body weight as body fat (84%), in those 8 weeks and after refeeding, than is assumed in the calculation of this "optimum rate". Other investigators report losses of fat mass of about 75% of the total weight loss (6,17,18). This figure is similar to the loss of body fat (73% of the weight lost) observed in our subjects after 8 weeks on a weight reduction diet, but before refeeding with the *100%* diet. Considering the experimental errors of measurement for body fat, the true value for body fat loss in our subjects may lie somewhere between those values.

Energy intake

No change occurred in digestibility and metabolizability of the *100%* diet before and after weight reduction. This suggests that easy weight gain after dieting is not due to a higher digestibility and metabolizability of the diet.

Energy expenditure

The observed mean 24hEE on the *100%* diet before weight reduction was similar to the mean 24hEE of 18 overweight women of the same body weight and body composition, measured in a previous study (19). The EE during sleep lies well within the range of sleeping energy expenditure or resting metabolic rates of overweight women with a similar body weight (18 - 21). However, the 24hEE was, in absolute terms, higher than that of lean women (19).
As expected 24hEE declined during and after weight reduction. Four factors are likely to have contributed to this decrease: (1) a lower dietary induced thermogenesis, due to the deficit of 5.8 MJ in ME intake when on the *4.2 MJ* diet; (2) loss of body weight and fat free mass; (3) lower energy costs of physical activities because of this weight loss and (4) adaptation to low energy intake. The observed decrease in 24hEE on the first day of the *4.2 MJ* diet is in agreement with the diminished dietary thermogenesis (factor 1)

predicted from the figures of Flatt (22) and with other decreases experimentally found (4,5). The continued decrease after 7 days on this weight reduction diet must be explained,however,by the other three factors. Factors 2 and 3 may have had little impact at this time, because weight loss was relatively small. Thus, an adaptation (factor 4) may already have occurred.

After 8 weeks on the weight reduction diets 24hEE had declined by 15% of the initial 24hEE on the *100%* diet. Other investigators have reported decreases of 12% in 24hEE of obese subjects after 6 weeks on a weight reduction diet with an energy deficit of 4.2 MJ/d, and of 16% after 14 weeks on a weight reduction diet with an energy deficit of 7.2 MJ/d (6,23).

On the first day of refeeding, 24hEE increased by 10% of the extra energy intake. This value is in agreement with the expected dietary thermogenesis due to increased food intake. The increase, however, did not continue over the next 7 days of refeeding, contrasting with the continued decrease observed at the start of the 4.2 MJ diet. This suggests that the adaptation had not disappeared after 7 days of refeeding.

The overall decrease in 24hEE on the *100%* diet before and after weight reduction was 1.08 MJ. This may be attributed to factors 2 - 4. To eliminate the effect

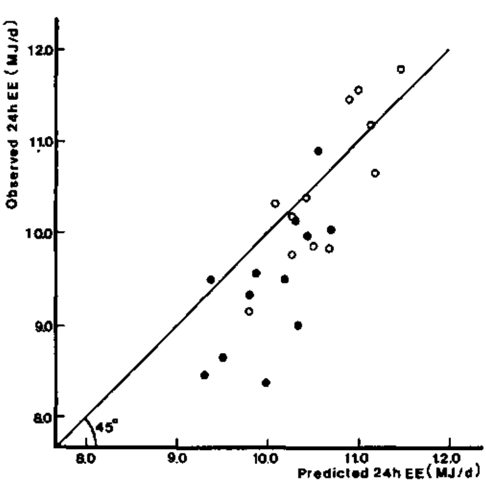

Figure 3A.
Observed and predicted 24 hour energy expenditure (24hEE) of 12 overweight women before (open circles) and after (dotted circles) weight reduction. 24hEE was predicted using the equation (19):
24hEE (MJ)=4.45+0.09xbody weight(kg)-0.05xbody fat(%)

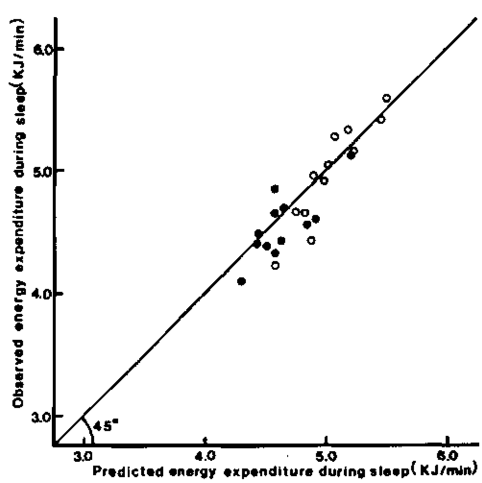

Figure 3B.
Observed and predicted energy expenditure during sleep (EE sleep) of 12 overweight women before (open circles) and after (dotted circles) weight reduction. EE sleep was predicted using the equation (19):
EE sleep(kJ/min)=1.50+0.02xbody weight(kg)+1.27xcreatinin(g/d)+0.37xsmoking*
* no smoking=0; smoking=1

of the reduced body weight and change in body composition, we predicted 24hEE, before and after weight reduction, from body weight and body fat percentage. A regression equation was used, that resulted from a previous study on the 24hEE and energy requirements of lean and overweight women (19). These women followed a 100% diet for 8 days. Their 24hEE expenditure was measured in the calorimeter, following the same physical activity pattern as the subjects of this experiment. Figure 3A shows the observed 24hEE and the predicted 24hEE on the *100%* diet before and after weight loss. The ratio of observed to predicted 24hEE before weight reduction was close to 1 (0.987 ± 0.013; mean ± SEM), but decreased to 0.943 ± 0.016 after weight reduction, and was significantly different from the ratio before weight reduction ($p < 0.005$). This indicates that 24hEE had declined more than could be explained by the change in body weight and body fat, and that an adaptation had occurred. When calculated for the EE due to sleep, which may be considered to be similar to resting metabolic rate (18,21) the ratios did not differ from 1 or from each other (see also figure 3B). Doré et al (24) reports similar findings on resting metabolic rate of 19 obese women after massive weight loss. Our results suggest that the adaptation is effective mainly during daytime hours. This adaptation of 24hEE may be due to (a) a change in behaviour and spontaneous activities in the calorimeter or (b) a metabolic adaptation to low energy intake. We did not (subjectively) observe any change in behaviour and spontaneous activities of the subjects in the calorimeter during the four calorimetry sessions. We cannot, however, support this with actual measurements on physical activity. Bessard et al (18), who measured 24hEE of overweight women before and after weight loss report that the mean diurnal percentage activity measured by radar was unchanged. Ravussin et al.(23) report that the spontaneous physical activity of 7 obese patients was unaltered by a diet of 3.4 MJ/d in the calorimeter.

With regard to a metabolic adaptation, it has been shown that caloric restriction in overweight subjects reduces serum triiodothyronine (T_3) levels (25,26,27) and the activity of the sympathetic nervous system (27,28). However, the relationship between energy expenditure and these changes in sympathetic activity and T_3 concentrations are still to be conclusively demonstrated. Recently, Ravussin et al. (23) reported that in their study with obese patients on a low energy diet no evidence was found for any adaptation mechanism in 24hEE apart from the factors 1-3 mentioned above.

During 72 hour refeeding of four obese women, after a semistarvation period of 24 days, a rapid rise in T_3 and sympathetic activity has been found, while the

RMR increased only slowly (27). The investigators state that "this might indicate that some adaptation has occurred during energy restriction, which is only slowly reversed on refeeding". Our data, however, suggest that the adaptation after refeeding occurs mainly during daytime, and not at night. The thermogenic response due to energy intake and/or other stimuli may well be reduced after refeeding. It has been suggested that the sympathetic nervous system of obese subjects with a history of childhood onset obesity is less stimulated by a meal than that of lean subjects (18). This reduced response did not disappear after 11 weeks of refeeding (18). Whether the observed adaptation in our experiment is temporary is not known.

Energy requirement.

The body energy used to meet energy requirements during the 8 weeks on the weight reduction diets was calculated from the change in body composition:
1.6 (loss of fat free mass) x 4.2* MJ + 8.3 (loss of fat mass) x 38.9* MJ = 330 MJ.
Energy balance in the calorimeter showed negative values of -5.4 MJ/d and -4.7 MJ/d on the *4.2 MJ* diet, resulting in a mean negative energy balance of 5.1 MJ/d or 5.1 x 56 = 286 MJ. The difference of 44 MJ (0.8 MJ/d) between the two values may be explained by errors of measurement of body fat or by a higher energy expenditure at home. Part of this higher expenditure at home may have been caused by the different composition of the prescribed diet from that of the *4.2 MJ* diet. The higher protein content of the prescribed diet may have increased daily energy expenditure by 0.1 MJ.
The k_m values(table 6) before and after weight reduction are lower than those found by Van Es et al. (5) in 13 (mainly lean) male and female subjects. This suggests that overweight subjects utilize their body energy more efficiently than lean subjects, to maintain their bodies. The observed adaptation to low energy intake violates the assumption that energy requirement is not affected by changes in energy intake level. We did, however, calculate energy requirement before and after weight reduction, from the k_m values determined,which were incorrect. The error in the calculated energy requirement caused by an incorrect k_m value is, at most, 1 %. Energy requirement decreased by 1.2 MJ/d after 10 kg weight loss. At the end of the study our subjects still showed a higher energy requirement than lean women (19). The overweight subjects nad, however, not returned to normal weight. Their energy

* the energy equivalent of 1 kg body fat was taken to be 38.9 MJ (9300 kcal) and that of 1 kg fat free mass 4.2 MJ (1000 kcal).

requirement decreased by 0.12 MJ/kg weight lost, a value higher than that found by Bessard et al. (18), but similar to that which can be calculated from the data of Leibel and Hirsch (29). Energy requirement as a function of fat free mass was also observed to decrease,as shown also by Bessard et al. (18). However, when expressed as a function of body weight, energy requirement remained unchanged. The observed energy requirement per kg body weight was lower in overweight than in lean women (19). The fact that the energy requirement per kg body weight did not change towards the value of the lean women suggests that overweight subjects may indeed have a lower energy requirement per kg body weight when their body weight has returned to normal. When the value from table 6, i.e. 113 kJ/kg, is used, the absolute energy requirement of an overweight woman who has reduced her body weight to normal (60 kg) is 60 x 113 = 6800 kJ/d. This is 1.8 MJ lower than the observed energy requirement of lean women (19).

One reason for this lower energy requirement may be that overweight subjects show a reduced dietary induced thermogenesis, even after weight reduction (18,21,30). However, contradictory findings have also been reported (20,31). It is suggested in the literature that the differences in results are to be attributed to differences in the overweight subjects. Jéquier (32) states that one third of the obese subjects appear to show this reduced thermogenesis but when the obese subjects are selected on the basis of a childhood history of obesity and frequent unsuccesful attempts to lose weight, reduced thermogenesis is observed in the majority of patients. It is not known whether this reduced thermogenesis is a consequence of many slimming attempts or a cause of becoming overweight.

It is clear from this study that overweight women who have reduced their body weight to normal cannot return to their original energy intake without gaining weight. This may be the cause of the complaints, often heard from overweight individuals, that they have to stick to their diet for the rest of their lives. However, the energy requirement remains well above the 4.2 MJ/d of the commonly prescribed slimming diets. If slimming diets of 6.3 MJ/d are prescribed, some women may indeed have to continue with this diet as after weight loss their energy requirement may approach this energy level. This may also explain the "resistance to slimming" on a 6.2 MJ diet observed by Miller and Parsonage (33) in women with a long previous history of dieting and with a low basal metabolic rate.

In conclusion, an adaptation seems to occur at low energy intake in overweight women, thus hindering weight loss. This adaptation did not disappear after

7 days of refeeding. It remains to be investigated whether this adaptation is a consequence of many slimming attempts or a causal factor in becoming overweight. In view of this adaptation, dietary practice may require re-evaluation with regard to the long-term prescription of energy restricted diets.

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7. GENERAL DISCUSSION

The object of the experiments described in this thesis was to measure 24 hour energy expenditure and to estimate energy requirement of humans in a situation that resembled everyday life. The method used was indirect calorimetry with whole body open circuit calorimeters. Energy metabolism studies with whole body calorimeters are time-consuming, costly and can be performed only with a small number of subjects. Therefore it is important to have more insight into (a) the *validity* and (b) the *reproducibility* of the method.

The *validity* of a method is the extent to which it measures what it is intended to measure. Using indirect calorimetry, oxygen (O_2) consumption and carbon dioxide (CO_2) production are measured. The calorimeters used were calibrated several times during the experiments; these calibrations indicated that O_2 consumption and CO_2 production were very accurately measured. Several equations are used to calculate energy expenditure from O_2 consumption, CO_2 production and urinary nitrogen excretion. The equations are based upon the energy yield of oxidation of carbohydrates, fats and proteins.The equation used in this study was recommended by a subcommittee on the constants and factors to be used in calculations of energy metabolism (Brouwer, 1965). Schutz (1985) mentions that the impact on the calculation of energy expenditure by the different equations is small. The close agreement found between measurements with direct and indirect calorimetry (Dauncey, 1980) also demonstrates that the method and equations used are valid for measuring energy expenditure. However, the extra dimension given to the term validity in these experiments was that it should reflect 24 hour energy expenditure (24hEE) during everyday life. Therefore, the daily physical activity pattern in the calorimeter was chosen to simulate the normal workingday of a person engaged in a sedentary occupation. The good agreement between the estimated energy requirement (using 24hEE measured in the calorimeter) of the lean female subjects (chapter 3) and the recommended daily energy intake of lean adult women engaged in light work (Dutch food composition table, 1981) suggests that this was succesful. On the other hand, the discrepancy of 0,8 MJ/d between estimates of the energy lost from the body during an eight week weight reduction diet, calculated from the change in body composition and from calorimetric data on the energy balance (chapter 6) argues somewhat against this. Part of this discrepancy may be explained, however, by errors in the measurement of body fat (see chapter 2).

The *reproducibility* of the method was studied in chapter 2. The small within-subject coefficient of variation in 24hEE observed over intervals of 2 to 24 months is in agreement with the findings of other investigators who used time-intervals of one week (Dallosso et al.,1982; Garby et al., 1984). The high reproducibility of 24hEE lends credibility to the data of the subsequent chapters. The findings suggest that under similar conditions 24hEE is fairly constant. In everyday life, however, 24hEE and energy requirement may be less constant than observed in chapter 2. Factors such as physical activity, stress, level of energy intake, diet composition, phase of menstrual cycle, drugs, smoking, cold or heat exposure may influence 24hEE. Energy requirement should therefore be considered a dynamic, and not a static, phenomenon (Sukhatme and Margen, 1982). Extrapolation of the data presented in the preceding chapters to everyday life thus requires caution. One promising method, which is used to measure energy expenditure over longer periods in everyday life, with all its thermogenic stimuli, is the doubly labeled water method (Schoeller and Van Santen, 1982; Coward et al., 1985; Westerterp et al., 1985). The validity and precision of this method, however, are still under study.

The coefficient of variation in the *24hEE* of the *lean* and *overweight* female subjects studied (chapter 3) was 18%. However, when 24hEE was expressed per kg body weight or per kg fat free mass, this coefficient of variation decreased to 15% and 10%, respectively. These figures indicate that some women have a 20-30% higher or lower 24hEE (and energy requirement) than average.

The 24hEE, in the calorimeter, of the overweight subjects was found to be higher than that of the lean subjects (chapter 3). This agrees with the findings of other investigators (Irsigler et al., 1979; Ravussin et al., 1982; Blaza and Garrow, 1983). However, the 24hEE was higher in both lean and overweight subjects than reported by these investigators. This may be attributed to the higher physical activity and possibly to exposure to other thermogenic stimuli, such as coffee consumption, smoking and social contact with the subject in the adjoining calorimeter.

When 24hEE was expressed in relation to body weight, the 24hEE of overweight subjects was lower than that of the lean subjects. Expressed per kg fat free mass, 24hEE was similar in the two groups. However, the use of such parameters to express 24hEE or energy requirement is questionable, because (a) by using the parameter body weight the difference in metabolic activity of the various body tissues is neglected and (b) the parameter fat free mass does not include the influence of movement and carrying of the fat mass and the energy costs of its metabolism. The regression equation calculated in chapter 3 shows that body weight (positively related) and body fat percentage (negatively related) both contribute to the prediction of 24hEE (and energy requirement) of the female subjects. Doré et al. (1982) found that, in addition to body weight and body potassium (an estimate of fat free mass), age also contributed significantly to the prediction of resting metabolic rate in women with various degrees of obesity. That age does not appear in the regression equation in the experiment reported here is probably a consequence of the selection of subjects between 20 and 47.

The observed higher 24hEE, which is an estimate of energy requirement (in the case of energy equilibrium), of overweight subjects compared to that of lean subjects disagrees with several investigators' findings on *energy intake in normal life* in lean and overweight people (Beaudoin and Mayer, 1953; Baecke et al., 1983; Kromhout, 1983). The data of those studies show that the (*usual*) energy intake of overweight people is similar to that of lean people. The comparison of data on 24hEE and energy intake in everyday life, assessed by the 7 day weighing record method (chapter 4), showed that the dietary assessment method used was not valid for estimating the *usual* energy intake of the overweight subjects. It is suggested that the discrepancy between the calorimetric findings and the foodconsumption data is due to an unjustified extrapolation of the results of a short term food consumption study to *usual* foodconsumption, and/or to a systematic error introduced by the dietary assessment method used. Reported intakes should therefore be checked against an independent method such as urinary nitrogen excretion over 24 hour (Isaksson, 1980), or related to the energy requirement predicted from body weight or from body weight and body fat percentage (chapter 3).

Since 24hEE is increased more by short-term overfeeding (about 15% of the extra energy intake; Dauncey, 1980; Van Es et al., 1984) than it is decreased by short-term underfeeding (about 5-10% of the energy deficit; Dauncey, 1980; Van Es et al., 1984) an attempt was made to *increase energy requirement by alternating the daily energy intake* (chapter 5). This did not succeed: this result is in agreement with that of Kirchgessner and Müller (1981) in sows. This does not mean, however, that alternating energy intake has no impact on energy requirement. Longer periods (2-4 days) of under-eating alternated with longer periods (2-4 days) of over-eating may well increase energy requirement, because the relatively inefficient conversion of dietary carbohydrate into body fat may occur during a longer period of over-eating.

The findings on *the energy requirement of overweight subjects* (chapter 6) before and after weight reduction show that after 5-7 days on a low energy intake the 24hEE had already decreased by 15% of the deficit in energy intake. This is a larger decrease than found by Van Es et al. (1984) in normal weight subjects. It may indicate that overweight subjects "adapt" their 24hEE more readily to a low energy intake than normal weight subjects. The energy requirement of the overweight subjects observed after 8 weeks on a slimming diet and one week of refeeding was lower than that predicted from the change in body weight and body fat percentage. Whether this "adaptation" is a consequence of slimming, a temporary phenomenon, or the cause of being overweight is not known. It may indicate that the energy requirement of overweight women who have reduced their body weight to normal is lower than that of women of the same normal body weight, who have never been overweight. An explanation for this lower energy requirement may be that some overweight subjects show a reduced dietary induced thermogenesis, which persists after weight reduction (Bessard et al., 1983). When,however, the overweight subjects are selected on the basis of a history of childhood onset of obesity and frequent unsuccessful attempts to lose weight, this reduced thermogenesis is observed in the majority of patients (Jéquier, 1984).

The combination of an extra reduction in 24hEE during under-feeding (chapter 6) and a possible reduced dietary induced thermogenesis during over-feeding in overweight people may result in a more difficult weight loss and an easier weight gain, compared to lean people. Over-eating, however, results in weight gain in both lean and overweight people. The question is, therefore, why do overweight people overeat, and why don't lean people overeat. The answer to this question must be sought in the regulation of energy intake rather than in the regulation of energy expenditure.

This does not mean, however, that *studies on energy expenditure in lean and overweight subjects* are superfluous. It may be possible, using calorimetry, to identify individuals with a low energy requirement, who are likely to become overweight, and individuals with a high energy requirement. The responses of those two groups to over- and under-feeding, or to various thermogenic stimuli, can then be investigated. Furthermore, experiments that focus on increasing energy requirement, as suggested in chapter 5, or on counteracting the decrease in energy requirement during and after weight reduction, may be helpful in the prevention and treatment of overweight and obesity.

It is advised for *dietary management of overweight* to prescribe diets that result in an energy deficit of about 4 MJ/d (Garrow, 1981).Often prescribed slimming diets contain between 4 and 6 MJ/d. Overweight subjects with a body weight of 100 kg or more showed energy requirements, that were about 11 MJ/d or higher (chapter 3). Initially, in view of these high total energy requirements, slimming diets with a higher energy content than the usual 4 or 6 MJ/d could better be be prescribed to such overweight individuals. The energy level should, however, be adjusted downwards during slimming, because the energy requirement decreases during slimming (chapter 6). The observed adaptation of the energy requirement after weight loss (chapter 6) may be an indication that long term prescription of low energy diets needs re-evaluation. One might try, for instance, to counteract this adaptation by alternating the energy intake from a few days low to a few days normal, or by instalment of an energy deficit that is not as large as the one induced in the subjects of chapter 6 (about 5.1 MJ/d). The data of chapter 6 show that plateauing of body weight during long term low energy intake (4.2 MJ/d) cannot be attributed to a complete adaptation of energy expenditure to energy intake, and should therefore be sought in a larger energy intake than prescribed or in a change in body composition without change in body weight.

The findings here summarized and presented give more insight in energy metabolism of overweight subjects and they may be useful for dietary management. However, it is recommended that studies are carried out in further detail on the effect of slimming diets and refeeding on energy metabolism of overweight individuals. This is being done in a research project which has started in succession of this project, e.g. the effect of slimming diets with an alternating energy intake on energy requirements of overweight women.

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APPENDIX

DESCRIPTION OF EXPERIMENTAL DIET AND INDIRECT CALORIMETRY

The diet and the indirect calorimetry have also been summarized by Van Es et al. (1984)

EXPERIMENTAL DIET

The experimental diet was composed of foods commonly eaten in the Netherlands (table 1). These were chosen after testing their suitability for handling, weighing, sampling, analysis and storage. Special attention was given to the

TABLE 1
Purchase, weighing and packaging in daily portions, and storage of foods for the experimental diet

	purchase			weighed and packed			duration of storage	type[1]
	before series 1 large batch		during series	before series		during series		
1. whole wheat bread	+			+			4-5 months	F
2. whole wheat cookies	+			+			4-5 months	F
3. spiced cake	+			+			4-5 months	F
4. sugar	+			+			4-5 months	C
5. soy sauce	+			+			4-5 months	C
6. chocolate strands	+			+			4-5 months	C
7. non dairy coffee creamer	+			+			4-5 months	C
8. powdered coffee	+			+			4-5 months	C
9. mashed potato flakes	+			+			4-5 months	C
10. white rice	+			+			4-5 months	F
11. carrots	+			+			4-5 months	F
12. leeks	+			+			4-5 months	F
13. white cabbage	+			+			4-5 months	F
14. green beans	+			+			4-5 months	F
15. cucumber			+			+	2 days	C
16. meatballs	+	or	+	+	or	+	2-3 months	F
17. cold sausage	+	or	+	+	or	+	2-3 months	F
18. orange juice	+					+	3-4 days	R
19. soft margarine	+					+	8 days	R
20. cheese (Gouda 48+)			+			+	8 days	R
21. whole fat milk			+			+	3-4 days	R
22. whole fat yoghurt			+			+	3-4 days	R
23. custard			+			+	3-4 days	R

[1] type of storage; F - Freezer, C - Cupboard, R - Refrigerator.

homogeneity of the foods. When possible the foods were bought for a whole experimental series (approximately 4 months duration) in one large batch. Table 1 shows the time of purchase, weighing and packaging in daily portions, and storage of the foods. Some foods (nos 15, 18, 21-23) could not be stored, after weighing in daily portions, for the full duration of an experiment (8 days) and were therefore bought fresh (nos 15, 21-23), weighed and packaged during the experiment. The foods were weighed out to the nearest 0.1 g, using an electric scale incorporating an automatic zeroing button and a large digital read out (Sartorius, 1406 MP). During the weighing of the foods, duplicate samples were taken for analysis of dry matter, energy and nitrogen.

The diets were designed to simulate the current Dutch eating pattern with regard to the composition of protein, fat and carbohydrate. Table 2 gives the composition of the diet in more détail. These values were obtained by using a computerized foodtable (Hautvast, 1975). The values thus obtained differ somewhat from the figures given in the preceding chapters for protein (14%), fat (40%) and carbohydrate (46%). This may be attributed to the use of a different foodtable and the use of our own values for some products that were not included in the composition table (Dutch Food Composition Table, 1981), when designing the diet.

TABLE 2

Composition of the experimental diet relative to metabolizable energy (ME) intake, computed with a computerized Dutch food composition table (Hautvast, 1975).

Metabolizable energy intake	100%
Total protein (ME %)	13
animal protein	8
vegetable protein	5
Total fat (ME%)	38
saturated fatty acids	17
mono-unsaturated fatty acids	10
poly-unsaturated fatty acids	8
Total carbohydrates (ME%)	49
sugars	24
polysaccharides	25
Dietary fiber (g per 4.2 MJ ME)	13
Dietary cholesterol (mg per 4.2 MJ ME)	89

The experimental protocol was such that the subjects had to consume the diet for five days prior to the calorimetry session. For this purpose the food was supplied in batches of three days and two days or, occasionally, five days, all packed in daily portions. After those five days the subjects occupied the calorimeter for 2-4 days; they were then provided each morning with all the food for one day. The subjects were free to choose which food was eaten for breakfast, lunch and dinner. To increase palatability, a two day rotating menu for dinner and a four day rotating menu for the dinner vegetables were designed. On one day the subjects were supplied with a dinner of mashed potatoes, cheese, a vegetable and for dessert yoghurt and custard, on another day with white rice, meatballs, a vegetable, soy sauce and yoghurt. The food for the dinner was already cooked and needed only to be warmed up. This was done by the subjects themselves on a hot plate "au bain marie", so that nothing would be burned and left stuck to the cooking pans. Everything provided had to be eaten. Tapwater, mineral water and tea were provided ad libitum.

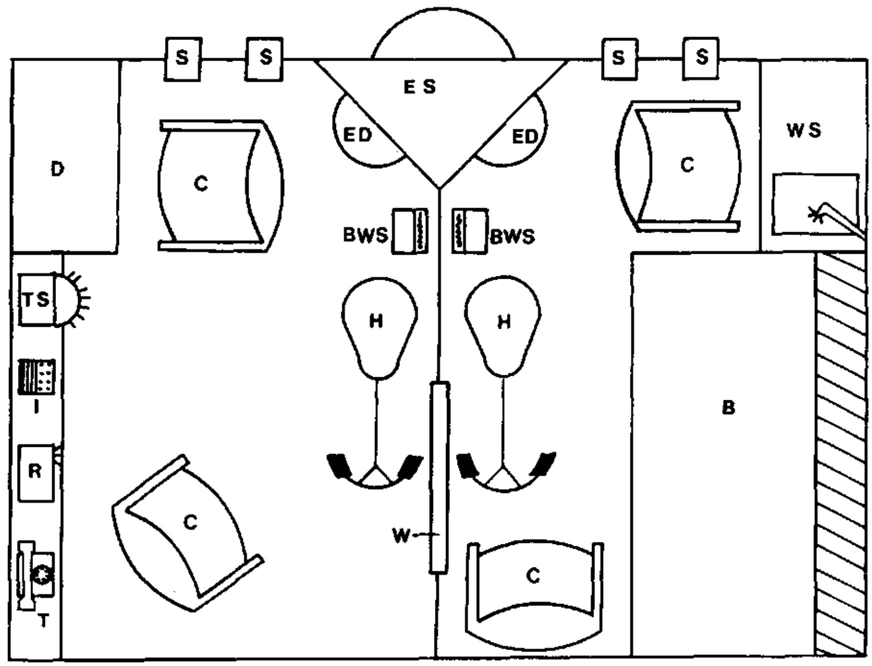
Treatment of foodsamples for analyses.

Samples of bread and cake were dried in a stove at 60° C, for one to two days, before grinding. White rice and vegetables were first freeze-dried and then ground. Soy sauce, orange juice, milk, yoghurt and custard were sampled in small polyethylene bags and, for the analysis for energy, dried in a vacuumstove. Samples of cheese, meatballs and sausages were mixed with silica gel (Gasil 23D) to prevent loss of fat during preparation of the pill for the bomb calorimetry, and to homogenize the sample. The other samples required no further treatment besides grinding prior to analysis.

Analyses

Weight loss due to drying at 60° C or to freeze-drying was measured. Moisture content of the dried material was determined by drying for 4 hours at 101° C. The analysis for energy was performed with a static bomb calorimeter. The samples of the dry and dried products were pressed to pills before combustion in the calorimeter. The samples, that were weighed into the polyethylene bags and then dried, were combusted together with the bag. The observed heat of combustion was corrected for the heat of combustion of the bag.

Analysis for nitrogen was performed using the Kjeldahl method (International Organization for Standardization, 1979) with mercury-oxide as catalyst.



B — bed (folding bed)
BWS — body weight scale
C — chair
D — desk
ED — entrance door
ES — entrance sluice
H — hometrainer
I — intercom
R — radio
S — sluices
T — telephone
TS — television set
W — window
WS — washstand

Figure 1. Interior of the calorimeters.

INDIRECT CALORIMETRY

Procedure in the calorimeter

Figure 1 shows the interiors of the calorimeters. The two calorimeters (11 m^3) were used simultaneously. The subjects could see each other through a large window in the connecting wall. They could speak with each other and with the caretaking staff through an intercom. During the day the bed was folded away, so that more room was available. There were two chairs, of which one (the desk chair) could also be used as a toiletchair. The two airsluices served as an inlet for food and papers, and as an outlet for faeces, urine and refuse.

The subjects entered the calorimeter in the evening, 8 to 9 hours before the gas exchange measurements started. The daily schedule in the calorimeter is presented in figure 2. In addition to providing the food in the morning, the caretaking staff visited several times, usually around 10.15, 13.00, 15.00, 17.00 and 22.15, to talk with the subjects. There was no supervision at night.

TIME	ACTIVITIES
7.45	Rise after call (through intercom); recording of body weight and body temperature.
8.00 - 8.15	Supply of food and morning paper.
8.15 - 8.45	Breakfast.
8.45 - 9.00	Bicycling on hometrainer (velocity 24 km/h, no load).
9.00 - 12.15	Sedentary activities (coffee break with cake or cookies).
12.15 - 12.30	Bicycling.
12.30 - 13.30	Lunch.
13.30 - 13.45	Bicycling.
13.45 - 17.30	Sedentary activities (tea break with cake or cookies).
17.30 - 17.45	Bicycling.
18.00	Dinner (preparation).
18.00 - 22.30	Sedentary activities (coffee or tea with cake or cookies).
22.30 - 22.45	Bicycling.
22.45 - 23.15	Prepare for bed; recording of body weight and body temperature.
23.15	In bed.

Figure 2. Daily schedule in the calorimeter.

The subjects' safety was maintained by alarms for powerfailure, high carbon dioxide concentration and fire. Fire alarms were installed in all rooms around the calorimeters. All alarms were connected to the telephones of three employees of the department and were audible outside the calorimeter. Only the fire alarm was heard inside the calorimeter, so that the subjects could flee as soon as they heard the alarm. In case of doubt they could always call the staff at all times.

The environmental temperature in the calorimeter was set at 21°- 22° C during the day and at 19°- 20° C during the night. The subjects wore their own clothes and adjusted their clothing to feel comfortable in the set temperature. The temperature was adjusted if the subjects still felt uncomfortable after adjustment of their clothes. The relative humidity was kept between 60 and 80%.

Gas exchange measurements

Figure 3 gives an outline of the gasflow and the systems used in the analysis for oxygen (O_2) and carbon dioxide (CO_2).The gas exchange measurements for each 24 hour started at 7.30 and ended the next day at 7.30. At the start of the

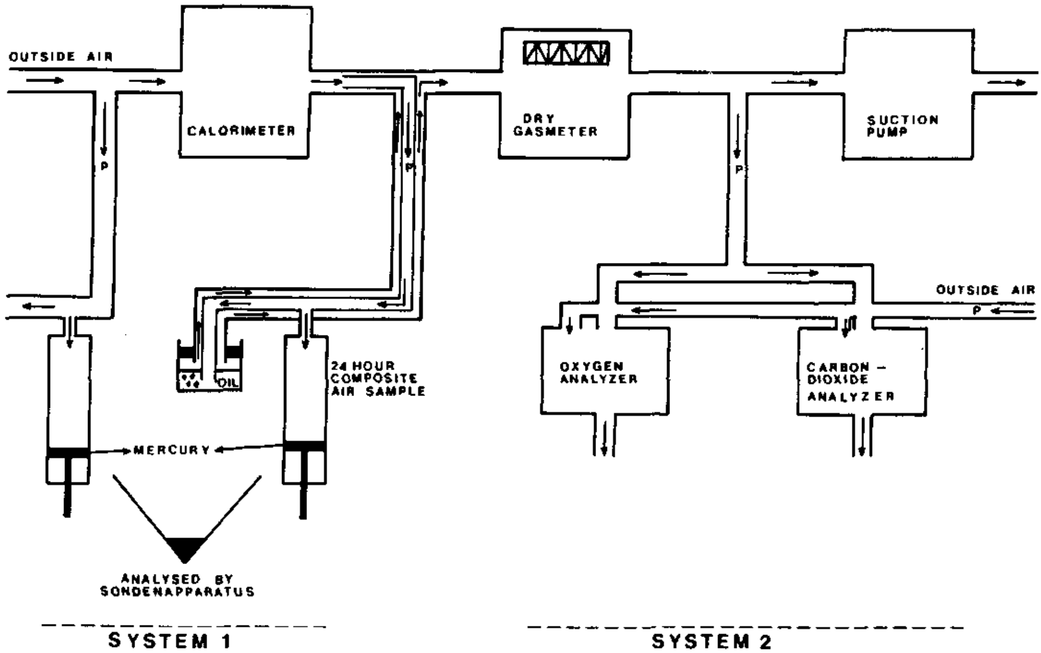


Figure 3. Outline of gasflow and systems used in the analysis for oxygen and carbon dioxide. P indicates pumps that draw air samples from the main stream to the systems for analysis.

calorimetry session the air suction pump was set at a constant speed for the total period of 2-4 days. The speed was so chosen that the mean CO_2 concentration in the calorimeter was about 0.5 - 0.8%. This suction pump maintained a slight under-pressure in the calorimeter throughout the experiment, so that, if leakage occurred, air would leak into and not out of the calorimeter.

Two systems were used to measure the gas exchange. With system 1 samples of the air entering and leaving the calorimeter were collected over 24 hours. These airsamples were analysed using a Sondenapparatus; this yielded the mean O_2

consumption and CO_2 production over 24 hours. The other system (no 2) was used to determine O_2 consumption and CO_2 production over shorter periods. The outside air was analyzed and recorded for 10 minutes every 3 hours (in some experiments this used to be 6 hours). For the rest of the time, the air leaving the calorimeters was analyzed and recorded alternatingly for the two calorimeters over 10 minutes.

Calibration

The dry gas meters used were calibrated before and after each calorimetry session, making use of a Blakeslee piston pump with mercury seals. The pump was calibrated with a calibrated wet gas meter or a calibrated dry gas meter before and after each series of experiments.

The analyses of the Sondenapparatus were checked daily against the O_2 and CO_2 concentration of the outside air. The analyzers of system 2 were calibrated daily against air samples analyzed for O_2 and CO_2 by Sondenapparatus.

The overall calibration of the calorimeters was checked by infusing pure nitrogen (N_2) or CO_2, or by burning alcohol, for 24 hours. The measurements by the calorimeter were 101% and 99.5 ± 1.0% (mean ± sd) of those predicted from the N_2 and CO_2 infusion, respectively, and were 99.5 ± 1.0% for O_2 and 99.7 ± 1.2% for CO_2 of those predicted from burning alcohol.

Calculation of energy expenditure

The equation given by Brouwer (1965) was used to calculate the mean 24 hour energy expenditure over 2 - 4 days from O_2 consumption, CO_2 production and urinary nitrogen (N_u):

$$EE\ (kJ) = 16.18 \times O_2 \text{ consumption (l)} + 5.02 \times CO_2 \text{ production (l)} - 5.99 \times N_u \text{ (g)}$$

The protein correction term ($5.99 \times N_u$) of this equation was neglected in calculating 24 hour energy expenditure per day and energy expenditure over shorter periods than 24 hour.

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ABBREVIATIONS

BMI	-	body mass index (weight (kg)/ height^2 (m^2))
BMR	-	basal metabolic rate
BW	-	body weight
C	-	constant regime
CO_2	-	carbon dioxide
CV (cv)	-	coefficient of variation
CV_w	-	within-subject coefficient of variation
d	-	day
EE	-	energy expenditure (24hEE- energy expenditure over 24 hour)
FE	-	energy in faeces
FFM	-	fat free mass
GE	-	gross energy
h	-	hour
kJ	-	kilojoule
L/H	-	low/high regime
ME	-	metabolizable energy
ME_{cal}	-	metabolizable energy calculated from foodtables
ME_{obs}	-	metabolizable energy intake assessed from analyses on energy of food, faeces and urine
min	-	minute
MJ	-	megajoule
N_2	-	nitrogen
N_u	-	nitrogen in urine
O_2	-	oxygen
RMR	-	resting metabolic rate
RQ	-	respiratory quotient
SD (sd)	-	standard deviation
SEM (sem)	-	standard error of the mean
UE	-	energy in urine

CURRICULUM VITAE

De schrijfster van dit proefschrift werd op 20 september 1954 te Arnhem geboren. In 1973 behaalde zij het diploma gymnasium-ß aan het toenmalige Westfries Lyceum te Hoorn. In datzelfde jaar begon zij haar studie aan de Landbouwhogeschool te Wageningen en in september 1980 behaalde zij het doctoraalexamen met Voedingsleer als hoofdvak en Dierfysiologie en Gezondheidsleer als bijvakken. Van februari tot mei 1981 was zij werkzaam op de vakgroep Gezondheidsleer van de Landbouwhogeschool. Van september 1981 tot maart 1982 was zij als statistisch analiste verbonden aan een onderzoeksproject van de vakgroep Humane Voeding van de Landbouwhogeschool. In maart 1982 werd zij aangesteld als wetenschappelijk assistente bij de vakgroep Dierfysiologie van de Landbouwhogeschool. Tot juni 1985 verrichtte zij daar het in dit proefschrift beschreven onderzoek met financiele steun van de Nederlandse Hartstichting en de Landbouwhogeschool.