Effects on lipid and glucose metabolism
of diets with different types of fat and sugar
in male fatty Zucker rats

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Effects on lipid and glucose metabolism of diets with different types of fat and sugar in male fatty Zucker rats

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
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Drukkerij Giethoorn-Huisman, Meppel

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STELLINGEN

1. De daling van de plasmacholesterolconcentratie die wordt waargenomen bij personen gedurende de eerste weken nadat zij op een linolzuurrijk dieet zijn overgegaan, kan, ook al neemt in die periode de uitscheiding van sterolen en galzuren met de faeces toe, het best worden verklaard op grond van het feit dat cholesterol van de snel uitwisselbare „pool" A naar de langzaam uitwisselbare „pool" B verschuift.
   S. M. Grundy & E. H. Ahrens Jr. (1970), *Effects of unsaturated dietary fats on absorption, excretion, synthesis and distribution of cholesterol in man*, J. Clin. Invest. *49*, 1135.
2. Bij een voeding *ad libitum* tendeert de stofwisseling van diverse soorten knaagdieren, en kennelijk ook die van de mens, naar diabetes mellitus van het „maturity onset" type.
   D. L. Coleman (1978), *Diabetes and obesity: thrifty mutants?*, Nutr. Rev. *36*, 129.
3. Het verschil in hypolipidaemisch effect van (−)-hydroxycitraat *in vitro* en *in vivo* tussen de rat en de kip kan worden toegeschreven aan het ontbreken van de enzymactiviteit van citraatlyase („cleavage enzyme", EC 4.1.3.8) in het vetweefsel van de laatstgenoemde diersoort; wat dit betreft zijn de mens en het rund (citraat in melk!) vergelijkbaar met de kip, en is het varken vergelijkbaar met de rat.
   H. Chee et al. (1977), *Influence of (−)-hydroxycitrate on lipogenesis in chickens and rats*, J. Nutr. *107*, 112.
4. De taxonomische verwarring die bestaat ten aanzien van het geslacht *Anabaena* (klasse der *Cyanophyceae*) zou kunnen worden opgeheven door bij het determineren van de soorten uit te gaan van de specificiteit van hun DNA-restrictie-enzymen.
5. Tegen de blindheid die in tropische gebieden herhaaldelijk wordt waargenomen bij kinderen die recentelijk mazelen hebben doorgemaakt, en die waarschijnlijk berust op een reeds bestaande xerophthalmie, is het toedienen van vitamine A een juistere preventieve maatregel dan immunisatie tegen mazelen.
   H. A. P. C. Oomen (1977), *Xeroftalmie en mazelen in Kenya*, Voeding *38*, 90.
6. Op grond van voedingskundige overwegingen en de te verwachten vraag naar zuivelprodukten met een verlaagd vetgehalte verdient het aanbeveling om bij het berekenen van het melkgeld dat aan de producent wordt uitbetaald het melkvet minder en het melkeiwit hoger te waarderen.
   Men kan in deze richting nog verder gaan door bij het fokken van melkvee een extra premie uit te keren voor de selectie van fokdieren op basis van het eiwitgehalte van de melk.
7. De vrees, geuit door Pitkin, voor een ongewenste verkleining van het circulerend bloedvolume als gevolg van de behandeling van prae-ecclamptische zwangeren met een normale dosering van saluretische diuretica is niet gerechtvaardigd, evenmin als deze volumeverkleining optreedt bij een dergelijke behandeling van diabetes insipidus.
   R. M. Pitkin (1977), *Nutritional influences during pregnancy*, Med. Clin. North Am. *61*, 3.

8. In tegenstelling tot hetgeen wordt opgemerkt door Kolb en door Kanis et al., treedt de toename van de intestinale calciumabsorptie na toediening van 24, 25-dihydroxycholecalciferol pas op na 1-hydroxylatie.
   E. Kolb (1976), *Zur Aktivierung des Vitamin D_3 und zum Wirkungsmechanismus des 1,25-Hydroxycholekalziferols und des 24, 25-Hydroxycholekalziferols*, Z. Gesamte Inn. Med. Ihre Grenzgeb. *31*, 561.
   J. A. Kanis et al. (1978), *Is 24,25-dihydroxycholecalciferol a calcium-regulating hormone in man?* Br. Med. J. i, 1382.
9. Het vaststellen van het „ideale gewicht" op grond van slechts één lichaamsafmeting (de lengte) is fysiologisch onjuist en kan daarom tot verkeerde gevolgtrekkingen leiden.
10. In het verleden is bij de meting van het energieverbruik ten gevolge van grote lichamelijke inspanning te weinig rekening gehouden met het toenemen van de warmteproduktie (thermogenese) gedurende de uren na deze arbeidsprestatie.
11. De bestaande gewoonte om de kamertemperatuur tot boven de behaaglijkheidsgrens te verhogen door centrale verwarmingssystemen toe te passen kan in ongunstige zin hebben bijgedragen tot de prevalentie van vetzucht, aangezien de omgevingstemperatuur invloed uitoefent op de ruststofwisseling.
12. De legende die St. Caecilia afschildert als patrones van de muziek berust op een onjuiste vertaling van de woorden „Cantantibus organis" uit de eerste antifoon van de eerste vesper die ter ere van haar feestdag (22 november) wordt gehouden; het orgel kwam als begeleidingsinstrument pas in gebruik aan het einde van de vijftiende eeuw, een duizendtal jaren na haar leven.
13. Bij het ontstaan van de „krankzinnigheid" van Robert Schuman (1810-1856) heeft de genegenheid van zijn vrouw voor Johannes Brahms (1833-1897) ongetwijfeld een zeer voorname rol gespeeld.

Proefschrift van H. de Waard:
„Effects on lipid and glucose metabolism of diets with different types of fat and sugar in male fatty Zucker rats."
Wageningen, 22 november 1978

Voorwoord

As the Irishman said, when asked the way to Dublin,
„If I were you, I wouldn't start from here".

Bij het overdenken van mijn loopbaan in het algemeen en van het in dit proefschrift beschreven onderzoek in het bijzonder, bekruipen mij soms de in bovenstaande uitspraak opgetaste gevoelens. Om welke redenen en langs welke wegen komt iemand tot een uitgangspunt? En door welke mensen wordt men daarop gewezen? Ongetwijfeld zijn er zekere redenen voor het innemen van een bepaald standpunt geweest en heeft het bereiken ervan niet geheel op toeval berust. Een analyse van deze redenen is echter uiterst moeilijk en de uitkomst ervan ingewikkeld. Laat ik daarop hier dan ook verder niet ingaan.

Wel moge ik dan allereerst mijn dank uitspreken aan allen die mij op enigerlei wijze behulpzaam zijn geweest bij mijn wetenschappelijke vorming en nu bij het totstandkomen van dit proefschrift.

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Behalve de medewerking die ik heb ondervonden bij het onderzoek voor dit proefschrift, wil ik ook nog de steun memoreren die ik tijdens mijn vroegere wetenschappelijke opleiding heb mogen genieten. Ik ga hierbij niet verder terug dan tot mijn specialisatie. Helaas zijn enkele van mijn voornaamste opleiders niet meer in leven: Prof. Dr. W. K. Dicke, Prof. Dr. H. A. Weyers en Prof. Dr. M. C. Verloop; ik wil hen met eerbied hier gedenken.

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Dan wil ik niet nalaten ook het bestuur van het NIZO mijn erkentelijkheid te betuigen voor de gelegenheid die het mij bood om deze promotie tot een feit te maken.

Ik doe beslist niemand van mijn medewerkers te kort wanneer ik hier met name Ir. G. Schaafsma dank zeg voor de enorm grote hulp die ik van zijn kant heb ondervonden. Niet alleen zijn kennis en vaardigheden, maar ook zijn zorgvuldigheid en daarenboven zijn enthousiasme en vriendschappelijkheid zijn van essentiële betekenis voor mij geweest; mijn erkentelijkheid daarvoor is bijzonder groot.

Verder moge ik de toewijding noemen waarmee onze proefdieren steeds zijn omringd door de dierenverzorgers van het Centrum Kleine Proefdieren van de Landbouwhoge-

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Hugo de Waard

Aan mijn ouders
Voor Anneke

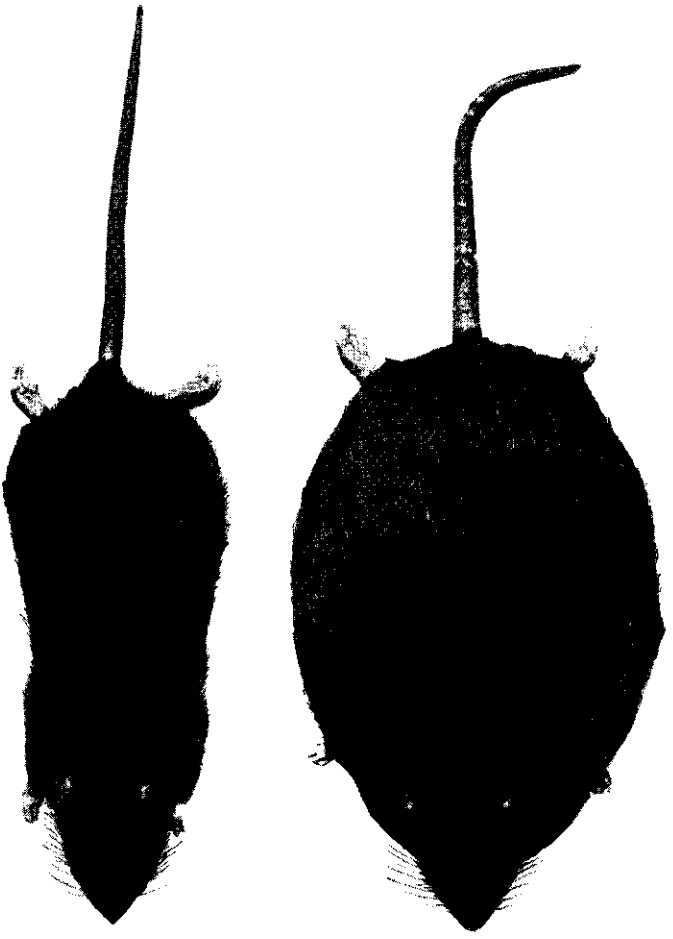

Figure 1. The phenotypes of the Zucker rat: obese and lean

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Chapter 1

Introduction

1.1 Dietary fat and serum cholesterol

It has been known for a long time that dietary fat influences lipid metabolism, notably that of cholesterol, both quantitatively and qualitatively. Already in 1952 Groen et al. and Kinsell et al. independently discovered, in man, the effect of the composition of the dietary fat on the serum cholesterol concentration. It is now generally accepted that a fair proportion of saturated fat in the diet can increase blood cholesterol levels, in contrast to poly-unsaturated fatty acids (particularly linoleic acid) in the dietary fat, which can lower these levels; the use of low-fat diets (which are mostly essentially rich in carbohydrates) and mono-unsaturated fatty acids (mainly oleic acid) are regarded as exerting an intermediate effect in this respect. Increased blood cholesterol concentrations should provoke or facilitate the development of atherosclerotic processes, in man and in a number of animal species. A mass of literature on this subject is available, mainly dealing with this connection to atherosclerosis, and most of it put forward from the viewpoint of atherogenesis and the clinical complications of the atherosclerotic process. As recent references may be mentioned: May 1974, Hautvast et al. 1975, Wissler et al. 1976, Paoletti & Gotto 1976, Schettler et al. 1977.

1.2 Other dietary effects on serum cholesterol

There are several other factors involved in atherosclerosis, nutritional as well as non-nutritional; of the former, dietary fibre may be mentioned here (Trowell 1972), because of its presumed relatively great significance. Balmer & Zilversmit (1974) found that undigestible dietary components lower plasma cholesterol concentrations and increase faecal sterol excretion — in this way influencing the turnover of cholesterol —, but do not inhibit intestinal absorption of cholesterol. A review of the sole dietary influence of dietary fibre on serum lipids has been given by Kritchevsky (1976).

1.3 Effect of dietary sucrose on serum lipids

The possible role of dietary sucrose in exerting hyperlipidaemias, as put forward by Yudkin (1957 and 1964) has resulted in extensive study on the effects of this sugar on lipid metabolism. The transient nature of this hyperlipidaemic effect was already noticed by Harper et al., in as early as 1953. The effect of sucrose on the liver (fat accumulation) compared with dextrin was described by Litwack et al. (1952) and by Marshall & Womach (1954) in weanling rats and in adult rats respectively and by Macdonald (1962) in adult rabbits. Early studies on the differences in metabolic properties between sucrose and corn starch were published by Portman et al. in 1956.

The initial increasing effect of sucrose — in contrast to the effect of starch — on blood lipids, cholesterol as well as triglycerides, has been demonstrated several times, in rats (Fillios et al. 1958, who again stressed its temporary nature, and Staub & Thiessen 1968) and in hyperlipidaemic man (Macdonald & Braithwaite 1964; Winitz et al. 1964. Kuo & Bassett 1965; Kaufmann et al. 1966a; Kuo et al. 1967; Szanto & Yudkin 1969) and more

recently, in normal man, by Mann et al. (1971), Mann & Truswell (1972), Laube et al. (1973), Naismith et al. (1974) and Rath et al. (1974).

The effects of the lipogenic properties of sucrose, particularly on the liver, were described by, amongst others, Bailey et al. (1968), Bender & Thadani (1970) and, in a depletion-repletion study, by Aoyama & Ashida (1972). These effects are related primarily to the fructose moiety of the sucrose molecule (Kaufmann et al. 1966b, Bar-on & Stein 1968). The diabetogenic properties of sucrose compared with those of starch were studied by Cohen & Teitelbaum (1964); later, these autors also dealt with fructose consumption and lipogenesis (Cohen & Teitelbaum 1968). Reviews on the subject of effects from sucrose were further provided by Bender & Damji in 1972 and by Macdonald in 1973.

1.4 Combined effects of dietary fat and sugar

Relatively little attention has been given to the combined influence of dietary fat and sucrose on serum cholesterol levels, in rats (Carroll 1963 and 1964, Carroll & Bright 1965) and in man (Antar et al. 1964, Hodges & Krehl 1965, Hodges et al. 1967, Macdonald 1967, the combined studies of Little et al., Birchwood et al. and Antar et al., all in 1970, and Mann et al. 1973). In this respect, the work of the Dutch scientist Groen (1967) may also be mentioned.

On the same combined influence on serum triglyceride concentrations was published by, amongst others, Nikkilä (1969) and Nestel et al. (1970). McGandy et al. (1966) indicated the lack of interaction between the two dietary factors. McGandy et al. published a review on the subject in 1967. Anderson et al. (1963) considered the effect of dietary sucrose of little importance.

1.5 Experiments on cholesterol metabolism with rats

On account of a number of unsolved problems related to the duration and the significance of the combined effects of dietary fat and sucrose on lipid and glucose metabolism (Bender & Damji 1972) which involved many implications for recommendations to be made with regards to the influence of nutrition on health, we were highly interested in this subject. Owing to the huge problems which are concerned with experimentation on humans and to the limitation of our facilities, we had to restrict ourselves to studies on animals.

The rat, a small laboratory animal which is easy to handle, did not appear to be a very suitable model for this kind of studies, because it has a number of physiological properties, which differ considerably from those of man. Moreover, the serum cholesterol concentrations in the rat are difficult to influence to any considerable extent by dietary treatment, though they can be elevated by adding 1% or more of cholesterol to the diet. This is particularly effective in combination with addition of either cholic acid or thyroid blocking agents (Malinow et al. 1954).

1.6 Obese rats as a model for studies on serum lipids

As there exist some strains of obese rats, whose serum cholesterol concentrations are similar to those of man, it was tempting to investigate this type of rat as a model for studies on the effects of dietary fats and sugar on lipid and glucose metabolism. Besides the BHE-rats*), which are reported to be obese, susceptible to sucrose and to have a

*) BHE is the abbrevation of „Bureau of Home Economics", a predecessor of the Nutrition Institute of Beltsville, Md. (USA). This strain of rats is a cross between the Pennsylvania State College strain and the Osborne-Mendel (Yale) strain.

tendency towards diabetes mellitus (Berdanier 1974), and an obese rat strain derived of the hypertensive Kuoto-Wistar and the Sprague-Dawly strain (Koletsky 1973 and 1975 a and b*), it was the genetically obese Zucker rat which we regarded as the most fitting model for studies on hyperlipidaemia, obesity and hyperinsulinism. The rat model as described by Cohen et al. (1972) is not obese, but has been selected from albino rats of the Hebrew University strain, for studies on diabetes mellitus.

A great deal of work has been done concerning the biochemical differences between these obese Zucker rats and lean litter-mates, but relatively little has been published on the influence of the diet on their metabolism.

In Zucker rats, obesity, decreased physical activity and increased lipid metabolism (Simonelli & Eaton 1978), as well as the occurrence of overt or latent diabetes, in particular with insulin resistance, are described. These factors are among those which are regarded as being related to atherosclerosis.

A comparison between these obese rats and man is thus attractive and promising, at least as far as lipid metabolism is concerned. It remained to be seen to what extent this comparison would also hold for atherosclerosis, a process which occurs very rarely in rats.

On account of our interest in this matter, which is fundamentally but also practically related to the diet of man, it was decided to make a study of the effects of dietary fats and sugar and their possible interrelationships in the genetically obese Zucker rat.

What can be found in the literature on studies with this rat strain will be referred to in the following chapter, in order that a clear picture of this animal is given before the design and the results of our investigations are dealt with.

It was considered useful if the study would not be confined to the qualitative aspects of fat consumption, such as, for instance, the various effects of nutritionally saturated towards poly-unsaturated high-fat diets, but would also be concerned with quantitative aspects (high-fat *versus* low-fat diets), which approach includes problems connected with the intake of energy and other food components. Moreover, we decided that attention would be given to the effects of sucrose in comparison with (wheat) starch, the other type of dietary carbohydrate of quantitatively primary importance.

As we wanted to restrict our study primarily to experiments with rats of one sex, only male rats were chosen, in accordance with the fact that in clinical medicine the atherosclerotic complications are more frequently found in males. The correlation between obesity, plasma insulin and triglyceride levels is reported to be lower in female than in male humans (Farquhar et al. 1973).

The extent to which this investigation will be of interest from a viewpoint of comparative physiology and, besides, will be relevant to human nutrition in practice, is expected to have been made clear at the end of this detailed report.

*) See also Nutr. Reviews (1978) and Chapter 10, Section 4.

Chapter 2

The genetically obese Zucker rat; literature review

„For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied . . . I have no doubt that there is quite a number of animals which are similarly created for special physiology purposes, . . ."

August Krogh (1929)
Am. J. Physiol. 90, 243-251

2.1 Introduction

In this chapter the information from all known studies about the genetically obese „Zucker" rat will be referred to. The references dealing with this laboratory animal, up to the beginning of 1978, are marked in the literature list with an asterisk. A few papers were discarded since they were abstracts which had been published elsewhere in a more extensive form.

2.2 Genetics

The obesity in this rat strain was originally discovered by Zucker & Zucker (1961) within their breeding colony of the brown rat (*Rattus norvegicus*), which was a crossbred from the Sherman and Merck strains (Cruce et al. 1974). The inheritance of the obesity in these rats appeared to be autosomal-recessive: approximately 25% of the progeny of carriers of the gene for fatness (fa), either male or female, become obese after about three weeks; this part of the offspring is designated as fa/fa.

Amongst their lean litter-mates there are twice as many heterozygotes for this gene, designated as Fa/fa, than there are lean rats with the normal allele couple, designated as Fa/Fa. The heterozygotes, however, cannot be distinguished phenotypically from the latter. When their genotype is not known, lean Zucker rats are designated as Fa/-.

2.3 Nutritional intake

2.3.1 Food consumption

Hyperphagia and obesity can be established before weaning (Bell & Stern 1976). The fatty rats show an increased food consumption of about 40%, compared with their lean litter-mates (Zucker & Zucker 1962, Barry & Bray, Bray & York 1972, Becker & Grinker 1977). When obese rats are pair-fed with lean rats, their gain in weight is approximately 40% lower than when they are fed *ad libitum*; even then they become heavier than the lean controls and develop an abnormally high proportion of body fat, but this goes at the cost of their muscle mass (Zucker 1967, Bray et al. 1973).*)

Recently Dilettuso & Wangsness (1977) found that food intake relative to body weight is higher in obese than in lean rats only during the early weeks of life, and even lower at an older age. The sex difference in the quantities of food consumption occurred also in obese rats, although to a lesser extent than in lean rats (Radcliffe and Webster 1978).

From the age of 35 to 90 days, male fatty rats pair-fed with lean litter-mates deposited

*) As in the development of obesity in Bar Harbor mice, hyperphagia must be regarded as a secondary phenomenon (Dubuc 1976a, P. Y. Lin et al. 1977).

60% less body protein, this figure being somewhat lower for females (Pullar & Webster 1974).

In obese rats fed to appetite, the energy retention was much higher, in contrast to the nitrogen retention, which was lower. According to Deb & Martin (1975) exercise decreases the fat content and increases the protein content to some extent, in obese as well as in lean rats. Park & Hershberger (1973), with a view on the higher fat deposition of the obese rats, suggested that thermogenesis from the diet, rather than its energy content, determines the voluntary food intake of both phenotypes.

As a continuation of the work of Pullar & Webster (1974), who hypothesized that there is, during growth, an inverse relation between food efficiency and protein deposition, Radcliffe (1977a) supposed that food intake is normally regulated by the attainment of maximal individual protein deposition. Fat deposition was much higher in the obese rats, with the exception of those which were fed on very low-protein diets. He observed that in rats between 34 and 66 or 98 days of age, at least in females, protein deposition was similar in obese and lean rats, if they were offered normal diets *ad libitum* which contained all essential nutrients. Obese male rats were found to lay down more protein than females, but lean males surpassed fatty males in this respect, even those which were fed on high-protein diets.

Fatties did not reach their maximal protein deposition at the level of 15% dietary protein (casein) or with restricted intake of high-protein diets. On low-protein diets or, also, on low-quality protein diets with a subnormal percentage of protein (15% gluten) — and at all levels of zein — the obese rats ate less than should have accorded with the otherwise attractive theory of Radcliffe on the regulation of food intake. This discrepancy is presumably another part of the problem of food consumption, occurring when qualitatively inadequate diets are given. This subject was studied further by Radcliffe (1977b), who had already published some of his data earlier (Radcliffe et al. 1975, Radcliffe & Webster 1976).

Radcliffe, on the basis of the results obtained with his experiments, denies the importance of such factors as the rate of energy retention or the storage of lipids in the regulation of food intake. This conflicts with at least one of the current theories on this subject, noticeably with the lipostatic theory (Nutr. Rev. 1977).

Becker & Grinker (1977), when studying meal patterns of Zucker rats observed enlarged meal size and decreased meal frequency in obese rats, which also failed to show the typical pattern of nocturnal eating. Their finding of a higher proportion of diurnal consumption with obese than with lean rats was confirmed by Wangsness et al. (1978). Obese Zucker rats respond more alertly to food supply than do their lean litter-mates, in contrast to ventromedial hypothalamic lesioned fatty rats, which react less on food supply than do normal rats (Greenwood et al. 1974, Cruce et al. 1974). As a consequence of their obesity, fa/fa rats are physically less active (Zucker 1965 and 1972, Pullar & Webster 1974, Stern & Johnson 1974, Drewnowski & Grinker 1978). Recently Martin & Gahagan (1977a) showed that the hyperphagia of obese rats is not an essential feature either in lipogenesis or in hyperinsulinism.

2.3.2 *Food efficiency*

Food efficiency, closely related to food intake, because of the influence of the requirements for maintenance, is higher in obese rats than in lean litter-mates (Zucker 1967 and 1975, Pullar & Webster 1974, Deb et al. 1976). Even before the obesity can be detected by observation an enhanced oxygen uptake is measured, at least when no correction is made for body surface (Bray 1969a, Pullar & Webster 1974). The latter authors observed that heat loss and nitrogen balance were similar in fatty and lean rats

when fed *ad libitum*, but that these properties were lower in obese rats, when they were pair-fed with lean controls.*)

The efficiency of metabolizable energy for growth in fatties was found to be higher than that in lean rats, namely 60 and 50% respectively. This increased food efficiency is connected with a decreased thermogenesis and increased lipid synthesis, as the result of a decreased protein synthesis. The energetic efficiency of net protein synthesis was estimated as 43% and of net fat synthesis as 65%, these figures being similar for lean and for obese rats. Pullar & Webster (1977) and Webster (1977) proposed animal feeding and selection based on the insights regarding energy losses obtained from the studies on Zucker rats. Recently, Webster et al. (1978) revealed the close correlation in Zucker rats of heat loss with protein synthesis, compared to that with either protein mass or body weight. According to Lobley et al. (1978) the low deposition of body protein of the obese Zucker rat is not due to a decrease in the fractional rate of protein synthesis.

Jenkins & Hershberger (1978) confirmed the higher food efficiency in obese rats as compared with that in lean controls. They concluded that obese as well as lean rats eat to attain a constant heat increment — independent of the composition of the diet —, and that obese Zucker rats apparently have no defect in the regulation of their food consumption.

2.3.3 *Effects of dietary composition*

Most studies on genetically obese rodents have been restricted to determinations of biochemical differences between these animals and lean controls or rats rendered obese by some different means, but relatively little attention has been paid to dietary composition. As far as such studies have been undertaken — apart from quantitative restriction — they have been performed mostly by the addition of some fat, usually soy bean oil, to the diet (Johnson et al. 1973, Park & Hershberger 1973, Lemonnier et al. 1974, Stern et al. 1975, Wahle & Radcliffe 1975, Martin 1976, Radcliffe & Webster 1976 and Thenen & Mayer 1977). Comai et al. (1978) compared the effects of 20% corn oil in the diet with those of an equal percentage of hydrogenated soybean oil.

On the other hand, Radcliffe (1977a), in studying the regulation of food intake, not only varied the fat content of the diet, but also varied the quantity of the protein as well as its quality, the cellulose content and even the glycerol content. This last compound had, as a dietary component, also been studied by Barry & Bray (1969). The observation of Radcliffe (1977a) that a very high proportion of dietary protein reduces the food intake of obese and of lean rats was confirmed by Wangsness et al. (1978) and by Jenkins & Hershberger (1978).

2.4 Lipid metabolism

2.4.1 *Fat mass*

The fat percentage of obese rats of the age of three to four months, which mostly approximates 50%, does not increase further, although the rats still gain weight during the first year of their lives (Zucker & Zucker 1963). Body weight can rise to over 1000 grams. The differences in body weight between obese and lean rats are mainly caused by a different fat mass (Zucker 1967, Johnson et al. 1971, Bray et al. 1973).

It appears that the increase in fat mass is not simply the result of an increased food intake, but is due to (a) increased food efficiency, which means a lower heat production,

*) Oxygen consumption was measured to be lower in obese than in lean mice of the Bar Harbor strain already at a very early age (Dubuc 1976b, Boissonneault et al. 1978).

and (b) enhanced lipogenesis in early life as a consequence of an inborn error in protein metabolism with a tendency to a lowered deposition of body protein (Pullar & Webster 1974, Bray et al. 1974). Martin (1976) suggested that the defect in protein metabolism induces an increased and, in terms of energy, efficient conversion of proteins into glucose and fatty acids.

Opsahl & Powley (1974) failed to reverse the obesity of four-month old Zucker rats by vagotomy, this in contrast to ventromedial hypothalamic obesity. Hypophysectomy performed on 150-day old rats blocked further development of obesity, but did not reduce existing adiposity (Powley & Morton 1976).

Godbole & York (1978) observed, in obese Zucker rats of 13 weeks, in comparison with younger ones (5 weeks old), an increase in hepatic lipogenesis but a decrease in adipose tissue lipogenesis.

2.4.2 *Cellularity of adipose tissue*

The number and size of the fat cells of obese rats have been measured. The adipose tissue appears to be both hypertrophic and hyperplastic (Bray 1969b, Bray et al. 1970a, Bray & York 1971a, Johnson et al. 1971, Lemonnier 1971a). This has also been observed in the genetically obese Bar Harbor mouse (Johnson et al. 1973). Rats rendered obese by lesioning of the ventromedial hypothalamic nuclei of the mid-brain did not show — connected with the time of onset of the obesity — hyperplasia of the adipose tissue (York 1975a).

The number of fat cells does not increase in obese Zucker rats after the age of about 26 weeks, which is later than in lean rats for which this period is 14 weeks. From that age onwards, the fat mass will increase only by a rise in fat cell size.

Stern & Johnson (1977) recently studied the relationship between the cellularity of the adipose tissue and the spontaneous activity of obese and lean Zucker rats. The decreased physical activity of obese rats follows the onset of hyperphagia and obesity. At 8 weeks of age exercised lean and obese rats have less total fat and fewer adipocytes than controls. Adipose cell size is decreased only in exercised lean rats. Rats exercised until 8 weeks and then confined until 6 months of age have similar body weights and total fat stores compared with control rats. Adipose cell number is permanently decreased only in formerly active lean rats. Exercise has no long-term effect in decreasing cell number in obese rats.

2.4.3 *Adipose tissue metabolism*

The metabolism of body fat of obese rats has been studied extensively by Zucker & Zucker (1963), Zucker (1972) and in particular by Bray (1968 and 1969b), Bray et al. (1970a and b), York & Bray (1973a) and Bray et al. (1974). Lipogenesis, in early life, is enhanced if either glucose or pyruvate was used as substrate. This occurs at the expense of the oxidation of these substrates (Bray et al. 1970b).

Bray et al. (1974) and Martin (1976) stated that the metabolic defect of the obesity of this rat strain is not localized in the adipose tissue. The increased voluntary food intake of the obese rats is regarded as a normal response to an abnormal growth pattern due to an error in protein metabolism.

The lipoprotein lipase (LPL) activity of adipose tissue in obese rats was found to be increased — especially in early life (De Gasquet et al. 1973, De Gasquet & Péquignot 1974, Greenwood & Hietanen 1976). Schonfeld et al. (1974) demonstrated an increased concentration of some lipase activator in the plasma of fatties. Nevertheless, these increases are not sufficient to clear the blood plasma quickly of triglycerides.

2.4.4 *Liver metabolism*

Studies on the hepatic lipid metabolism of Zucker rats have been carried out by Lemonnier et al. (1974), Martin (1974), Taketomi et al. (1975), York & Godbole (1977) and Bloxham et al. (1977). Zucker (1967) reported that the livers of these obese rats had been enlarged and fattened. Lemonnier et al. (1974) established that glucose incorporation into triglyceride fatty acids was seven times larger in liver tissue and twice in adipose tissue of obese Zucker rats than in the respective tissues of lean controls. Lipogenic enzymes in the livers of obese Zucker rats (glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, acetyl-CoA carboxylase, malic enzyme citrate lyase and the fatty acid synthetase complex) were enhanced (Bray et al. 1970b, Martin 1974).

Recently, Bloxham et al. (1977) studied hepatocytes of obese and lean Zucker rats (at an age of 90 to 120 days) fed on either a fat-free high-sucrose diet or a low-fat control diet. They established a much smaller contribution from glucose than from lactate in lipogenesis, particularly in obese rats, and on feeding the experimental diet. They stressed the little importance of glucose as a substrate for lipogenesis in the liver, even when this process is greatly enhanced, as is the case with diets very rich in carbohydrates.

In a study in which isolated livers of obese Zucker rats were compared to those of Wistar rats, which were perfused with either ^{3}H-lauric acid or with ^{3}H-oleic acid and ^{14}C-glycerol, Chanussot & Debry (1977a) measured two pools of synthesized labelled compounds. From the former substrate, trilaurin was synthesized only in the obese rat livers. Triglycerides released into the perfusate of Wistar rat livers contained one or two labelled lauric acid molecules at most. From oleic acid and glycerol, the livers of obese rats synthesized more dipalmitoylolein, dioleoylpalmitin and triolein than the livers of Wistar rats. Linoleoyloleoylpalmitin was formed in the livers of both strains, a larger quantity of this compound was detected in the perfusate of the obese rats. Dioleoyllinolein and dilinoleoylolein appeared to be equally present in both strains. After 30 minutes perfusion, less hepatic phospholipid was found in the Zucker rats than in the Wistar rats.

In a further study (Chanussot & Debry 1977b) with perfused livers of these rat strains, on the synthesis of phospholipids and triglycerides, the former substances appeared not to be synthesized from lauric acid. The triglycerides synthesized from lauric acid — trilaurin being formed in the Zucker rat livers only — were more rapidly oxidized by the livers of the obese rats than by those of the normal rats. From oleic acid and glycerol, the phospholipid synthesis was less significant with the obese rats than with the Wistar rats but, from these substrates, the Zucker rats synthesized more triglycerides than did the Wistar rats. So, with regards to fatty acid metabolism, qualitative as well as quantitative differences were found between normal Wistar rats and obese Zucker rats.

In an investigation carried out by Subbiah & Connelly (1976) a significantly increased bile acid and sterol excretion with the faeces was observed in obese Zucker rats in comparison with lean rats.

Novikoff (1977) mentioned the increased tendency towards the development of fatty livers in obese Zucker rats fed on a high-sucrose diet containing 1% orotic acid, for the purpose of experiments on hepatic steatosis.

2.4.5 *Fatty acid composition*

The fatty acid composition of body fat has also been studied by several workers (Bray 1969b, Schonfeld et al. 1974, Wahle 1974, Wahle & Radcliffe 1975 and 1976). Obese Zucker rats were found to have increased percentages of palmitoleic ($C_{16:1}$) and oleic

($C_{18:1}$) acid in their body fat, and decreased proportions of linoleic ($C_{18:2}$) and arachidonic ($C_{20:4}$) acid, in comparison with lean litter-mates. This is in agreement with the higher tissue desaturase activities on the monoene fatty acids, which are observed in obese rats (Wahle 1974).

Although the liver lipid content in obese males does not differ from that in females (it is four times higher than that in lean rats), obese females have similar proportions of linoleic and arachidonic acids as lean females, in contrast to obese males, which have lower percentages of poly-unsaturated fatty acids than lean males.

The ratios of mono-unsaturated to saturated fatty acids of both a chain length of 16 and 18 carbon atoms are similar in obese males and females, and are higher than in lean rats (Wahle & Radcliffe 1976). These reflect increased desaturase activities, but also increased lipogenesis and increased liver weights (Zucker 1967).

Upon the supply of additional dietary linoleic acid, the monoenes in the body fat of obese rats declined, whereas the linoleic acid content of the body fat increased. The percentage of linoleic acid of lean rats remained higher than that of the fatties, both in adipose tissue and in liver lipids. The arachidonic acid content, however, increased in the lean rats only. Accordingly with the rise in the content of poly-unsaturated fat in the tissue, the hepatic desaturase activity was found to decrease with the provision of additional linoleic acid. This extra amount of linoleic acid decreased the liver lipid content of obese rats, contrary to its effect on that of lean controls (Wahle & Radcliffe 1975).

2.5 Protein metabolism

Apart from the influence of the quantity and the composition of dietary protein on food and energy utilization and growth, as mentioned in a former section (2.3.3, Radcliffe 1977a), one could study separately the influence of dietary protein on protein utilization, as was undertaken by Chu et al. (1978). Besides a 5/3 times higher efficiency for energy utilization in obese rats than in lean Zucker and Charles River rats, they found a similar efficiency for protein utilization in the obese and the lean rats.

The hypothesis of Martin (1976) that an inborn error of protein metabolism would be the cause of the genetic obesity in Zucker rats has already been mentioned.

Protein synthesis in the livers of obese rats appeared to be higher than that in the livers of lean controls (Fillios & Saito 1965). Females of both phenotypes exhibited this ability more clearly than males, at least in the microsomal fraction of the cells. Cholesterol feeding (1.5% with 0.5% cholic acid) depresses this protein synthesis (Fillios & Yokono 1966 and 1968) and causes a larger decrease of protein synthesis in obese than in lean rats. Relating to this, Fillios et al. (1969) also established a difference in cholesterol esterification between the obese and the lean rats: in fatties a part of the cholesterol in the microsomal fraction was esterified, whereas normally only free cholesterol occurs in these organelles. They concluded that the esterification of cholesterol in these rats, which also showed hypercholesterolaemia, is very efficient. The significance of the increased protein synthesis in the livers of the obese rats remains unclear.

2.6 Blood lipids

The obese Zucker rats show an increased lipid content in the blood (Zucker & Zucker 1961 and 1962, Zucker 1965 and 1972); in particular, the triglyceride concentrations are very high. Zucker (1974) even proposed on the base of the lactescence of the blood plasma an assay for hyperlipidaemia. The high triglyceride levels in the blood of these rats may be a consequence of the increased lipogenesis both in adipose and in liver tissue (Bray 1968, Bray et al. 1970b). Even after overnight fasting, the rats were found to

have triglyceride concentrations of 1000 mg per 100 ml blood or more (Zucker & Zucker 1962, Barry & Bray 1969, York et al. 1970).

Sullivan et al. (1977a and b) studied the hypolipidaemic effect of (-)-hydroxycitrate; this substance also in Zucker rats suppressed triglyceridaemia and lipogenesis.

In contrast to lean litter-mates — which have, as do rats of non-obese strains, cholesterol concentrations of 80-90 mg per 100 ml blood — obese Zucker rats have cholesterol levels up to about 250 mg per 100 ml blood. This value depends to some extent on the composition of the food. This dependence is also greater than that in lean rats.

Contradictory to an earlier paper (Zucker & Zucker 1962), later articles reported higher free fatty acid concentrations in the blood of obese rats (Zucker 1972, Schonfeld et al. 1974). This contradiction is presumably related to the very young age of the rats in the first experiments.

In addition, the mobilisation of fatty acids from adipose tissue was found to be increased (Bray et al. 1970a). This enhanced lipolysis is also expressed by increased plasma glycerol concentrations in obese rats of several weeks of age, although this compound can very easily be (re)utilized — in the form of glycerol-3-phosphate — in fatty acid esterification besides in gluconeogenesis (Barry & Bray 1969, Bray et al. 1970a and b, Martin & Lamprey 1975). Already at the age of six weeks, obese rats clearly show an increased release of glycerol after an injection af adrenalin (York & Bray 1973b).

Very low-density lipoproteins (VLDL) form by far the largest blood lipid fraction of these rats (Schonfeld & Pfleger 1971). In fatties the VLDL fraction is increased several-fold, and it contains 78% of triglycerides, as compared with 60% in lean controls. Low-density lipoproteins (LDL) and high-density lipoproteins (HDL) are about twice as high in fatties as in leans. In obese rats fed on a low-fat stock diet VLDL constitute 52% of the total lipoprotein mass, whereas in lean controls HDL is the predominant representative, making up 62% of the plasma lipoprotein mass (Schonfeld et al. 1974). Abnormal apolipoproteins were not detected.

Recently, Redgrave (1977) reported that the clearance of chylomicrons from the blood of obese (Zucker) rats was slower than that from the blood of lean controls, of cholesterol ester more so than of triacylglycerides. Triton 1339 also had an accumulating effect on the chylomicrons in obese rats.

Biery & Evarts (1975) used obese Zucker rats to study the relationship between hyperlipaemia and the concentrations of α-tocopherol (vitamin E), supplied with the diet, in plasma and tissues. They observed higher levels of tocopherol in the plasma and lower concentrations in the tissue of obese rats, although these rats had, because of their larger adipose mass, more total body α-tocopherol than the lean ones.

2.7 Endocrinal aspects

2.7.1 *Insulin and glucagon*

Fatty rats have, probably as a result of their obesity, increased insulin levels in the blood and a resistance of their adipose tissue to insulin (York et al. 1970 and 1972, Lemonnier 1971b, Zucker & Antoniades 1972, Stern et al. 1972 and 1975). Shino et al. (1973) mentioned hypertrophic pancreatic islets in obese Zucker rats; Boder et al. (1972) described, in addition, hyperplasia of the islets of obese Zucker rats of 28 to 300 days of age (referred to by Boder & Johnson 1972), with the clearest expression between 9 and 20 weeks of age. Schade & Eaton (1975) reported increased insulin secretion in an *in vitro* study. Mikuni (1974) injected radioactively labelled porcine pro-insulin in Zucker rats and concluded that it was utilised in a normal manner. Zucker & Antoniades (1972), in a study with the diaphragm muscle of Zucker rats, found that in obese contrasting to

lean rats insulin injections did not promote further conversion of ^{14}C-glucose into glycogen.

Blood glucose concentrations in fatty rats were reported to be within the normal range (Zucker & Zucker 1962, Zucker & Antoniades 1972, York et al. 1972a, Stern et al. 1972) and glucosuria does not occur. In contrast to Malewiak & Griglio (1978, to be published), who found lower ketonaemia levels in obese Zucker rats than in lean controls, Bach et al. (1977a) reported that ketone bodies were found to be only slightly higher than in lean rats.*) However, Bach et al. (1977b) observed lower ketogenic activities in obese Zucker rats than in their lean litter-mates at the provision of medium-chain triglycerides. These are quickly oxidized, whereas long-chain triglycerides being incorporated in body lipids appeared to be non-ketogenic.

Thus, only a tendency to diabetes mellitus can be observed (Zucker & Antoniades 1972), which is different from most other obesity syndromes in rodents (Bray & York 1971a). Larsson et al. (1977) mentioned the reduction to normal of the islet morphology by dietary restriction and the subsequent decrease in serum insulin levels; the latter was also obtained by streptozotocin injection. After the dynamic phase of the obesity (in rats of 3 to about 16 weeks of age), insulin levels even tend to decrease (York & Bray 1973a).

Lemonnier et al. (1974) observed no difference in pancreatic glucagon content between obese and lean Zucker rats. Laburthe et al. (1975) found no differences in the amino acid composition of either insulin or glucagon between lean and obese rats. Martin & Gahagan (1977a) observed no significant decrease in hepatic lipogenesis upon provision of glucagon in obese rats, in contrast to the results of experiments with lean litter-mates.

Eaton et al. (1976a) found reduced plasma glucagon levels in fatties compared with lean rats. These values were further reduced during fasting, and increased subnormally when stimulated with arginine injections. On the other hand, a decreased insulin/glucagon ratio was observed in male obese Zucker rats upon administration of halofenate, a hypolipaemic drug (Eaton et al. 1976b). Also Bryce et al. (1977) found reduced glucagon release in obese rats in comparison with lean Zucker rats, besides higher plasma insulin levels in the former, independently of the (high-fat or high-carbohydrate) diet. Mahmood et al. (1978) studied the effect of induced hyperglucagonaemia on the metabolism of the fatty Zucker rat.

Broer et al. (1977) studied the hormone receptor binding and the corresponding cyclic adenosine monophosphate levels in hepatocytes of obese Zucker rats.

2.7.2 *Other hormonal aspects*

With regard to a number of different aspects of hormonal balance in obese rats, attention has been given to the water metabolism (Bray 1968, Yrok & Bray 1971), which showed polyuria as a result of polydipsia in fatties and to the function of the thyroid gland, which was found to be impaired (Bray 1968 and 1970, Bray & York 1971b, York et al. 1972, Bray et al. 1973, 1974 and 1976).**) The basal metabolic rate of fatty Zucker rats is

*) A lower ketone body production by livers — perfused with albumin-bound oleate — of obese mice than that by livers of lean Bar Harbor mice was reported by Assimacopoulos et al. in 1974.

**) It may be that an impaired thyroxin metabolism is due to a difference in proportional conversion of thyroid hormone into triiodothyronine and in the metabolically less active (energenetic) „reverse" triiodothyronine (Hirsch & Schneider 1976). There are also indications that a reduced activity of a thyroid-dependent, ouabain-suppressible Na^+ and K^+-adenosine triphosphatase (ATP-ase, EC 3.6.1.3) in obese mice, in comparison with lean Bar Harbor mice (M. H. Lin et al. 1978) and Zucker rats (York et al. 1978), is responsible for the metabolic properties in the obesity of these strains.

lower than after injury to the hypothalamic region in rats of the same strain, which points to pituitary abnormalities in obese Zucker rats.

Martin & Gahagan (1977b) recently studied a number of hormonal levels in Zucker rats (insulin, growth hormone, prolactin and thyroid-stimulating hormone) at the age of 5 to 11 weeks, and Martin et al. (1978) the diurnal changes in serum metabolites and hormones in lean and obese Zucker rats.

Insulin levels in obese rats remained, even during fasting, much higher than in lean rats. Growth hormone was lower in obese rats; it increased only slightly with age and was reduced by fasting. Serum prolactin was decreased in the obese rat but similar at 11 weeks of age. Corticosterone levels decreased with age and were only at 11 weeks of age higher in obese rats. TSH increased with age and was lower in obese than in lean rats at 9 weeks of age.

With respect to gonadal functions obese females appear to be sterile, although they show infrequent estrous cycles; the uterine weight is decreased (Bray et al. 1973, 1974 and 1976). Obese males have a diminished reproductive capacity (Saiduddin et al. 1973). The pituitary gland is found to be smaller and less active in obese rats (Bray & York 1971b, York et al. 1972b, Bray et al. 1973 and 1974).

The size and activity of the adrenals is increased in the fatty rats.

2.8 Prolonged fasting and heat production: the maintenance of the energy balance

Obese rats, like obese mice, can withstand prolonged fasting very well (Zucker 1967, Bray et al. 1970a, Zucker & Antoniades 1972, York & Bray 1973a). In these conditions they obviously feel quite comfortable, at least at normal room temperature. Their weight losses then are fairly small, even after a period of some 80 days.

Plasma insulin levels as well as body fat stores decrease immediately after the beginning of fasting, but a long time is needed before the normal values of lean litter-mates are reached (Zucker & Antoniades 1972). Plasma triglycerides normalize in one week to the normal full-fed ranges for lean rats (Zucker 1967).

Connected with the fact that the obese animals have an increased food efficiency under normal circumstances, they have an impaired heat production and consequently behave less favourably when exposed to cold stress (York et al. 1972b, Trayhurn et al. 1976). Neither in obese nor in lean rats did there appear to be a thermoneutral zone in this respect. This means that at no air temperature was heat loss found to be independent of it (Pullar & Webster 1974). There is a renewed interest in thermogenetic problems (B. G. Miller et al. 1977), in which studies on Zucker rats also play a role (James & Trayhurn 1976).

2.9 Comparative results of experiments with other rodents

There is a number of extensive literature reviews on genetically obese rodents, in which Zucker rats are also mentioned (Bray & York 1971a, Bray et al. 1974, Bray 1974, York 1975a, Hunt et al. 1976, Bray 1977). The review of Herberg & Coleman (1977) is mainly restricted to mice. Some of the strains of obese mice have a polygenic inheritance, so that lean litter-mates do not occur and other strains of mice have to serve as controls.

Comparisons have, in several cases, not only been made of obese rats with lean controls, but often also with rats rendered obese either with high-fat diets or by means of gold thioglucose, or by surgical lesioning of the so-called satiety centre in the hypothalamic region of the mid-brain (Barry & Bray 1969, Bray 1969a, Bray et al. 1970b, Bray & York 1971b, Johnson et al. 1971, York et al. 1972b, York & Bray 1973a). Such comparisons also involved obese mice, especially the already mentioned genetically obese Bar Harbor mouse (C57-Bl/6J), designated as ob/ob (Bray & York 1971a, Bray

et al. 1973, Lemonnier et al. 1974, De Gasquet & Péquignot 1974, York 1975a).

Besides a number of similarities in all strains mentioned, there are many small differences, pointing to a large metabolic diversity (Bray 1974). Depending on the available information and the purpose of the research, a choice can be made with respect to the type of experimental animal required.

Although obese rats are hardly discussed in the review of Herberg & Coleman (1977), highly interesting is their conclusion that most of the symptoms of all these obese rodents tend after a certain time to decrease or to stabilize. This points to a spontaneous amelioration of the diabetic state in particular, which is not regularly observed in man. Certainly this is one of the differences between Zucker rats and man.

2.10 Miscellaneous aspects

Obese Zucker rats have also been used in several other experiments in which the study of obesity as a phenomenon was regarded as important. In this respect some papers may be mentioned here briefly: one article by Chesters (1975), who studied the effect of zinc deficiency on food consumption, one by Redgrave & Snibson (1975), who looked at the clearance of chylomicron cholesterol from the plasma, and two by Cruce et al. (1976 and 1978), on measurements of the catecholamines in the brains of Zucker rats. Crowley et al. (1978) recently measured the biological active peptides of the pituitary gland and hypothalamic nuclei. The sensitivity of diabetic and genetically obese (Zucker) rats to a number of pharmacological agents was investigated by Foy & Lucas (1976), who also studied the regional blood flow in various tissues of these rats (Lucas & Foy 1977). Coffey et al. (1978) studied the differences in collagen composition of the skin from obese and lean Zucker rats.

2.11 Conclusion

In conclusion, the obese Zucker rat was thought to be a convenient animal as an experimental model for the study of lipid metabolism on different diets. The most obvious biochemical differences of this rat from the genetically obese mouse are shown by the hyperglycaemia and glucosuria of the latter, and the mainly dietary origin of the plasma triglycerides of obese rats on high-fat diets (Bray et al. 1974, York 1975a). On low-fat diets these triglycerides appear to be derived, as usual, from predominantly endogenous sources (Lemonnier et al. 1974, Martin 1974). Thus, high-fat diets clearly depress fatty acid synthesis in these rats, particularly in the liver (Wahle & Radcliffe 1975, Martin 1976).

The underlying study was designed to investigate the effect of qualitative as well as quantitative differences in the dietary fat, and of the dietary carbohydrates, starch and sucrose, which are — from a quantitative point of view — by far the most important energy providing carbohydrates in the human diet.

2.12 Summary

1. The increased fat mass of obese Zucker rats is not simply the result of a higher food intake, but is due to an increased food efficiency combined with a lower heat production and to an enhanced lipid synthesis in early life because of a less efficient protein metabolism („inborn error"?) and a tendency to a lower deposition of body protein.
2. The cholesterol concentrations in the blood of the obese rats are moderately increased and the triglycerides substantially elevated, in comparison with those of lean rats.
3. The insulin levels in the blood of the obese rats are enhanced, whereas the blood glucose levels are normal or only slightly elevated.

Part One of the study on male Zucker rats

Chapter 3

Design of the study

I think this consideration is very important in the human problem. We look upon the spectrum of different genetic strains (. .) as representing the real and potential variations of the physiologic states observed in man in terms of lipid deposition and mobilization. We can select the strain that best can permit one to answer a specific question.

R. A. Liebelt (1963),
Ann. N.Y. Acad. Sci 110, *723-748*
(Addendum)

3.1 Introduction

The total investigation to be described here consisted of various parts. Young male Zucker rats at the age of approximately six weeks were given different diets for a period of sixteen weeks. During this time, a number of blood parameters was studied: plasma cholesterol and triglycerides, blood glucose and plasma insulin. All dietary groups, in total eight groups, consisted of at least twelve rats. This study on young rats formed the first part of our total investigation.

The Zucker rats were originally obtained from the „Centre de Sélection et d'Elevage d'Animaux de Laboratoire" of the „Centre National de la Recherche Scientifique" in Orléans (France), and were further bred within the „Centre for small laboratory animals" of the Agricultural University in Wageningen.

Since in our experiments only male Zucker rats were used, for their higher response with regard to plasma cholesterol, it appeared to be impossible to launch the number of animals desired at the same time. Since, on average, one out of four young rats will become obese, only one out of eight rats will be obese and male.

3.2 Grouping

For our experiments no more than approximately fifteen male obese rats were obtainable each fortnight, so that they had to be launched in several series in order to ensure that all eight groups consisted of a number of at least twelve rats. Thus, usually two rats from each of the seven series were placed in each dietary group. It was intended that the averages and variabilities of all groups of obese rats would be similar with regard to age, body weight, plasma cholesterol and triglyceride concentrations. For the lean rats this was only possible, compared with obese rats, as regards age. Details of all these data will be given in chapter 4. In order to exclude within-litter influences, no obese rats from one litter were placed in the same group.

During the study concern was given continuously to the fact that the rats within each group were not of the same age. In the course of this part of the investigation blood was taken from all rats on a fixed number of days after their launching. This was an important prerequisite with regard to sample collection. The results of the assays of the blood (and the faeces) collected during this part of the study are therefore fully comparable.

3.3 Housing

At the start of the study the rats were 39 days of age on average. From that time they were individually housed in metal cages of adequate proportions, with wired floors without further bedding materials. Room temperature was kept at 21 ± 1°C and relative humidity at 65 ± 10%. The illumination of the room was entirely artificial, with a day and night periodicity changing at 6.00 a.m. and at 6.00 p.m. every 24 hours. The ventilation of the animal house is designed for a capacity of at least 15 times the total air content of the rooms per hour.

The animals were fed to appetite, with the exception of the fasting experiment with a duration of four days, but at all times they had free access to drinking water.

3.4 Diets

3.4.1 *The semi-synthetic diets*

The aim of the study was to obtain knowledge of the influence exerted by the dietary fat and also of the carbohydrate on some parameters of the lipid and glucose metabolism of obese Zucker rats. For this purpose, a number of six semi-synthetic diets was used. Two groups were given low-fat diets, to which either wheat starch or sucrose was added. The four other semi-synthetic diets were high-fat diets (with approximately 40 energy %); two of these contained saturated fat and the other two highly poly-unsaturated fat. To each of these two pairs of diets, again either starch or sucrose was added. Naturally, the proportion of carbohydrate in the high-fat diets was considerably lower than in the low-fat ones. The design of the study in relation to the diets is given in Table 1.

Table 1. Grouping and global composition of the diets of the Zucker rats.

group	number of rats	phenotype	dietary fat	dietary carbohydrate
I	12	obese	low-fat	starch
II	12	obese	low-fat	sucrose
III	13	obese	high-saturated fat	starch
IV	13	obese	high-saturated fat	sucrose
V	12	obese	high-poly-unsaturated fat	starch
VI	12	obese	high-poly-unsaturated fat	sucrose
VII	13	obese	commercial ration	
VIII	12	lean	commercial ration	

As it is well known that rats on high-fat diets will gain more weight than those on low-fat diets (Lemonnier et al. 1971 and 1974), although they will eat more of the latter, the diets were designed to be iso-energetic as well as iso-nitrogenous, as will be clear from Table 2. It was estimated that approximately 85 parts of the high-fat diets would be consumed compared with 100 parts of the low-fat diets. It was expected nevertheless that the rats on the high-fat diets, when fed ad libitum, would gain weight more rapidly than those on the low-fat diets. As it was aimed to obtain similar weight curves for all obese rats, we were prepared to restrict the food intake of the rats that were fed on the high-fat diets. It appeared that, for reasons which will become obvious in Chapter 4, only

Table 2. Designed proportional composition of the semi-synthetic diets, (iso-energetic, iso-nitrogenous), on weight basis.

	I	II	III	IV	V	VI
casein + 0.5% DL-methionine	20	20	20	20	20	20
wheat starch	67	—	37	—	37	—
sucrose (powdered)	—	60	—	33	—	33
cellulose	4	11	7	11	7	11
cocoa butter/palm oil 2:1	—	—	13	13	—	—
sunflower oil	1	1	—	—	13	13
fat-soluble vitamins, in palm oil + 5% cholesterol	2	2	2	2	2	2
water-soluble vitamins	1	1	1	1	1	1
mineral mixture	5	5	5	5	5	5
	100	100	85	85	85	85
energy (kcal)	340		340		340	
metabolizable energy (kcal/g)	3.4		4.0		4.0	
proteins	22 energy %		22 energy %		22 energy %	
carbohydrates	70 energy %		39 energy %		39 energy %	
fats (linoleic acid included)	8 energy %		39 energy %		39 energy %	
linoleic acid	3 energy %		3 energy %		24 energy %	

the animals fed on the high-poly-unsaturated-fat diets had to be restricted for some time, to a maximum of 130 g and later of 120 g per week.

In order to prevent undesirable effects from the cholesterol content of the foods, vegetable fats were used exclusively in the diets. The saturated type of fat was a mixture containing two parts of cocoa butter*) and one part of palm oil**). This mixture provides a fatty acid composition very similar to that of most saturated fats of animal origin, in contrast to that of coconut oil which is frequently used in such experiments.

The poly-unsaturated type of fat used was sunflower oil with a linoleic acid content of approximately 70%***). In the high-fat diets of which this oil formed part, a linoleic acid content was provided which was clearly sufficient to reach the maximal effect of the fatty acid in lowering the plasma cholesterol concentration: this maximum is in the range of 17-23 energy % (Brown 1971, Vergroesen 1972).

A small quantity of this fat was added to the low-fat diets, in order to prevent any deficiency in essential fatty acids (Holman 1970). The presence of a reasonable amount of palm oil (8% linoleic acid) in the high-saturated-fat diets was regarded as being sufficient to prevent any such deficiency for the rats fed on these diets. The quantities of linoleic acid present in the low-fat and the high-saturated-fat diets were designed to be similar.

The fatty acid composition of all these diets was such that only a small number of different fatty acids was largely involved (see Table 3). Apart from a very small quantity of myristic acid, this concerned the saturated palmitic acid, the mono-unsaturated oleic acid and the poly-unsaturated linoleic acid. The proportions of oleic acid differed to a small extent only, so that the effects of the various diets would be largely independent of differences with respect to this fatty acid.

*) Obtained from „De Zaan" Cocoa Company, Wormerveer.

**) Refined palm oil, gratuitously provided by the „Unimills" Company, Zwijndrecht.

***) This oil was, through the good offices of „Unimills", gratuitously provided by the „Union" Company, Kleve (Federal Republic Germany).

Table 3. Actual fat content and fatty acid composition of the diets.

groups	fat content (%)	energy % from fat	percentage distribution of fatty acids					linoleic acid in the total diet	
			$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	content (%)	energy %
I and II	5	12	1	30	6	33	30	1.6	4
III and IV	17	38	1	34	21	37	7	1.2	3
V and VI	17	38	—	12	5	23	60	10.1	25
VII and VIII*)	5	12	2	21	10	32	30	1.4	4

*) The fat in the commercial ration also contained 5% other fatty acids.

The different indications for the fat content of the diets in Table 2 and Table 3 reflect deviations to some extent in preparing these diets. The former table gives designed figures and the latter actually measured ones. The differences, however, are not very significant for the aim of our study, because we did not try to provide extremely low-fat diets.

To all of these semi-synthetic diets a very small quantity of cholesterol (0.1% in the low-fat diets) was deliberately added. To improve the absorption of crystalline cholesterol this compound was dissolved, at a temperature of approximately 160°C, in the fat which was used for the preparation of the fat-soluble vitamin mixture.

With a view to the difference in purity to be taken in account (Nikkilä 1969) between wheat starch — consisting of approximately 90% of carbohydrate — and sucrose, the quantity added of the former carbohydrate was 10% larger. This difference was compensated for by the addition of different amounts of cellulose. In preliminary work with obese Zucker rats, the cellulose in question*) had been found not to influence plasma cholesterol concentrations.

Casein was used as the main source of protein, with the addition of 0.5% DL-methionine, in order to provide a maximal protein quality.

Table 4. Fat-soluble vitamin mixture added to the semi-synthetic diets (per kg of low-fat diet).

vitamin A (retinyl acetate)	12500 I.U. (= 3.75 mg)
vitamin D_3 (cholecalciferol)	2500 I.U. (= 62.5 μg)
vitamin E (α-tocopheryl acetate)	50 mg
vitamin K (menaphthone)	2 mg
made up with palm oil and 5% cholesterol to 2% of the final diet*).	

*) The quantities of minerals and vitamins administered are given per kilogram of diet, at least as far as the low-fat diets are concerned.

In Tables 4, 5 and 6 the various vitamin and mineral additions to the diets are mentioned. With regard to vitamin E it was considered that, despite an increased requirement for this vitamin when highly poly-unsaturated fat diets are used, the supply of this vitamin with the diets was already sufficiently high, so that no further increase was deemed necessary. Sufficient quantities of *myo*-inositol (Hasan et al. 1970) and choline chloride (Lombardi et al. 1968) with special regard to liver metabolism were added. Para-amino-benzoic acid was discarded from the vitamin mixture, because it is regarded as being only part of the folic acid derivatives.

*) Akufloc, AKU, Arnhem.

Table 5. Water-soluble vitamin mixture added to the semi-synthetic diets (per kg of low-fat diet).

vitamin B_1 (thiamine)	3	mg
vitamin B_2 (riboflavin)	4	mg
vitamin B_6 (pyridoxin)	3	mg
nicotinamide (niacin)	25	mg
DL-Ca-pantothenate	20	mg
folic acid (pteroylmonoglutamic acid)	0.8	mg
vitamin B_{12} (cyanocobalamin)	0.04	mg
biotin	0.12	mg
myo-inositol	100	mg
choline chloride	1800	mg
made up with glucose to 1% of the final diet.		

The mineral mixture (see Table 6) was made according to Williams & Briggs (Cohen et al. 1967).

Table 6. Mineral mixture per kg low-fat diet.

calcium carbonate	($CaCO_3$)	10.25 g
secondary calcium phosphate	($CaHPO_4$)	16.25 g
secondary sodium phosphate	(Na_2HPO_4)	9.25 g
potassium chloride	(KCl)	10.25 g
magnesium sulphate	($MgSO_4.H_2o$)	3.5 g
manganese sulphate	($MnSO_4.H_2O$)	225 mg
ferric citrate	(16.7% Fe)	217.5 mg
copper sulphate	($CuSO_4$)	18.75 mg
zinc carbonate	($ZnCO_3$)	37.5 mg
potassium iodate	(KIO_3)	1.25 mg
making 50 g (= 5% of the final diet).		

3.4.2 *The control diet*

Two control groups were formed, one consisting of obese and one of lean rats of the same strain. These animals were treated similarly to those fed on the semi-synthetic diets, but they were given a commercial ration*) without sucrose, which was the same as that which had been supplied to the other rats before the start of the experiment.

3.5 Blood sampling

Unless indicated otherwise in the following chapters, all blood sampling was performed on rats which had fasted overnight, by orbit puncture under slight ether anaesthesia, with a heparinized capillary glass tube. Usually, the animals resumed eating very soon after this procedure.

3.6 Statistics

The statistical evaluation of the results of most of our experiments were carried out by analysis of variance (R. A. Fisher). When the effect of either dietary fat or sucrose *versus* starch was concerned, a two-way analysis of variance was applied. When the numbers of rats in the various groups were different, corrections to obtain orthogonal polynomials were made.

*) RMH-B, from Hope-Farms Company, Woerden; according to the supplier (H. Morse), this ration contains approximately 22% protein, 6.5% fat, 4.2% crude fibre and traces of cholesterol; metabolizable energy (calculated) approximately 3.3 kcal/g.

If statistically significant differences occurred in this way, possible group effects were further studied by means of „Student's"-test (W. S. Gosset) on the differences between the group means *versus* pooled error variance. For the differences between the lean and the obese rats we restricted to this t-test.

If other methods were used, this will be mentioned at the appropriate places. In general, a special effect was accepted to occur, when the level of significance for such an effect exceeded 95% (p being < 0.05).

3.7 **Discussion**

This discussion will be confined to only one remark. In lean rats an increased plasma cholesterol concentration can only be established after addition to their diet of large quantities of at least 1-2% cholesterol, with preferably a small amount of cholic acid.

In our experiments it was designed to add only a small quantity of cholesterol, in order to reflect the situation in man, who consumes cholesterol with his food in quantities of approximately 200-600 mg per day. On an energy basis this corresponds with an amount of about 0.1% in the diet of these rats. So this was the percentage aimed to be present in the low-fat diets. The percentage of the high-fat diets was adapted to this, on an iso-energetic base.

The figure of 0.1% was the same as that used by a.o. Green et al. (1976) in their studies with guinea pigs and by Corey et al. (1976) in the diets of their monkeys.

Chapter 4

Body weight, food consumption and apparent digestibility

4.1 Introduction

In this chapter the results will be given of body weight gain, food consumption and digestibility of the various diets. The figures deal with rats of initially 39 days old (with a standard error of the mean of 0.5 day, n = 108) given the experimental diets during sixteen weeks.

The rats had been initially screened for age, body weight, and plasma cholesterol and triglyceride concentrations, and five days later had been put on the experimental diets. With a view to the problems of recognition of the obese animals shortly after birth, it was hardly possible, nor necessary for the aim of the study, to start with the diets at any appreciably earlier age of the rats. At the end of this part of the experiments the rats were approximately five months of age.

In all seven series in which the rats were used, blood was drawn — each time after they had fasted overnight at two, four, nine and fifteen weeks from the start of the experimental diets — for the determination of plasma cholesterol, triglycerides, blood glucose and insulin concentrations.

4.2 Methods

Body weights were recorded weekly. Food was provided three times a week, in a weighed quantity, so that food consumption could be calculated, on a weekly basis, from the difference between the quantity of food provided and the quantity left by the rats. Spillage of the food was minimal and was estimated. The diets were prepared once every month. The diets were provided *ad libitum*, except to groups V and VI during several weeks (see below).

In order to determine the apparent digestibility of the diets, we collected the faeces of all rats during four days of the twelfth week of the experiment. The faecal contents were pooled per group and assayed for crude protein and fat by the colorimetric method of Koops et al. (1975) and by the method of Roese-Gottlieb (Horwitz 1970) respectively.

4.3 Results

4.3.1 *Body weight*

Table 7 shows that the body weight of the obese rats at the start of the investigation was approximately 100 g on average, whereas that of the lean rats was somewhat lower.

Sixteen weeks later, at the end of part one of the experiments, the lean rats had a mean body weight of just below 300 g. The body weights of the obese rats had increased to approximately 450 g in groeps I, III, IV, V and VI, whereas in groups II and VII they appeared to be somewhat lower. Body weight curves are given in Figure 2.

4.3.2 *Food consumption*

From the recorded figures of the food consumption of all rats in this part of the study, the mean consumption for all eight groups was calculated. These figures, together with their standard errors of the mean are also given in Table 7.

Table 7. Mean body weights and food consumption of rats in eight groups during the first part of the study (in g ± s.e.m.*).

group	number	at the start	sixteen weeks later	food intake (g per week)
I	12	100 ± 8	463 ± 8	144 ± 2
II	12	97 ± 9	417 ± 10	145 ± 3
III	13	97 ± 7	456 ± 12	141 ± 3
IV	13	102 ± 6	452 ± 14	141 ± 4
V	12	101 ± 5	459 ± 8	116 ± 1
VI	12	104 ± 5	452 ± 6	117 ± 1
VII	13	96 ± 7	433 ± 10	157 ± 3
VIII	12	90 ± 5	295 ± 4	106 ± 2

*) Standard error of the mean.

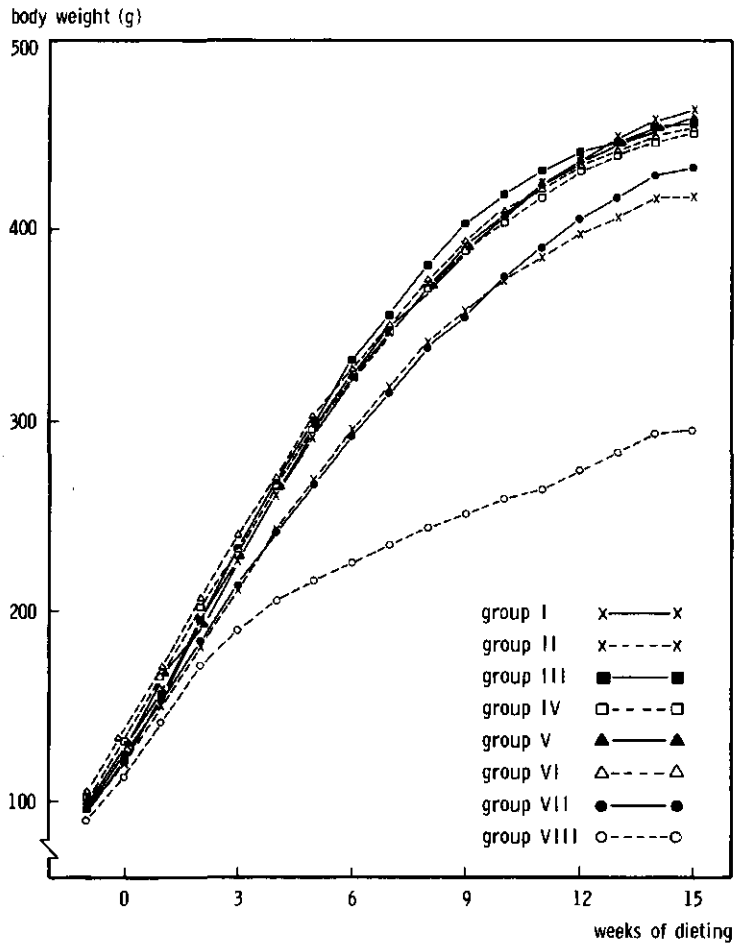

Figure 2. Body weight curves of the eight groups of Zucker rats (cf. Table 7).

It can be seen that the obese control rats ate about 50% more than the lean rats and that the food intake of the rats fed on the high-poly-unsaturated-fat diets was lower. This was due firstly to the higher digestible energy of these diets (see below) and secondly to the restriction of the food intake which was applied in these groups in order to obtain similar body weights, as had been foreseen to be necessary (see Chapter 3.4.1, the semi-synthetic diets).

Sucrose in the diets substituted for starch had no influence on the quantity of food eaten.

4.3.3 *Apparent digestibility*

During four days of the twelfth week of the experiment the faeces of all rats in the various groups were collected. The faecal contents were pooled per group and assayed for fat and crude protein. The results of these measurements, together with relevant indices for food consumption, are given in Table 8.

The digestibility mentioned is called apparent because it is based on determinations of faecal losses of protein and fat without considering whether their sources are endogenous or not. Such endogenous losses may originate from secretion products, either biliary or from the intestinal wall, from desquamated cells or from microbial material in the gut. When endogenous losses are taken into account, the digestibility is called true digestibility. (This can be determined only when special techniques, such as labelling techniques, are used for measuring the endogenous faecal losses.)

Because of the restricted precision of measured and calculated data it was decided to give the apparent digestibility for fat and for protein as a combined percentage. From the other figures in the table it can be concluded to what extent losses must be attributed to a restricted capacity for the digestion of either fat or protein.

Table 8. Apparent digestibility of the various experimental diets.

group	pooled faeces (g/day)	fat content (%)	protein content (%)	faecal energy losses per day (kcalories)			energy*) intake (kcalories per day)	apparent digestibility for fat and protein(%)
				fat	protein	total		
I	2.6	5	13	1.3	1.3	2.6	70	96
II	5.0	5	16	2.1	3.2	5.3	70	92
III	4.6	28	15	11.6	2.7	14.3	81	82
IV	6.1	28	7	15.3	1.6	16.9	81	79
V	3.2	6	10	1.7	1.3	3.0	66	96
VI	3.9	6	1	1.9	0.2	2.1	67	97
VII	6.8	9	26	5.6	7.0	12.6	74	83
VIII	5.6	8	2	4.1	0.4	4.5	50	91

*) Food consumption (g/day) × metabolizable energy (kcal/g).

Table 8 shows higher figures for the fat content in the faeces in groups III and IV (fed on the high-saturated-fat diets), and for the faecal protein content in group VII (the group of obese rats fed on the commercial ration). From these higher figures, lower values were calculated for the apparent digestibility in these groups than in the other groups, which showed calculated values for this laying in the normal range.

4.4 Discussion

4.4.1 *Body weight*

The lean rats, fed on the commercial ration, naturally gained much less weight than did the obese ones.

If it is assumed that the body fat content of the lean rats is approximately 10% (Bray et al. 1973, Farkas et al. 1973, Deb & Martin 1975, Jenkins & Hershberger 1978) and that the fat-free mass of lean and obese rats fed *ad libitum* on a balanced diet is approximately equal, the body fat content of the obese rats can be calculated to be approximately 40%. These figures correspond well with those given by Bray et al. (1973). In fact, the latter supposition is not completely justified, because it has only full validity for female Zucker rats (Radcliffe 1977a).

It was expected that the groups of rats fed on the high-fat diets, when fed to appetite, would gain weight more rapidly than would the groups on the low-fat rations. This expectation was realised only as regards the high-poly-unsaturated-fat diets and not as regards the high-saturated-fat ones (groups III and IV). This will correspond with the steatorrhoea that occurred in groups III and IV (Table 8: second and last columns).

The groups V and VI could be kept on average at the same weight gain as groups I, III and IV by a simple restrictive measure as indicated above. The smaller weight gain in group II may have to do with the occurrence of pronounced fatty livers in this group, which will be discussed in Chapter 9.

The mean weight of the rats in group II at the end of the first part of the study was not much different from that of the rats in group VII which had been fed on the commercial ration. The composition of the latter diet, however, was completely different from that of the semi-synthetic diets.

It may also be mentioned that the sucrose-fed groups of rats did not show higher weight gains than those fed on starch, contrary to what has been described in man (Szanto & Yudkin 1969). The finding of these investigators may have been due to the difference in degree of purity of sucrose and starch, which was not taken into account. On the contrary, rats fed on low-fat diets containing very high proportions of sucrose, such as were given to our group II, are known to show lower weight gains than those fed on diets rich in starch (Al-Nagdy et al. 1970).

4.4.2 *Food consumption*

The consumption of the commercial diet by the lean rats was, according to their smaller weight gain, approximately 50% lower than that by the obese rats on this diet. This confirms earlier findings (Bray & York 1972, Pullar & Webster 1974).

The difference between the intake of the low-fat diets (groups I and II) and the high-saturated-fat diets with a higher energy density (groups III and IV) was strikingly small. The body weights, however, of groups I, III and IV were equal. This energetic discrepancy will be due to the saturated fat being poorly absorbed by the intestines.

The mean energy consumption of the low-fat diets by rats in groups I and II and that of the high-poly-unsaturated-fat diets by rats in groups V and VI was not the same, as had been intended when the diets were designed. The expected intake of the high-fat diets — approximately 85% of that of the low-fat diets — turned out to be approximately 81%, including the dietary restriction applied, so that the intake of the nutrients other than fat will not have been exactly the same.

The intake of the high-poly-unsaturated-fat diets was about 83% of that of the high-saturated-fat diets, so that fairly considerable differences in the intake of all nutrients will have occurred between these groups. Our observation was in disagreement with that of Comai et al. (1978), who did not find that the food intake of a diet with

hydrogenated (soybean) oil differed from that of a diet with corn oil in obese Zucker rats.

The proportional intake of the commercial ration related to that of the semi-synthetic diets is less deserving of mention, because of the clearly different composition of the former diet.

4.4.3 *Apparent digestibility*

With respect to the apparent digestibility of the diets used in this study two striking features can be observed:

a. The first is the decreased apparent digestibility for protein of the obese animals in group VII fed on the commercial ration. This decrease was not found in the lean rats fed on the same diet (nor in the groups fed on the semi-synthetic diets). This finding was at variance with the observations of Pullar & Webster (1974), who reported similar digestibility in obese and lean Zucker rats as an explanation of the obesity of the former.

As our result was just a single observation on the basis of pooled faeces which had been collected in one limited period of four days during the course of an experiment of several months of duration, we repeated the determination with four individual male obese rats of approximately four months of age. All of these rats showed a crude protein content in the faeces, which had been collected for four days, of 21%, which largely confirms our data in Table 8.

We reinvestigated also a pooled faecal sample of four lean rats, of about four months of age, collected over four days, and this time obtained a figure for the protein content of the faeces of approximately 20%. This will indicate that our initial observation was incorrect, and that obese and lean Zucker rats have similar abilities for protein digestion, according to the observations of Pullar & Webster (1974) and Radcliffe & Webster (1978). The digestibility of well-tolerated semi-synthetic diets will be slightly higher, because of the smaller losses of crude protein under these conditions.

b. The second feature of interest inferred from Table 8 is that the faecal fat contents were very high in groups III and IV, being the groups fed on the high-saturated-fat diets. This fat was not of animal origin, but was composed of two parts of cocoa butter and one part of palm oil.

On the one hand, it is known from the literature that saturated fatty acids from dietary fats are absorbed to a somewhat lesser degree than are poly-unsaturated fatty acids (Deuel 1955, Carroll 1958, Ockner et al. 1972, Mansbach 1976, Clark et al. 1977); nevertheless, under healthy conditions these differences are small and regarded as of no practical significance. Triscari et al. (1978) report a lower absorption of stearate (65%) than of oleate and linoleate (86% and 84% respectively) in female Charles River rats.

On the other hand, the mixture of saturated fat used in these experiments, which seemed favourable with regard to its fatty acid composition in comparison with other types of fat given to the other groups of obese rats, will have had a composition different from that of natural animal fats. These differences might concern the positioning of the fatty acids to glycerol such as occur in different types of fat.

Such differences, for example, are observed in pork fat with regard to palmitic acid, which occupies predominantly the 2-position of the glycerol moiety of the fat, in contrast with other fats and oils, which have their palmitic acid mainly at the outer positions 1 and 3 of glycerol (Kuksis 1972). It may well be that variations of this nature occur in the positioning of saturated or mono-unsaturated fatty acids in either animal fats or cocoa butter and palm oil.

4.4.4 *Concluding remarks*

For the study in general it is important to raise the question whether the groups of rats fed on the high-saturated-fat diets, which showed steatorrhoea may be considered as to have actually used a high-fat diet.

A first approach to answering this question is the calculation of the proportion of fat absorbed, from the measurements of the faecal fat content (Table 8) and the food consumption data (Table 7), together with the figures for the dietary composition. In this way a net absorption of the dietary fat can be calculated of 44%. This would come down to a fat energy percentage from the food absorbed of approximately 17. This figure is about twice as high as the fat energy percentage of the low-fat diets calculated (Table 2) and 1.5 times as high as the one actually measured (Table 3).

The conclusion that the saturated-fat diets could be really considered metabolically as high-fat diets is confirmed by a second approach regarding the clear differences in the blood lipid levels between the groups fed on these diets and those fed on the low-fat diets, particularly with respect to triglycerides (see Chapter 5).

With the exception of the two features mentioned above, the values for the apparent digestibility of the Zucker rats were normal (approximately 95%).

4.5 Summary

1. The body weight curves of the groups of obese Zucker rats fed on various diets during fifteen weeks (from on average the 7th to the 22nd week of life) were similar, with the exception of the somewhat lower figures for the group fed on the commercial ration and the group fed on the low-fat diet with sucrose.
2. In order to obtain this similarity in body weight gain, the food consumption of the animals fed on the high-poly-unsaturated-fat diets had to be restricted somewhat, as had been expected. The rats fed on the high-saturated-fat diets (cocoa butter/palm oil 2 : 1), however, showed a considerable degree of steatorrhoea.
3. The rats fed on the commercial ration had somewhat lower figures for protein digestion or absorption than those fed on the semi-synthetic diets.

Chapter 5

Plasma cholesterol, plasma triglyceride, blood glucose and plasma insulin concentrations

5.1 Introduction

In this chapter the results regarding the influence of the type of diet on the plasma cholesterol, triglyceride, blood glucose and insulin concentrations of male, obese Zucker rats will be presented. Blood was drawn for the first time at the age of the animals of 39 days ($\pm$ 0.5 day s.e.m., n = 108). The blood was assayed to randomize the rats into groups on the basis of age, body weight and blood lipid levels. Five days later the rats were offered their respective experimental diets. The blood determinations of cholesterol, triglycerides and insulin were carried out with heparinized plasma — glucose was determined in whole blood — taken at exactly two, four, nine and fifteen weeks after the feeding on the experimental diets had started. As significant changes in cholesterol concentrations of the plasma were expected during the first period of the study, a rather high frequency of blood sampling was chosen in the first weeks. The results of these determinations will be given in this chapter.

5.2 Methods

Plasma cholesterol was measured by an enzymatic method (Boehringer, Mannheim: Biochemica test combination). After saponification of the cholesteryl esters with cholesterol esterase, cholesterol is oxidized with cholesterol oxidase. By this reaction hydrogen peroxide is formed which, in the presence of catalase, transforms methanol into methanal. The latter compound then is converted with ammonia and acetylacetone into a lutidine (Hantzch condensation) which is measured colorimetrically at 405 nm.

Plasma triglycerides were measured by the method of Soloni (1971) with some minor modifications. After extraction of the triglycerides, sodium ethoxide in isopropanol is added, and the mixture is incubated for transesterification. The glycerol formed is extracted with sulphuric acid and chloroform. The liberated glycerol then is oxidized to methanal with sodium perjodate, and sodium arsenite is added to cope with the latter. From the formation of methanal onwards, the method is identical to that used for cholesterol. Because of the very high values which could be detected in the plasma of the obese Zucker rats, in several cases a suitable dilution of the samples had to be applied. The plasma samples often had a cloudy or even creamy appearance, although the rats had been in the fasting state after their food had been removed in the afternoon, at approximately 4.30 p.m., of the day before the blood was sampled, which took place at approximately 9 a.m..

Blood glucose was measured with the hexokinase method (Boehringer, Mannheim) and plasma insulin with a double antibody radio-immune assay system (Radiochemical Centre Amersham, Buckinghamshire, England), as described by Midgley et al. (1969).

The statistical analysis of the results of these experiments was carried out by two-way analysis of variance, as has already been mentioned in Chapter 3. With regard to the normal distribution of the figures obtained there was doubt in the case of the plasma triglyceride and the insulin concentrations; to these parameters, therefore, logarithmic

transformation was applied. When the original figures for these quantities are given, with standard errors of the mean, they are meant merely to give an idea of their variability.

5.3 Results

5.3.1 *Plasma cholesterol*

The results of the cholesterol determinations are given, together with standard errors of the mean, in Table 9 and, without any indication of their variability, in Figure 3.

Table 10 contains the results of the statistical analysis relating to the effects of the semi-synthetic diets, by two-way analysis of variance and subsequent t-tests for significant group effects; the levels of significance are also given for all four times of blood sampling. There was, in the course of time, a slight decrease in the number of observations within the groups, because some rats died in the meantime, mostly as a result of the blood sampling under anaesthesia. These observations were included in the calculations of which the results are contained in this table, but excluded from those for Table 9.

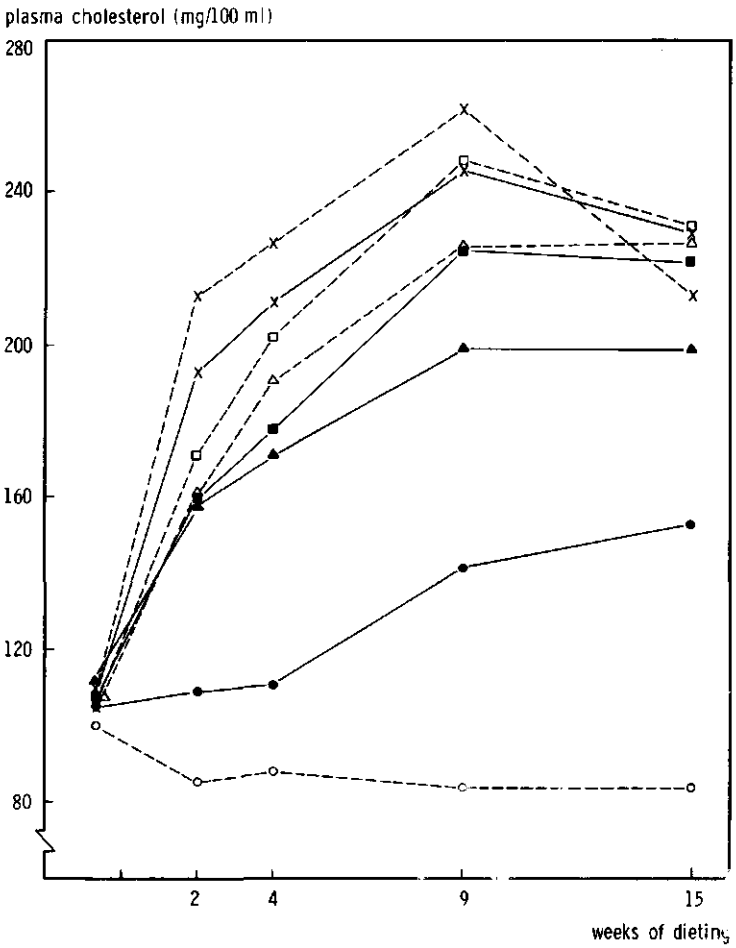

Figure 3. Mean plasma cholesterol concentrations of the groups of rats during 15 weeks of dieting (cf. Table 9). For symbols see Fig. 2 (page 25).

Table 9. Mean plasma cholesterol concentrations of the groups of rats on the various diets in the course of the first part of the study (in mg per 100 ml ± s.e.m.*).

group	number	at the start	after 2 weeks	after 4 weeks	after 9 weeks	after 15 weeks
I	12	108 ± 6	193 ± 5	211 ± 5	246 ± 8	230 ± 9
II	12	110 ± 4	213 ± 9	227 ± 9	262 ± 6	213 ± 8
III	13	108 ± 3	160 ± 5	178 ± 9	225 ± 11	222 ± 9
IV	13	107 ± 5	171 ± 7	202 ± 6	248 ± 10	232 ± 8
V	12	112 ± 4	158 ± 7	171 ± 5	200 ± 8	199 ± 8
VI	12	107 ± 5	161 ± 10	191 ± 8	226 ± 10	219 ± 11
VII	13	105 ± 5	109 ± 4	111 ± 4	142 ± 10	153 ± 10
VIII	12	100 ± 3	85 ± 2	88 ± 5	84 ± 3	84 ± 2

*) s.e.m.: standard error of the mean.

Table 10. Levels of significance (probabilities) from corrected orthogonal polynomials in two-way analysis of variance, followed by Student's *t*-test, for contrasts between plasma cholesterol concentrations, of obese Zucker rats fed on semi-synthetic diets, after 2, 4, 9 and 15 weeks.

		F-values							
treatment	dimension	2 weeks	p	4 weeks	p	9 weeks	p	15 weeks	p
dietary fat (A)	2	18.83	<0.001	10.90	<0.001	10.66	<0.001	2.53	<0.10
sucrose *vs* starch (B)	1	3.27	<0.10	12.22	<0.001	9.18	<0.005	0.81	n.s.
A × B (interaction)	2	0.43	n.s.	0.22	n.s.	0.21	n.s.	1.09	n.s.
variance (s^2)	d	61.0 (d = 74)		75.6 (d = 70)		100.4 (d = 70)		95.2 (d = 68)	
		t-values							
low *vs* high-saturated fat		5.03 (t_{52})	<0.001*)	3.05 (t_{48})	<0.005	1.91 (t_{48})	<0.10	0.02 (t_{48})	n.s.
low *vs* high-poly-unsaturated fat		5.72 (t_{50})	<0.001	4.58 (t_{48})	<0.001	4.62 (t_{48})	<0.001	1.95 (t_{46})	<0.10
saturated *vs* poly-unsaturated fat		0.67 (t_{52})	n.s.	1.58 (t_{50})	n.s.	2.74 (t_{50})	<0.01	1.95 (t_{48})	<0.10

*) two-sided n.s.: not significant

The plasma cholesterol concentrations of the obese rats fed on the commercial ration increased gradually to a level, which was significantly higher than that of the lean controls.

From the first weeks of the experiment onwards the obese rats fed on the semi-synthetic diets had higher plasma cholesterol concentrations than those fed on the control diet. During the last weeks of the experiments there appeared to be a decreasing tendency for the plasma cholesterol levels of the rats fed on the semi-synthetic diets.

At nine weeks of the experiment the highest mean cholesterol concentrations were observed in the plasma of the groups fed on the low-fat diets (groups I and II), followed in ranking by those of the groups fed on the high-saturated-fat diets (groups III and IV), the high-poly-unsaturated-fat diets (groups V and VI) and the obese control rats on the commercial ration. From Table 10 it can be seen that the influence of dietary fat was very significant, in particular during the „dynamic" phase, i.e. the first few weeks. The lower part of the table indicates the differences between the respective types of fat used in the semi-synthetic diets. It can be concluded from these data that the differences between the low-fat and the high-fat diets were significant during the dynamic phase.

It was found that, particularly from the time of four weeks after the commencement of the experimental diets, substitution of sucrose in the diets for starch significantly increased the mean plasma cholesterol concentrations (Table 10). This effect of sucrose, however, became significant somewhat later than that of the dietary fat used, and was of a shorter duration; the differences were found to decrease between the ninth and the fifteenth week.

Interaction between the effects of dietary fat and sucrose was absent.

At the highest levels measured, after the duration of the experiment of nine weeks, the plasma cholesterol concentrations of groups I and IV and those of groups III and VI were almost equal. This suggests that, at that time, the increase of the plasma cholesterol levels as the result of the presence of sucrose in the diets was about compensated for by the differences as the result from the type of dietary fat.

The only apparent deviation in the over-all pattern of the plasma cholesterol values occurred during the last weeks in group II; the rats in this group did not gain as much weight as those in the other groups fed on the semi-synthetic diets.

5.3.2 *Plasma triglycerides*

On all the blood samples taken in the first part of the investigation the triglyceride concentration of the plasma was determined.

The results are given, together with the standard errors of the mean, in Table 11 and, without the latter figures, in the form of a graph in Figure 4.

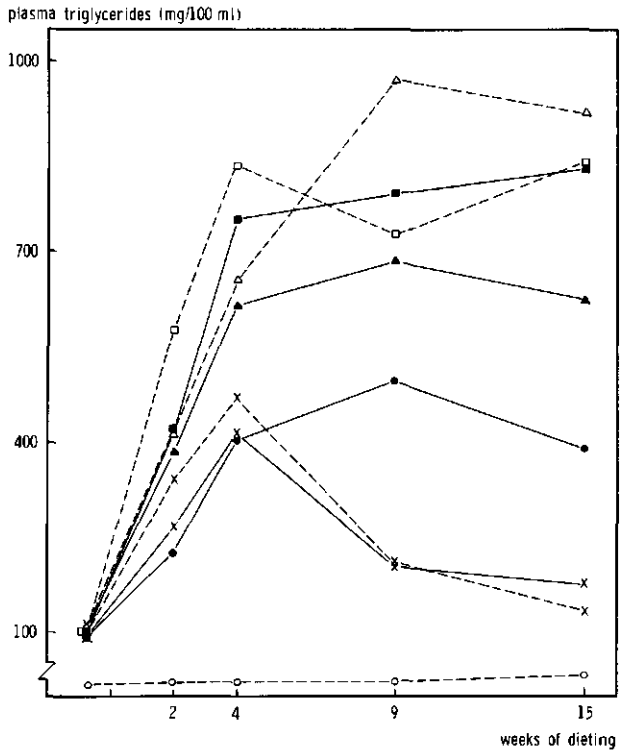

Figure 4. Mean plasma triglyceride concentrations of the groups of rats during 15 weeks of dieting (cf. Table 11). For symbols see Fig. 2 (page 25).

For statistical analysis, logarithmic transformation of these figures was applied, because of their apparently abnormal distribution. The results of this analysis are given in Table 12. (The number of observations, again, decreased slightly in the course of the study, because some rats died.) The large variability of these figures may be due in part to different time lags between the last food intake of the rats and the blood sampling: some rats may just have eaten before the food was removed on the day prior to blood collection, and others may have not.

Table 11. Mean plasma triglyceride concentrations of the groups of rats on the various diets during the first part of the study (in mg per 100 ml ± s.e.m.*).

group	number	at the start	after 2 weeks	after 4 weeks	after 9 weeks	after 15 weeks
I	12	94 ± 12	269 ± 25	416 ± 38	204 ± 23	117 ± 20
II	12	103 ± 20	338 ± 58	471 ± 58	207 ± 47	134 ± 20
III	13	97 ± 16	420 ± 50	750 ± 98	791 ± 110	830 ± 144
IV	13	97 ± 15	574 ± 41	835 ± 76	727 ± 102	838 ± 135
V	12	96 ± 10	386 ± 35	617 ± 93	685 ± 75	623 ± 82
VI	12	99 ± 12	417 ± 52	656 ± 91	970 ± 142	916 ± 133
VII	13	94 ± 11	225 ± 25	403 ± 46	495 ± 51	389 ± 57
VIII	12	18 ± 2	21 ± 2	23 ± 3	21 ± 2	32 ± 3

*) s.e.m.: standard error of the mean.

Table 12. Levels of significance (probabilities) from corrected orthogonal polynomials in two-way analysis of variance, followed by Student's *t*-test, for contrasts between plasma triglyceride concentrations (logarithmically transformed) of obese Zucker rats fed on semi-synthetic diets, after 2, 4, 9 and 15 weeks.

		F-values							
treatment	dimension	2 weeks	p	4 weeks	p	9 weeks	p	15 weeks	p
dietary fat (A)	2	13.39	<0.001	8.27	<0.001	21.13	<0.001	68.28	<0.001
sucrose *vs* starch (B)	1	8.79	<0.005	0.65	n.s.	0.13	n.s.	0.00	n.s.
A × B (interaction)	2	0.21	n.s.	0.01	n.s.	0.24	n.s.	1.55	n.s.
variance (s^2)	d	0.03 (d = 74)		0.05 (d = 70)		0.13 (d = 70)		0.06 (d = 68)	
		t-values							
low *vs* high-saturated fat		4.60 (t_{52})	<0.001*)	4.17 (t_{48})	<0.001	5.20 (t_{48})	<0.001	10.00 (t_{48})	<0.001
low *vs* high-poly-unsaturated fat		3.40 (t_{50})	<0.005	2.50 (t_{48})	<0.005	6.20 (t_{48})	<0.001	10.14 (t_{46})	<0.001
saturated *vs* poly-unsaturated fat		1.00 (t_{52})	n.s.	1.67 (t_{50})	<0.10	−1.00 (t_{50})	n.s.	−0.14 (t_{48})	n.s.

*) two-sided.
n.s.: not significant.

As can be seen from Table 11, the obese rats already at the start of the experimental diets had plasma triglyceride concentrations which were approximately five times higher than those of the lean rats.

The plasma triglyceride levels of all groups of obese rats had increased after they had been fed for two weeks on their respective diets, particularly on the high-saturated-fat diets. In the animals fed on the low-fat diets the rise in the plasma triglyceride concen-

trations was lower. After four weeks these concentrations of the groups fed on the low-fat diets (also on the commercial ration) had further increased, but they were still very significantly lower than those of the groups fed on the high-fat diets. The mean triglyceride levels in the plasma of rats on the low-fat diets decreased significantly after four weeks, in contrast to those of rats fed on the control diet which were fairly constant after that time.

No significant difference was found between the effects of saturated and poly-unsaturated fat.

In the first weeks of the experiment, sucrose in the diet had a significantly increasing effect on the plasma triglyceride concentrations. From the time of four weeks onwards this effect had disappeared.

There was no interaction between the effect of dietary fat and sucrose.

The over-all picture of Table 12 is more or less similar to that of Table 10: the influence of dietary fat dominates that of the type of carbohydrate. The dietary effect (lower part of the Table) is exerted by its quantity rather than by its quality. In this case the difference between the high-fat diets was at no occasion significant. The effect of sucrose was statistically significant only after two weeks.

5.3.3 *Blood glucose*

Table 13 shows the results of the determinations of the glucose concentrations, after one night of fasting, together with their standard errors of the mean. The means are graphically presented in Figure 5.

Table 13. Mean blood glucose concentrations (mg/100 ml) with standard errors of the mean, of the groups of rats fed on the various diets.

group	number	at the start	after 2 weeks	after 4 weeks	after 9 weeks	after 15 weeks
I	12	90 ± 4	84 ± 6	86 ± 4	74 ± 5	79 ± 3
II	12	75 ± 6	95 ± 7	83 ± 4	62 ± 4	76 ± 4
III	13	78 ± 6	88 ± 7	85 ± 4	86 ± 2	88 ± 3
IV	13	81 ± 4	100 ± 9	95 ± 5	84 ± 5	86 ± 3
V	12	76 ± 4	108 ± 8	106 ± 8	93 ± 4	90 ± 2
VI	12	82 ± 6	114 ± 6	110 ± 7	108 ± 7	101 ± 4
VII	13	83 ± 3	94 ± 4	95 ± 4	85 ± 3	90 ± 3
VIII	12	68 ± 5	61 ± 5	60 ± 5	78 ± 7	79 ± 2

The most important conclusions that can be drawn from these figures are the following:

a. The lean rats had blood glucose concentrations that were significantly lower than those of the obese rats fed on the same diet ($p<0.001$).

b. A statistically significant elevation of the blood glucose concentration as an effect of the type of dietary fat was observed between the groups V and VI (fed on the high-poly-unsaturated-fat diets) and the first four groups ($p<0.001$, after 4, 9 and 15 weeks). This may be connected with the higher fat content and utilization of the diets in the former groups, as will be discussed more extensively later in this chapter.
There were no significant differences in blood glucose levels between the groups fed on the high-saturated-fat and the low-fat diets.

c. The differences between the groups fed on either sucrose or starch were not statistically significant. There was no interaction between dietary fat and sugar.

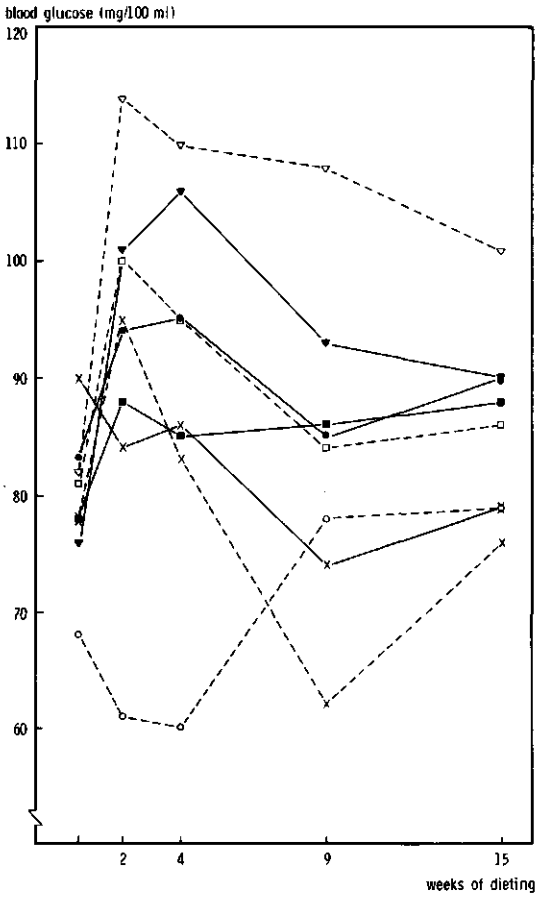

Figure 5. Mean blood glucose concentrations of the groups of rats during 15 weeks of dieting (cf. Table 13). For symbols see Fig. 2 (page 25).

5.3.4 *Plasma insulin*

The results of the measurements of the plasma insulin levels are shown in Table 14, together with the standard errors of the means and the number of observations. The group means are also presented graphically in Figure 6. A few samples which showed haemolysis were discarded, because this was known to give very high values. Of the samples that were taken before the diets were started only a restricted, randomized number was assayed, because of the large total number of samples drawn. They had been derived from obese as well as from lean rats.

Statistical analysis of these data was performed after logarithmic transformation, because there was doubt as to their normal distribution. Despite the very large variability of the plasma insulin levels, some significant differences could be observed. The main conclusions are:

a. the lean rats had statistically significant lower plasma insulin levels than the obese rats on the control diet ($p<0.05$);

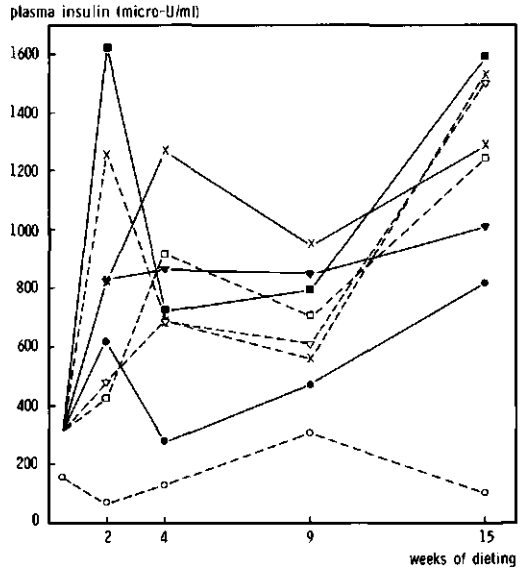

Figure 6. Mean plasma insulin levels of the groups of rats during 15 weeks of dieting (cf. Table 14). For symbols see Fig. 2 (page 25).

Table 14. Mean plasma insulin levels of the groups of rats fed on the various diets, in micro-U/ml, with standard errors of the mean, the number of observations is given between brackets.

group	at the start	after 2 weeks	after 4 weeks	after 9 weeks	after 15 weeks
I		830 ± 224 (13)	1271 ± 163 (13)	948 ± 127 (12)	1289 ± 228 (10)
II		1260 ± 241 (13)	693 ± 167 (11)	566 ± 47 (12)	1531 ± 226 (12)
III		1617 ± 411 (11)	724 ± 230 (11)	798 ± 138 (10)	1593 ± 260 (10)
IV	310 ± 180 (15)	426 ± 141 (14)	915 ± 186 (13)	707 ± 113 (10)	1245 ± 274 (12)
V		830 ± 226 (12)	866 ± 165 (13)	848 ± 182 (11)	1014 ± 185 (11)
VI		483 ± 90 (13)	692 ± 158 (13)	608 ± 80 (12)	1503 ± 239 (11)
VII		620 ± 304 (13)	276 ± 49 (13)	474 ± 104 (12)	818 ± 139 (12)
VIII	156 ± 107 (5)	70 ± 29 (14)	130 ± 80 (12)	309 ± 106 (13)	100 ± 49 (9)

b. in the groups fed on semi-synthetic diets no significant differences were found, neither between the low-fat and the high-fat diets, the saturated- and the poly-unsaturated-fat diets nor between the sucrose containing and the starch containing diets;

c. the obese rats fed on the commercial ration (group VII) showed a tendency to lower plasma insulin levels than those fed on the semi-synthetic diets (groups I-VI).

5.4 Discussion

5.4.1 *Cholesterol and triglycerides*

Obese Zucker rats appeared to have elevated plasma cholesterol and triglyceride concentrations.

It was already known from the literature that plasma triglyceride levels of obese Zucker rats on high-fat diets were higher than of these rats on low-fat diets (Schonfeld & Pfleger 1971, Lemonnier et al. 1974), in contrast to what occurs in most other animal species, including man (Nikkilä 1969, Nestel et al. 1970, Ginsberg et al. 1976). Hunt et al. (1976) agreed that this hyperlipidaemia is of dietary origin.

It may consequently be regarded as a striking feature that the low-fat semi-synthetic diets gave the highest cholesterol levels in the plasma of obese Zucker rats, at their maximal values measured*), but the lowest triglyceride levels. This may also be important from the viewpoint of the lipoprotein patterns of these rats, to be discussed directly.

As far as the timing of the effects from the various diets is concerned, the influence of sucrose in the diet on the triglycerides apparently precedes that of sucrose on the cholesterol levels of the plasma. This effect on the triglycerides also appears to decrease earlier than that on cholesterol (Tables 10 and 12).

These features are fully in line with the conclusions reached by Nestel & Goldrick (1976), which imply that a gradual change of lipoproteins in the blood is occurring from very low-density lipoproteins (VLDL) to low-density lipoproteins (LDL).

However, the lipoprotein patterns in the blood of conventional rats (Lasser et al. 1973) and of Zucker rats (Schonfeld et al. 1974) differ from those of man. Also the turnover of VLDL in rats is different from that in man (the half-life time of VLDL in rats is 4 to 5 days, whereas it is approximately 10 days in man). In particular, rats have only small proportions of LDL. Cholesterol from LDL, however, will be gradually transferred to HDL.

The tendency of the plasma cholesterol levels to decrease after the ninth week of the experiment will be related to the end of the „dynamic phase" of the obesity of the rats at the age of approximately 16 weeks (York & Bray 1973a) rather than to the transient character of this dietary effect. In man, the effect of dietary fat is accepted to be already fully clear after two weeks and lasting (Anderson 1963 and 1976), whereas that of sucrose is transient (Harper et al. 1953, Bender a Damji 1972).

5.4.2 *Glucose and insulin*

The significantly higher glucose concentrations in the blood of the obese controls compared with those of their lean litter-mates indicate that the obese Zucker rats are in a slight but definite diabetic state, which is in their case combined with hyperinsulinism and insulin resistance. In the literature, blood glucose levels of obese Zucker rats laying in the normal range were often reported (Zucker & Zucker 1962, Zucker & Antoniades 1972, Stern et al. 1972, Lemonnier et al. 1974). However, York et al. (1972a) mentioned the occurrence of hyperglycaemia in four months old obese Zucker rats on a low-fat stock diet. Also Comai et al. (1978) and Martin et al. (1978) found higher blood glucose levels in obese than in lean Zucker rats, at an age of over 5 months.

The tendency for the diabetic state to diminish and to normalize after the obese rats were approximately three months of age has already been mentioned (York & Bray 1973a, Herberg & Coleman 1977). Although the results of the blood glucose concentrations in this study do not contradict with this view, we are left with an increase of the

*) According to Comai et al. (1978), the plasma cholesterol values of the groups III and IV (fed on the high-saturated-fat diets, Table 9) may have been suppressed by the lower fat absorption on these diets (see Section 4.3.3 and Table 8 in the preceding chapter, and *confer* with Table 17 in Chapter 6, Table 22 in Chapter 7 and Table 27 in Chapter 8).

plasma insulin levels in all groups of obese rats between nine and fifteen weeks from the start of the experiment (corresponding with an age of approximately 3½ to 5 months), which contrasts to this tendency to amelioration of the diabetic state. Possibly the high fat content of the plasma samples was responsible for the very high figures of the insulin levels measured.

A striking feature was that the groups of obese Zucker rats fed on the high-poly-unsaturated-fat diets had significantly higher blood glucose concentrations. York (1975b) reported, from a study concerning 7 to 8 week old Bar Harbor mice, that a high-fat diet (72 energy % corn oil) reduces both blood glucose and plasma insulin levels in obese as well as in lean mice and increases the free fatty acid concentration of the blood. Lemonnier et al. (1974) observed, in Zucker rats fed on high-fat diets (72 energy % lard), lower blood glucose and plasma insulin concentrations than in Zucker rats fed on low-fat diets, but similar blood glucose levels in obese and lean rats. Their animals had been fed on their experimental diets for 7 months, from the age of 5 months onwards. Houtsmuller (1975) found that blood glucose levels in obese diabetics who did not require insulin supply, were lower when they were fed on a poly-unsaturated-fat diet than on a saturated-fat regimen.

York (1975b) observed, when obese and lean Bar Harbor mice fed on a high-fat diet were compared with similar mice fed on a lower-fat diet, a reduced utilization of glucose for conversion into fatty acids and oxidation to CO_2, in adipose tissue. The little importance of glucose as a substrate for lipogenesis, even when the diet is very rich in carbohydrates, was recently mentioned, also with regard to Zucker rats, by Bloxham et al. (1977).

Our rats in the groups V and VI, which showed the definitely higher blood glucose concentrations than those in the other groups, had been somewhat restricted in their food intake, in order to obtain similar body weight gains as the rats in most of the other groups (see Chapter 4), but they were in excellent condition, with regard to fat absorption (Table 8) as well as to their hepatic lipid contents when these were measured ultimately (see Chapter 9). These groups had no lower plasma insulin levels than the other groups fed on semi-synthetic diets. They all seem to have very high, possibly maximally stimulated insulin levels (York 1975b, Czech et al. 1977).

As a final remark it may be mentioned that the plasma insulin levels of rats that had fasted overnight showed a slight tendency to be somewhat lower when the animals were fed on sucrose-containing diets than when fed on diets with starch. This would agree with the observations made by Mann et al. (1971) and Mann & Truswell (1972) in man.

5.5 Summary

1. From eight groups of male Zucker rats, which initially were approximately six weeks of age and which were fed on different diets, blood was drawn after 2, 4, 9 and 15 weeks, and assayed for cholesterol, triglyceride, glucose and insulin concentrations.
2. Obese rats compared to their lean litter-mates showed overt hyperlipidaemia. The obese rats fed on the control diet had significantly higher plasma cholesterol levels than the lean rats from the age of approximately 8 weeks onwards.

   Consumption of low-fat diets was accompanied by high plasma cholesterol levels. These were significantly higher than those attained with saturated-fat diets after 2 and 4 weeks of dieting ($p<0.001$), but they tended to decrease between the 9th and 15th week.

   The high-poly-unsaturated-fat diets gave significantly lower plasma cholesterol concentrations than the low-fat diets at 2, 4 and 9 weeks of the experiment ($p<0.001$) and, only at 9 weeks, than the high-saturated-fat diets ($p<0.01$).

Significantly still lower levels, however, were found in obese rats fed on a commercial ration.

3. Plasma triglyceride concentrations of obese Zucker rats were many times higher than those of lean rats, and were very significantly higher in rats fed on high-fat diets than in rats fed on low-fat diets ($p<0.001$).

   The influence of a large quantity of linoleic acid in the high-fat diets was not significant in this respect.

4. Sucrose in the diets resulted, after 2 weeks of the experiment, in significantly higher plasma triglyceride levels than did starch ($p<0.005$); at 4 weeks this effect had disappeared.

   Somewhat later than the rise in the triglyceride levels, at 4 and 9 weeks of the experiment, a significant rise in plasma cholesterol concentration was seen in the rats fed on the sucrose-containing diets in comparison with those fed on starch ($p<0.005$); at 15 weeks from the start of the experiment this difference had disappeared.

5. Obese Zucker rats have slightly but, already at the age of six weeks, significantly higher blood glucose levels than lean rats ($p<0.001$).

   The high-poly-unsaturated-fat diets gave higher blood glucose concentrations than low-fat and high-saturated-fat diets ($p<0.001$). This will be related to, respectively, the lower availability and utilization of the fat in the latter types of diet.

   The presence of sucrose in the diets had no effect on the blood glucose concentrations.

6. Plasma insulin levels of obese rats fed on the control diet were significantly higher than those of lean rats ($p<0.05$), but they tended to be lower than those of obese rats fed on the semi-synthetic diets.

Part Two of the study on male Zucker rats

Chapter 6

The turnover of cholesterol

6.1 Introduction

Metabolic processes reflect regulatory mechanisms that are necessary to maintain, within certain limits, a metabolic equilibrium which provides the best chances fur survival of the living organism. To gain a deeper insight into these metabolic processes, it is possible to carry out kinetic studies. The following chapters — forming the second part of our investigations — will therefore deal with the effects of obesity and diet upon the cholesterol turnover (which is the result of its intake, absorption, synthesis, catabolism and excretion) and upon the lecithin : cholesterol acyl-transferase (LCAT) activity in plasma. LCAT is involved in the esterification and the transport of cholesterol (Glomset 1968). Furthermore the effects of fasting upon plasma lipid levels were investigated, and results will be given of studies on the liver lipid contents and on the fatty acid composition of liver and adipose tissue lipids as influenced by dietary treatment or as a consequence of obesity.

Finally, an attempt was made to establish whether or not a relationship exists between lipid metabolism and atherosclerosis in rats of the Zucker strain.

For the cholesterol turnover study the older half of the rats from six groups of part I of the experiment were chosen. The younger rats participated in the study on fasting and were — after refeeding — used for the LCAT determinations of the plasma and, after their sacrifice, for the investigations of liver and adipose tissue, whereas two subgroups, which were excluded from the cholesterol turnover study, were kept alive for another two months to be studied for the occurrence of atherosclerosis in their aortas. Each rat was given the same diet as that was used during the first part of the investigation (Table 2, Ghapter 3), *ad libitum*.

Details of the procedures applied in these experiments as well as the relevant data on body weights and food consumption will be given in the appropriate chapters.

The statistical evaluation of the results obtained was, in most cases, carried out in the same way as was indicated in part one (Chapter 3, p. 22), that is by two-way analysis of variance (R. A. Fisher) and subsequent *t*-testing for effects in the groups fed on the semi-synthetic diets, or by the latter method only, when effects in both phenotypes fed on the control diet were compared. When other methods were applied this is mentioned in the text.

6.2 Methods

6.2.1 *Animals*

For the cholesterol turnover study the older rats were chosen from the groups fed on the low-fat diet with starch (group I), the high-saturated-fat diet with starch (group III), the

high-poly-unsaturated-fat diet with starch (group V), the high-poly-unsaturated-fat diet with sucrose (group VI), and the commercial ration (groups VII and VIII consisting of obese and lean rats respectively.

The effect of sucrose on the turnover of cholesterol was investigated only in one group, because we had somewhat restricted facilities at our disposal with respect to studies with isotopes. For this purpose group VI was chosen, which had shown significantly higher plasma cholesterol concentrations than its opponent group with starch in the diet.

In short, it was possible to study the effects of obesity *versus* leanness in animals fed on the same diet (group VII *vs* group VIII), of low-fat *versus* high-fat diet (group I *vs* groups III and V), of the type of fat (group III *vs* group V) and of the presence of sucrose *versus* starch in the diet (group V *vs* group VI).

At the start of this experiment the age of the rats was 25 weeks on average.

6.2.2 *The two-pool model of Goodman & Noble*

There are two modern and reliable methods which can be used for measuring the cholesterol turnover: the one assaying the whole sterol balance after an intravenous injection of radioactively labelled cholesterol — including the measurement of faecal excretion of sterols (Grundy & Ahrens 1969) —, and the other, the one which we followed, in which only the decay of the radioactivity of the injected cholesterol in the blood is measured and the exponential functions obtained in this way are calculated.

In both cases the result can best be expressed as a hypothetical, imaginary model (Goodman & Noble 1968). In this model cholesterol is regarded to be present in two or three „pools" (Goodman & Noble 1968, Goodman et al. 1973), which are exchangeable to a certain extent, depending on the velocity constants for the transfer of cholesterol from one pool to another. The kinetic analysis of this model was derived from Gurpide et al. (1964).

For studies of a short duration such as we performed, the assumption of two pools suffices (Goodman & Noble 1968), a third pool being necessary to be assumed only in studies with a duration of more than twelve weeks (Goodman et al. 1973, Samuel & Lieberman 1973).

The first pool „A" is a pool of rapidly exchangeable cholesterol, comprising mainly the cholesterol in the blood, partly that in the liver, and some from the intestinal wall. The second pool „B" is a slowly exchangeable pool consisting predominantly of the

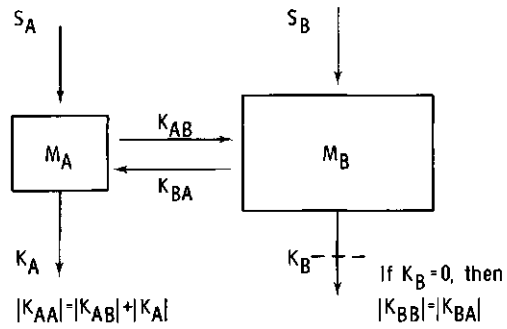

Figure 7. The two-pool model, with rate constants of the cholesterol metabolism, according to Goodman & Noble (1968).

remaining part of the cholesterol in the liver and the other tissues, including adipose tissue, muscle and brain. This division into pools, however, does not accord with the anatomical tissue division in any strict sense.

For the calculation of the kinetics of the cholesterol metabolism it is a prerequisite for the rats to be in a steady state with respect to cholesterol input and output. This condition is assumed to be fulfilled when the body weight and the plasma cholesterol concentrations are kept constant. Therefore, the body weights were carefully measured before and after this part of the study and the plasma cholesterol concentrations were determined frequently.

In Figure 7 a picture is given of the two-pool model of Goodman & Noble, with the rate constants which can be calculated from the decay curves of labelled cholesterol. In the equation fitting to these decay curves:

$$S_A = C_A \cdot e^{-\alpha t} + C_B \cdot e^{-\beta t}$$

S_A is the total amount of — synthesized and exogenous (dietary) — cholesterol entering pool A (S_B represents only, in pool B, synthesized cholesterol; C_A and C_B represent the intercepts with the vertical axis for the specific activity of ^{14}C-cholesterol (in, respectively, pool A and pool B at zero time), whereas α and β represent the slopes of either part of the decay curve, which can be derived directly from the semi-logarithmic graph of log S_A *versus* time (Figure 8).

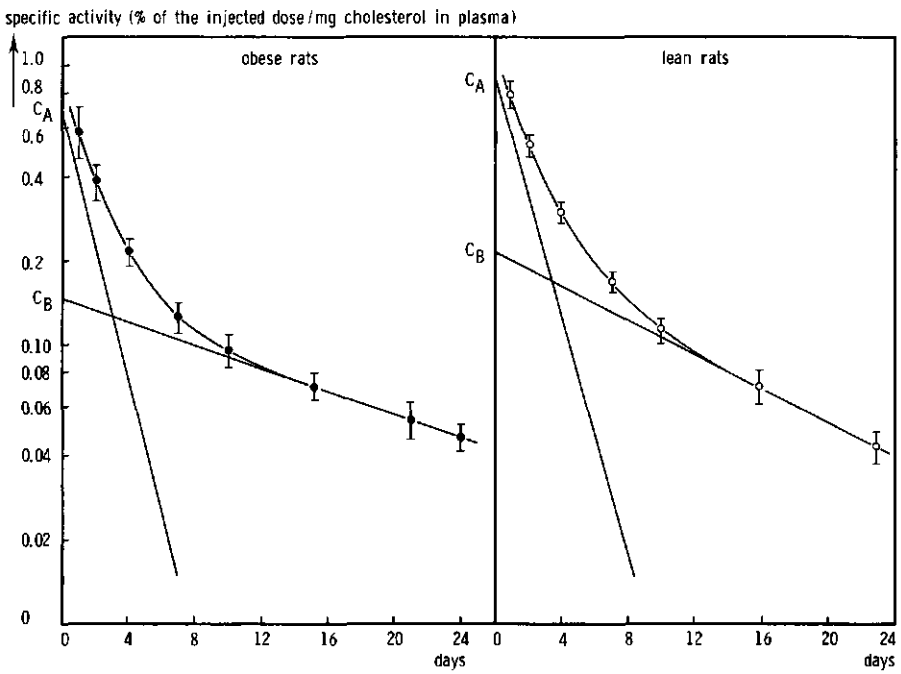

Figure 8. ^{14}C-cholesterol decay curves of the plasma in obese and lean Zucker rats (cf. Table 15, page 46).

From the equation presented one can primarily calculate:

K_{AA}: rate constant for total excretion of cholesterol from pool A, per day;

K_{BB}: rate constant for total excretion of cholesterol from pool B, per day;

M_A: magnitude (mass) of the rapidly exchanging pool A, in mg;

PR_A: production rate of cholesterol in pool A, i.e. the rate of entry of cholesterol in pool A (mg/day), with the exception of the cholesterol derived from this pool which returns to it from pool B (in other words it represents the rate at which cholesterol is entering pool A for the first time). Under steady-state conditions this rate represents the turnover of cholesterol and, consequently, equals the rate of the metabolic excretion of cholesterol.

Subsequently the following parameters can be calculated:

K_{AB}: rate constant for transfer of cholesterol from pool A to pool B, per day;

K_{BA}: rate constant for transfer of cholesterol from pool B to pool A, per day;

K_A: rate constant for external excretion of cholesterol from pool A, per day.

M_B, the magnitude of pool B, can be estimated as the average of the calculated minimal and maximal value of pool B (assuming $K_B = 0$). With this estimation the synthesis of cholesterol in pool B can be calculated, taking into account the absorption coefficient of exogenous cholesterol, the cholesterol content of the diet and the amount of cholesterol eaten. For the calculation of M_B, a daily food intake of 20 g was assumed in this experiment.

Further, one can estimate the total pool mass:

$$M_T = M_A + M_B;$$

the relative magnitude of pool A can be expressed as:

$$M_A / M_T.$$

From the rate constants mentioned above, the ratio

$$K_{AB} / K_{AA}$$

reflects the relationship between the quantity of cholesterol entering pool B from pool A and the total amount leaving pool A, independent of the latter pool mass.

With the general equation:

$$r = M \times K,$$

from the rate constants together with the pool masses the absolute amounts of cholesterol entering or leaving the pools can be calculated:

$$r_A = M_A \times K_A,$$

or

$$r_{AB} = M_A \times K_{AB} \quad \text{and} \quad r_{BA} = M_B \times K_{BA}.$$

6.2.3 *Measurement of the cholesterol turnover*

In order to be able to take into account also the absorption of cholesterol, which was a constituent in particular of the semi-synthetic diets used in our study, the dual-labelled isotope technique as described by Nilsson & Zilversmit (1972) was followed. A short exposition of this technique will be given.

After the rats had fasted for 24 hours, 0.5 ml of a freshly prepared colloidal solution containing 3 micro-Ci of 4-^{14}C-cholesterol in ethanol and saline was injected in their tail

vein, while they were under slight ether anaesthesia. To the groups fed on semi-synthetic diets containing cholesterol (groups I, III, V and VI), a dose of 5 micro-Ci of 1α, 2α-^{3}H-cholesterol*), emulsified in 1 ml trioleate-cholesterol-sodium taurocholate solution, was subsequently administered by gastric tube, in order to be able to determine the intestinal absorption of cholesterol, according to the plasma isotope ratio method. The principle of this method, its mode of application and validation in rats as well as the preparation of the solutions required are described in detail by Zilversmit & Hughes (1974). The coefficient of variation in the quantity of the injected and the oral doses provided was less than 1%.

Immediately after the provision of the isotopes, the rats were allowed to eat and drink freely. It was established that they started eating almost immediately.

The animals in the various groups had been divided into two series which, with a time space of one week, were included in the experiments. During the first days blood was drawn from all rats every day, later the animals were punctured with a gradually decreasing frequency until after three weeks a log-linear relationship between the specific activity of 4-^{14}C-cholesterol in plasma *versus* time had been achieved.

In this part of the investigation, plasma cholesterol concentrations were determined, for convenience, because the assay had to be carried out within the radioactive room of the animal house, according to Huang et al. (1961). The radioactivity was measured in 0.1 or 0.2 ml plasma after addition of 10 ml instagel (Packard) in a Mark 1 (Nuclear, Chicago) liquid scintillation counter. The efficiency of counting for ^{3}H and for ^{14}C was approximately 20% and 50% respectively. A correction was made for the quenching of the radioactivity by means of quench curves, obtained by the channel ratio method.

6.2.4 *Excretion of cholesterol with the faeces*

In an additional experiment an attempt was made to measure the amount of cholesterol excreted with the faeces which were collected per group (pooled samples) for four days in the twelfth week of Part I of our investigation for measuring the apparent digestibility (Chapter 4, Table 7).

Extraction was carried out by the following procedure: 1 g of dried faeces was boiled under reflux and magnetic stirring with 25 ml of a 0.5 N methanolic KCl and 1 g of sea sand for one hour. The raw extract was then filtered through a spun-glass pledget and the filtrate was shaken three times with portions of 15 ml petroleum ether 40 : 60 in a separation funnel. The petroleum ether fractions were collected in a 50 ml measuring flask, which was subsequently made up to the mark. 10 ml petroleum ether extract was dried under low pressure and the residue dissolved in 2 ml isopropyl-alcohol. Of this latter solution 0.1 ml was used for the enzymatic cholesterol determination as described in Chapter 5.

The enzymatic reaction used for the assay of cholesterol is susceptible to 3-β-OH-sterols, with the exception of coprostanol which compound is a degradation product of cholesterol, formed in the intestinal tract by microbial action. However, the measurement of cholesterol in this way could give a reasonable idea of the sterol excretion with the faeces of the rats.

6.2.5 *Statistics*

The statistical evaluation of this experiment was carried out by one-way analysis of

*) Both cholesterol isotopes were purchased from the Radiochemical Centre, Amersham, Buckinghamshire, Engeland. These compounds had a purity of 99 and 98% respectively, measured by thin-layer chromatography with the solvent system.

variance for the groups fed on the semi-synthetic diets, which could be followed by Student's *t*-test, and by this latter test only for establishing the differences between the control groups of obese and lean rats fed on the commercial ration.

6.3 Results

6.3.1 *Body weights and plasma cholesterol concentrations*

With regard to the establishment of steady-state conditions, there appeared to be only a small gain in body weight of the rats in the course of this experiment of on average 2.5% ± 1.2% (standard error of the mean, $n = 33$) with no significant difference in body weight change between the groups.

The individual variations in the plasma cholesterol concentrations were also small and had no influence on the group differences. Since plasma cholesterol concentrations will always be subject to small variations, it appeared justifiable to accept the occurrence of steady-state conditions in this experiment. The Huang method as used in this experiment gives values in measuring cholesterol that are approximately 10% higher than the results obtained by the enzymatic method used in the other experiments.

Apart from the magnitude of the plasma cholesterol concentrations in this experiment, a difference in the order of ranking of the groups occurred with regard to these concentrations in the present experiment and in that as described in Chapter 5 (see Table 9); this time these levels were higher in the group fed on the high-saturated-fat diet (group III) than in that fed on the low-fat diet (group I), as will be seen in Table 17.

6.3.2 *Effect of obesity on the turnover of cholesterol*

The decay curves of the specific activities of 4-^{14}C-cholesterol in the plasma are given in Figure 8. The parameters α, β, C_A and C_B are derived from these curves and are given in Table 15.

Table 15. Variables of the cholesterol metabolism derived from the decay curves of the ^{14}C-cholesterol in the plasma, together with the body weights and plasma cholesterol concentrations of the obese and lean rats fed on the commercial ration.

	obese rats ($n = 5$)	lean rats ($n = 5$)
body weight (g) *)	468 ± 21	303 ± 9
plasma cholesterol (mg/100 ml)*)	183 ± 19	92 ± 2
α	0.522 ± 0.042	0.499 ± 0.021
β	0.047 ± 0.003	0.073 ± 0.001
C_A**)	0.713 ± 0.093	0.956 ± 0.068
C_B**)	0.145 ± 0.011	0.221 ± 0.010

*) Mean values during the turnover study, with standard errors of the mean.
**) C_A and C_B expressed as percentages of the dose of ^{14}C-cholesterol given, per mg cholesterol in the plasma.

In Figure 8 the slope of the second exponential line is apparently less steep for obese than for lean rats. In addition, the lean rats had higher values for C_A and C_B. The significance of these findings becomes clear from the calculated parameters given in Table 16.

The most significant differences between obese and lean rats are the increase of the cholesterol pool masses M_A and M_B in the obese animals and the larger rate constants for the transfer of cholesterol from pool A to pool B (K_{AB}).

The relatively higher transfer of cholesterol from pool A to pool B in obese rats than in lean rats will be clear, particularly when the ratio K_{AB}/K_{AA} is considered. For obese rats

Table 16. Calculated parameters of the cholesterol metabolism, according to the two-pool model, for obese and lean Zucker rats fed on a commercial ration.

		obese rats (n = 5)	lean rats (n = 5)
K_A	(day^{-1})	0.19 ± 0.01*)	0.24 ± 0.01
K_{AB}	(day^{-1})	0.25 ± 0.02	0.18 ± 0.01
K_{BA}	(day^{-1})	0.13 ± 0.01	0.15 ± 0.01
M_A	(mg)	123 ± 16	87 ± 5
M_B	(mg)	320 ± 21	175 ± 4
PR_A	(mg/day)	22.5 ± 1.3	20.3 ± 0.7
PR_A	(mg/kg/day)	48.1 ± 1.9	67.1 ± 1.1

*) Standard error of the mean.

this ratio was 0.564 ± 0.018 and for lean rats only 0.425 ± 0.026 (means ± s.e.m., n = 5). The difference between these ratios is statistically significant ($p<0.01$).

The production rate (= turnover) of cholesterol in pool A (PR_A), when expressed as mg cholesterol per day, was more or less equal for obese and lean rats. However, because of the difference in body weights between the two phenotypes, the PR_A, when expressed per kg body weight per day, of 67 mg for the lean and of 48 mg for the obese rats showed a significant difference ($p<0.01$) in favour of the lean rats. We shall come back to this difference when the results are shown of all groups of obese rats studied.

Figure 9 presents the two-pool models for obese and lean Zucker rats. The pool masses are not given in combination with the rate constants — as was the case in Figure 7 —, but this time together with the figures representing the absolute amounts of cholesterol entering or leaving the pools, in mg per day. These figures were calculated

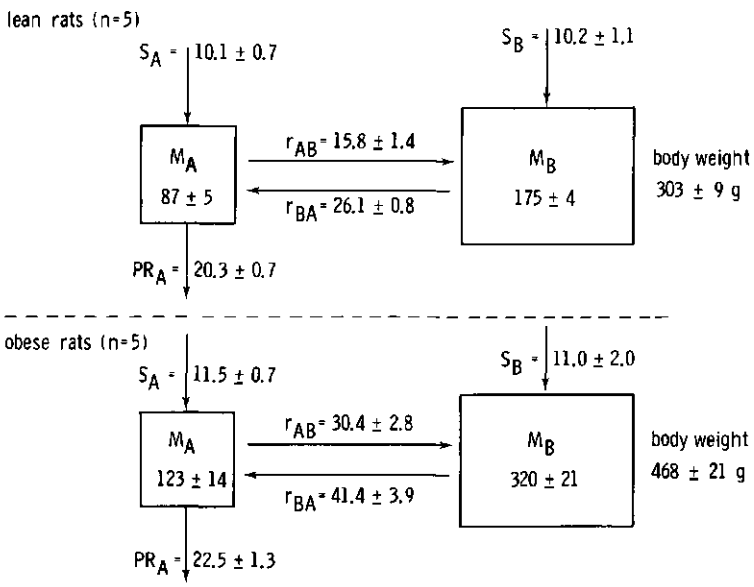

Figure 9. The two-pool model with variables of the cholesterol metabolism of lean and obese Zucker rats fed on the commercial ration (cf. Table 16). The pool masses are expressed as mg, the other quantities as mg/day, with standard errors of the mean.

by means of the general equation: $r = M \times K$, as mentioned above on page 44. As can be seen from this figure, the values for the input and output of cholesterol are identical for either phenotype. The obese rats show higher figures for all aspects presented.

6.3.3 *Dietary effects on the cholesterol turnover in obese rats*

In Table 17 are given the effects of dietary manipulation on the variables of the decay curves of ^{14}C-cholesterol in the plasma. The mean body weights and plasma cholesterol concentrations of the animals are also shown. As mentioned above, also in this part of the study significant differences were found in the plasma cholesterol concentrations of the rats, with a ranking order with respect to these concentrations of the groups which differed from that observed in Chapter 5 (Table 9). Similar features will be described in the next chapter for the other subgroups which participated in this part of the study, compared with part I.

In the present experiment there were no significant differences with regard to the body weights of the obese rats, as has also been mentioned already.

Table 17. Variables of the cholesterol metabolism derived from the decay curves of ^{14}C-cholesterol in the plasma of obese Zucker rats (means and standard errors of the mean) as influenced by dietary manipulation; body weights and plasma cholesterol concentrations per group are also given.

group	number	body weight*) (g)	plasma cholesterol*) concentration (mg/100 ml)	α	β	C_A**)	C_B**)
I	6	439	237	0.67	0.028	0.403	0.109
		± 7	± 20	± 0.02	± 0.004	± 0.053	± 0.019
III	6	483	285	0.46	0.036	0.392	0.122
		± 18	± 13	± 0.03	± 0.002	± 0.054	± 0.009
V	5	464	204	0.50	0.033	0.505	0.108
		± 9	± 9	± 0.05	± 0.002	± 0.038	± 0.007
VI	6	489	245	0.41	0.036	0.308	0.124
		± 4	± 9	± 0.02	± 0.002	± 0.017	± 0.005
VII	5	468	183	0.52	0.047	0.713	0.145
		± 21	± 19	± 0.04	± 0.004	± 0.093	± 0.011

*) Mean values during the turnover study, with standard errors of the mean.

**) C_A and C_B as percentages of the dose of ^{14}C-cholesterol given, per mg cholesterol in the plasma.

Table 18 presents the calculated kinetic parameters of the cholesterol metabolism in the groups of obese rats, as already indicated earlier in this chapter and presented in the same way as was done in Table 16. Group VII gave the highest figure for K_A, being the excretion of cholesterol from pool A, which is not transferred to pool B, with group V (poly-unsaturated fat with starch) in the second place. Group I shows the highest figure for K_{AB} (and for K_{BA}) representing the transfer of cholesterol from the one pool to the other. The ratio K_{AB}/K_{AA}, giving an idea of the relative transfer of the total amount of cholesterol from pool A to pool B — independently of the mass of pool A —, is lowest in group VII (on the control diet) and highest in group I (low-fat with starch), with intermediate figures for the other groups fed on the high-fat diets.

In agreement with what was calculated for K_A, group VII shows the lowest value for M_A (the mass of pool A), per kg body weight, whereas the average size of M_B (and

consequently of M_T, which is the sum of M_A and M_B) is highest again in group I. This latter group gives the lowest figure for the relative mass of pool A (M_A/M_T), which is even lower than that of group VII with its smaller magnitude of pool A in absolute amount.

Differences in plasma cholesterol concentrations did not always correlate with differences in pool masses (cf. Tables 17 and 18), e.g. the higher plasma cholesterol levels of group III did not correspond with a higher mass. This may be due to a different distribution of cholesterol over the blood and the tissues (Bieberdorf & Wilson 1965, Grundy & Ahrens 1970).

Table 18. Calculated parameters of the cholesterol metabolism, according to the two-pool model, of obese Zucker rats fed on different diets (means with standard errors of the mean).

	group I (n = 6)	group III (n = 6)	group V (n = 5)	group VI (n = 6)	group VII (n = 5)
K_A (day^{-1})	0.112 ± 0.013	0.119 ± 0.009	0.140 ± 0.012	0.103 ± 0.007	0.191 ± 0.019
K_{AB} (day^{-1})	0.420 ± 0.027	0.234 ± 0.020	0.278 ± 0.033	0.199 ± 0.011	0.248 ± 0.023
K_{BA} (day^{-1})	0.160 ± 0.007	0.144 ± 0.018	0.118 ± 0.011	0.145 ± 0.013	0.129 ± 0.012
K_{AB}/K_{AA}	0.787 ± 0.027	0.660 ± 0.020	0.661 ± 0.022	0.660 ± 0.011	0.564 ± 0.018
M_A (mg/kg)	482 ± 53	423 ± 33	359 ± 27	477 ± 14	261 ± 26
M_B (mg/kg)	1386 ± 243	780 ± 56	902 ± 55	737 ± 30	686 ± 47
M_T (= M_A + M_B)	1868 ± 295	1202 ± [53]	1262 ± 75	1214 ± 29	947 ± 52
M_A/M_T	0.27 ± 0.02	0.35 ± 0.03	0.29 ± 0.01	0.39 ± 0.01	0.28 ± 0.02
PR_A (mg/kg/day)	52 ± 1	50 ± 1	50 ± 2	49 ± 3	48 ± 2

The values for the production rate of cholesterol of the obese rats are remarkably equal, whereas they differ significantly from those referring to the lean rats, when expressed on a body weight basis, as mg per kg per day (see Table 16). This greatly similar rate of cholesterol entering pool A — including synthesized cholesterol — which is, under steady-state conditions, keeping up with its excretion, observed in the groups of obese rats with slightly different cholesterol contents in their various diets, forms a very important observation.

6.3.4 *Maximal production rate of cholesterol per kg body weight (PR_A max)*

In order to gain a better insight into the relationship between the production rate (PR_A, = turnover) and the magnitude of pool A (M_A), this pool mass was plotted *versus* the ratio of M_A and PR_A, of both the obese rats combined and of the lean rats, in either case

expressed per kg body weight (Figure 10). A similar plot was presented by Miettinen (1974) for the cholesterol metabolism of man and by Hermus (1975) for that of rabbits.

There appeared to be a very high correlation between these two parameters for both lean rats ($r = 0.95$, $n = 5$, $p < 0.02$) and obese rats ($r = 0.94$, $n = 28$, $p < 0.001$). Extrapolation of the regression lines in Figure 10 to $M_A/PR_A = 0$, that is at relatively very high, near-maximal levels for PR_A, provides a value for the mass of pool A of the lean rats of approximately 140 mg, which is about half of its magnitude as presented in Figure 10. For the obese rats, however, this value at the attainment of $M_A/PR_A = 0$ tends to be zero.

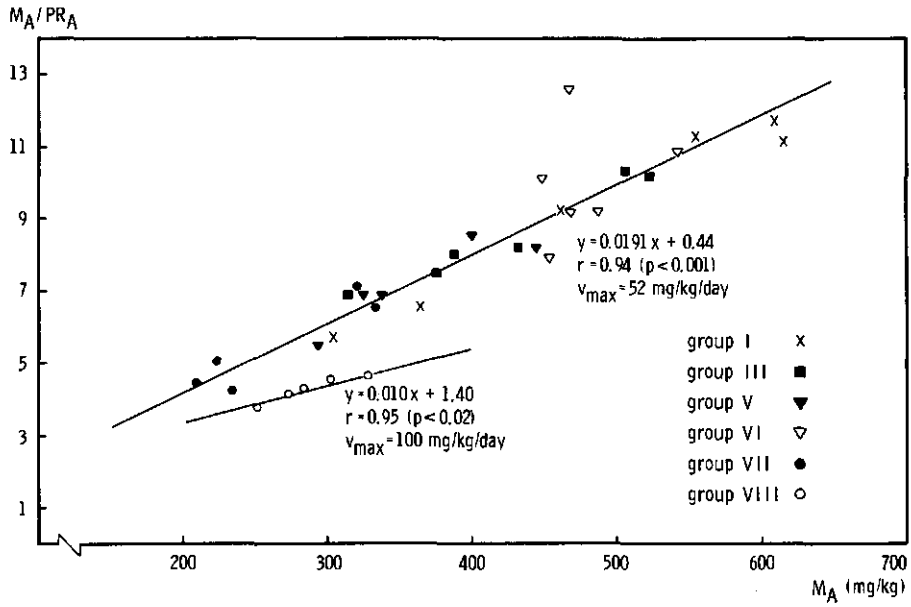

Figure 10. Relationship of the mass of pool A (M_A) with the ratio of M_A and the production rate (PR_A) of cholesterol in the various groups of Zucker rats (for text, see Section 6.3.4). The maximal excretion capacity for cholesterol (V_{max}) of obese and lean rats is calculated from the slope of the regression lines.

This result indicates that in obese rats, the observed production rate of cholesterol was maximal in all dietary groups; in this respect they contrast with their lean controls. As the production rate represents, under steady-state conditions, the turnover and simultaneously the metabolic excretion of cholesterol, this strongly suggests that the excretion of cholesterol in the fatty rats was maximal, and was fixed to approximately 50 mg/kg/day. This apparently occurred in all dietary groups of obese rats and explains the similarity of the figures for PR_A as given in Table 18. For lean rats a capacity for cholesterol excretion was calculated of 100 mg/kg/day, which is 1½ times the PR_A actually measured in this control group (see Table 16).

6.3.5 *Intestinal absorption of ^{3}H-cholesterol in obese rats fed on different semi-synthetic diets*

Table 19 shows the effect of the semi-synthetic diets used on the intestinal absorption of ^{3}H-cholesterol in obese Zucker rats.

Table 19. Intestinal absorption of ^{3}H-cholesterol in obese rats fed on various semi-synthetic diets.

group	number	intestinal absorption of ^{3}H-cholesterol (% of the dose given, with standard errors of the mean)
I	6	81 ± 5
III	6	64 ± 3[a])
V	5	84 ± 4
VI	6	66 ± 4[b])

[a]) Statistically significant difference from absorption in groups I and V ($p<0.05$)
[b]) Statistically significant difference from absorption in group V ($p<0.05$)

The differences in cholesterol absorption between the rats fed on the low-fat and those fed on the high-saturated fat diet, between those fed on the latter diet and their opponents fed on the high-poly-unsaturated-fat diet, as well as between those fed on the last-mentioned diet and those fed on the same type of fat with sucrose substituted for starch, were all statistically significant ($p<0.05$).

In Figure 11 the relationship is presented between the fractional intestinal absorption of ^{3}H-cholesterol (between 50 and 100%) and C_B, being the interception with the vertical axis of the second linear part of the decay curve of the injected ^{14}C-cholesterol. The level of this cut-off point is negatively correlated with the mass of pool B, and was already mentioned as giving lower values for obese than for lean rats (Table 15, Figure 7). From Figure 10 it appears that there is a significant negative correlation in the obese rats between the intestinal cholesterol absorption and the level of the cut-off points with the Y-axis of the decay curves for cholesterol ($r = -0.71$, $n = 23$, $p<0.005$).

This means that there is a positive relationship between the intestinal absorption of cholesterol and the mass of pool B ($r = +0.64$, $n = 23$, $p<0.005$).

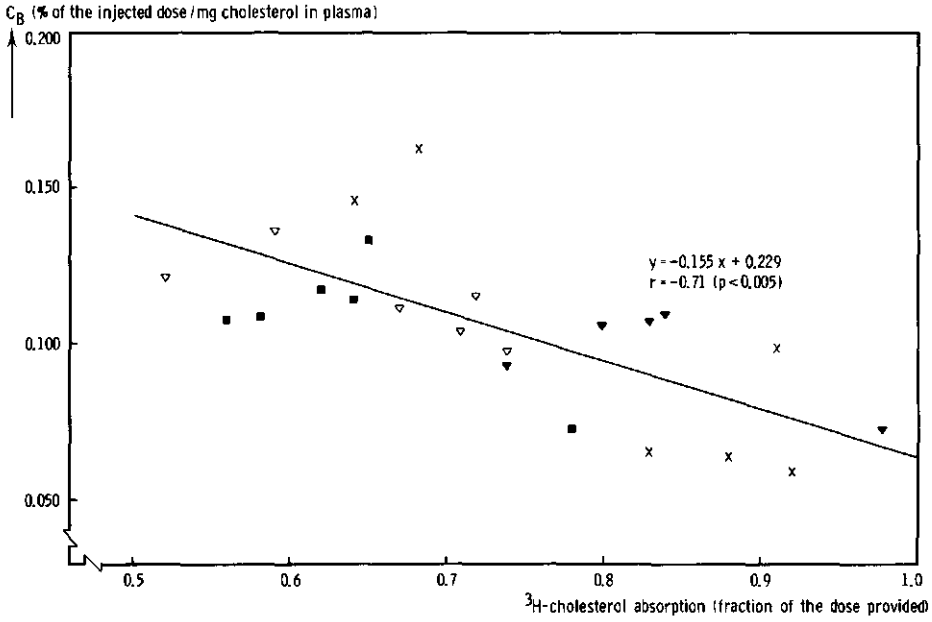

Figure 11. Relationship between the intestinal absorption of ^{3}H-cholesterol (cf. Table 19) and the specific activity measured for pool B (C_B, cf. Table 17). C_B correlates negatively with the mass of pool B (M_B, cf. Table 18). For symbols see Fig. 10 (page 50).

6.3.6 *Faecal excretion of cholesterol*

The results of the measurement of the 3-β-OH-sterols (with the exception of coprostanol) in the faeces of the rats, per group, are given in Table 20.

Table 20. Contents of 3-β-OH-sterols (with the exception of coprostanol) in the faeces from the groups of rats (pooled samples), in mg per day.

group	faeces	excretion of 3-β-OH-sterols	
	(g/day)	(mg/g faeces)	(mg/day)
I	2.6	2.2	5.7
II	5.0	2.6	12.6
III	4.6	4.7	21.9
IV	6.1	4.6	27.8
V	3.2	3.8	12.1
VI	3.9	3.7	14.5
VII	6.8	2.7	18.3
VIII	5.6	2.6	14.6

The 3-β-OH-sterol excretion with the faeces was slightly lower in the lean than in the obese rats. The lowest values were found in the rats fed on the low-fat diets, the highest in those fed on the high-saturated-fat diets (groups III and IV, presumably related to the steatorrhoea which occurred in these groups), with fairly low values in between for those fed on the high-poly-unsaturated-fat diets. All groups with sucrose in their diets yielded somewhat higher figures for the sterol excretion than did the corresponding groups with starch.

6.4 Discussion

6.4.1 *Turnover of cholesterol*

A study of the type as we performed can only be carried out with adult rats, because of the necessary condition of the occurrence of a steady state, although even adult rats continue to gain weight gradually during their whole life span. As this weight gain was only small and the plasma cholesterol levels were fairly constant — the other condition to be fulfilled for the acceptance of a steady state with regard to the cholesterol metabolism — we are allowed to draw conclusions from the results obtained.

Large differences were found between lean and obese rats regarding most of the calculated parameters of the cholesterol metabolism, rate constants as well as pool masses, which were all found to be higher in the obese rats. In particular the ratio K_{AB}/K_{AA}, being the relative transfer of cholesterol from pool A to pool B, was significantly higher in the fatties ($p<0.01$).

However, the production rate PR_A representing the turnover of cholesterol was significantly higher ($p<0.01$), when expressed per kg body weight, in the lean than in the obese rats. The values for the PR_A were very strikingly found to be similar in all groups of obese rats, thus irrespective of their diets and despite variations in several other parameters of the cholesterol metabolism in these rats: a higher mass of pool B as well as a higher total pool mass and a higher relative transfer of cholesterol from pool A to pool B (K_{AB}/K_{AA}) in the group fed on the low-fat diet (group I).

Regarding these similar PR_A figures for all the groups of obese rats, it appeared by extrapolation of the calculated mass of the rapidly exchangeable pool A (representing the blood pool to a fairly considerable extent) and the ratio of this pool mass M_A to the production rate (which is identical with the turnover and occurs simultaneously with the

metabolic excretion of cholesterol) that — again very strikingly — all obese rats, in contrast to their lean litter-mattes, had probably reached their maximal capacity for the excretion of cholesterol. This is not only an explanation of the similarity of their PR_A values, but also of their hypercholesterolaemia and higher pool masses in comparison with lean rats.

The impaired metabolic excretion of cholesterol and its possible accompanying effect of increasing plasma cholesterol levels is in line with generally accepted views on obesity. When becoming obese, man appears to have an increased risk of elevated plasma cholesterol concentrations — the degree of which depends on genetic predisposition — and consequently of atherosclerosis and its complications developing (Wilmore & McNamara 1974, Schreibman & Dell 1975 and Pelkonen et al. 1977). Conversely, Miettinen (1971) showed that the production of cholesterol, measured with a sterol balance technique, was diminished in people who had lost weight.

In the Zucker rats the influence of dietary composition was much smaller than that of genetic predisposition. Nevertheless, there were some significant dietary effects on the *cholesterol metabolism. Of the two possibilities for lowering plasma cholesterol levels* by dietary measures, either an increased excretion with the bile (Nestel et al. 1973b, Grundy 1975) or a different distribution of cholesterol over the tissues (Grundy & Ahrens 1970), the latter seems to be the effective mechanism in our study, in agreement with the conclusions of Bieberdorf & Wilson (1965) from their studies on rabbits.

Group VII (the obese rats fed on the commercial ration) had, relative to the other groups of obese rats, rather small cholesterol pools. Group V (high-poly-unsaturated fat with starch) had a fairly small pool A but a somewhat larger pool B than the other groups fed on high-fat diets; both groups V and VII had relatively low plasma cholesterol levels. Group III (high-saturated fat with starch) had high plasma cholesterol levels with a rather small mass of pool B; group I (the low-fat diet with starch) showed a fairly high plasma cholesterol level in combination with a very large mass of pool B.

The conclusion could be that the lower plasma cholesterol concentration of group V, in comparison with that of group III, was due to a different distribution of cholesterol over the pools rather than to a lower total pool mass in the rats of group V. It seems that sucrose in the diet compared with starch (group VI *vs* group V) had a similar effect as had the type of dietary fat (group III *vs* group V, see above).

6.4.2 *Excretion of 3-β-OH-sterols with the faeces*

The faecal excretion of the 3-β-OH-sterols of the various groups, however, was different. The faeces investigated were collected at the age of the rats of approximately 18 weeks. The contents measured will not have accounted for the excretion of all compounds derived from cholesterol, e.g. coprostanol. Nevertheless, the figures for the sterol excretion of about 20 mg per day appear to give a reasonable approximation when compared with those for the cholesterol turnover. The higher values measured in groups III and IV fed on the high-saturated-fat diets will be related to the steatorrhoea of the rats in these groups (Chapter 4, Table 8). The observed differences in sterol excretion with the faeces will have been compensated by adaptive cholesterol synthesis in the rats.

The relatively high sterol excretion of the rats on the control diet containing only traces of cholesterol (with the obese rats showing a slightly higher figure than their lean litter-mates, in agreement with their respective food intake) will be connected with an increased synthesis and a lower reabsorption of cholesterol. The intestinal absorption and the slightly higher figures for the groups with sucrose in their diets will be dealt with in the next section.

6.4.3 *Intestinal absorption of ^{3}H-cholesterol*

The significant differences in the intestinal absorption of cholesterol between the various groups (Table 19) may be explained as follows:

a) the different absorption of the groups III and V will have resulted from the type of dietary fat used and the steatorrhoea connected with this;

b) the differences between the groups I and III and between the groups V and VI will be related not so much to a difference in the proportion of starch in the former two groups or to the difference in the type of carbohydrate in the latter groups, but to the different cellulose content of the diets (see Table 2, Chapter 3).

The percentages of cellulose provided in the semi-synthetic diets were thought necessary to compensate for the differences in the percentage of starch in the diets, which contains some quantity of impure constituents. The type of cellulose used (Akufloc) had been shown not to influence plasma cholesterol levels in preliminary work, but this does not in any way prove that such differences in the absorption of cholesterol do not occur. These differences can be compensated for by differences in cholesterol synthesis, particularly in the liver (Kritchevsky et al. 1974).

As the mass of the slowly exchangeable pool B appeared to correlate positively with the absorption of cholesterol, the magnitude of this pool B was also influenced (decreased) by the cellulose content of the diet. The relationship between the presence of sucrose in the diets and the sterol excretion with the faeces (Table 20) will only have been an indirect one, *via* the cellulose content of these diets.

The quantity of roughage (crude fibre) in the semi-synthetic diets will have differed from that in the commercial ration; the latter will have contained more roughage (see the foot-note at the end of Chapter 3), which explains the slightly higher sterol excretion with the faeces of the obese controls (group VII) in comparison with that of the groups fed on semi-synthetic diets, as far as these rats did not suffer from steatorrhoea (groups I, II, V and VI).

With respect to the differences between the metabolic excretion of cholesterol (mainly by the biliary route) and the faecal excretion of sterols as mentioned, which is related to both dietary and absorptive factors, these point to the complexity of the cholesterol metabolism: dietary intake, absorption, endogenous synthesis (also in the cells of the intestinal wall) metabolic excretion with the bile (also in the form of cholic acids), reabsorption (the entero-hepatic cycle), conversion and degradation of sterols in the intestines, mainly by microbial action, and the ultimate faecal excretion (Kaplan et al. 1963, Wilson & Lindsey 1965, Grundy et al. 1969, Connor & Connor 1972, Lutton & Chevallier 1972, Chevallier 1977, Glueck & Connor 1978).

Therefore, the significance of the intake of dietary cholesterol for the plasma concentration of this compound can be easily, and often is, misinterpreted. This will be discussed also in Chapter 11 (general discussion).

6.4.4 *Comparison with other studies on the cholesterol turnover in lean rats*

The results obtained from this study on the cholesterol turnover of lean Zucker rats are in reasonable agreement with those of other workers, as far as they are concerned with lean rats of other strains. As can be seen in Table 21a, we found a rather low turnover of cholesterol. The differences observed may be related to different body weights of the rats used and to strain differences, apart from those in the techniques applied.

For further comparison Table 21b contains, at the bottom, the variables of the equations from the decay curves of ^{14}C-cholesterol in the plasma, obtained in our study and in that of Nilsson & Zilversmit (1972), who used the same method. As these latter authors expressed their figures for C_A and C_B as a percentage of the dose of ^{14}C-cho-

lesterol provided per ml plasma, whereas we gave them (Tables 15 and 17) as the percentage of the dose provided per mg cholesterol in the plasma, we now give the figures for C_A and C_B as the ratio between these variables to eliminate any differences arising from the mode of expression. A fairly good agreement was found to exist between the two studies.

Comparisons between some animal species and man will be discussed at the end of the study, in Chapter 11.

Table 21a. Comparison of the cholesterol turnover of lean Zucker rats with that of other rat strains, as given in the literature.

authors	rat strain (only males)	number	body weight (g)	food	cholesterol turnover (mg/day)	method used
Raicht et al. (1975)	Sprague-Dawley	9	225-250	commercial ration	21 ± 2(s.e.m.)*	sterol balance
Zilversmit & Hughes (1974)	Sprague-Dawley	8	411 ± 1	commercial ration	33 ± 2	isotope dilution
Kellogg (1974)	Wistar	12	391 ± 6	semi-synthetic (cholesterol-free)	34	sterol balance
this study	lean Zucker	5	303 ± 9	commercial ration	20 ± 1	isotope dilution

*) Standard errors of the mean.

Table 21b. Comparison of the variables from the decay curve of ^{14}C-cholesterol in the plasma obtained from lean Zucker rats (this study) with those in the plasma from Sprague-Dawley rats (means with standard errors of the mean).

authors	number	C_A/C_B	α	β
Nilsson & Zilversmit (1972)	5	5.16 ± 0.41	0.59 ± 0.03	0.071 ± 0.005
this study	5	4.32 ± 0.23	0.50 ± 0.02	0.073 ± 0.001

6.5 Summary

1. The turnover of ^{14}C-cholesterol — measured under acceptable steady-state conditions — was found to be significantly lower in obese than in lean Zucker rats ($p<0.01$) when expressed on a body weight basis (48 and 67 mg/kg/day respectively).

   The obese rats had higher pool masses of cholesterol and a significantly higher ratio for the relative transfer of cholesterol from the rapidly to the slowly exchangeable pools A and B respectively.
2. All groups of obese rats studied — irrespective of their diets and in contrast to the lean rats — appeared to have reached their maximal capacity for the metabolic excretion of cholesterol, which was calculated to be approximately 50 mg per kg body weight per day. In lean rats this was approximately 100 mg/kg/day.
3. Significant differences in the intestinal absorption of ^{3}H-cholesterol were measured between the groups fed on the semi-synthetic diets. They were related either to the quantity of cellulose in the diets (a higher absorption in group I than in group III ($p<0.05$) and in group V than in group VI ($p<0.05$), or to the type of dietary fat: a higher absorption in group V than in group III ($p<0.05$).

There was a significant positive correlation between the mass of the slowly exchangeable pool B and the intestinal absorption of cholesterol ($r = 0.64$, $n = 23$, $p < 0.005$).

4. The excretion with the faeces (pooled samples) of 3-β-OH-sterols (coprostanol not measured) was somewhat higher in the obese rats fed on the commercial ration than in the lean rats.

   This faecal sterol excretion was higher in the groups fed in the high-saturated-fat diets (probably because of the steatorrhoea occurring in these groups) than in the other four groups fed on the semi-synthetic diets.

   These latter four groups all had smaller figures for their faecal sterol excretion than the one of the obese rats fed on the control diet, presumably as a result of the larger proportion of roughage in the commercial ration.

   The seemingly increasing effect on the sterol excretion of the presence of sucrose in the diets will also have been caused by the proportion of cellulose added to these diets being larger than that added to the respective starchy diets, rather than by the mere sucrose.

Chapter 7

Effects of four days fasting on circulating cholesterol, triglyceride, glucose and insulin levels

7.1 Introduction

In order to obtain additional insight into the dynamics and the regulation of the lipid and the glucose metabolism, a fasting experiment with a duration of four days was designed. It was aimed in this way to collect information about the course of plasma cholesterol and triglycerides as well as about blood glucose and plasma insulin levels during a short period of fasting so that effects from the previous diets could be observed (Angel & Farkas 1974). Under certain conditions, the type of diet may have a long-lasting, and sometimes even permanent, influence on the nutritional status of the experimental animals involved. As an example of this latter effect, intermittent fasting and re-feeding at an early age may alter adipose tissue accretion in later life (Angel & Farkas, 1974).

As we had observed with regard to cholesterol accumulation, that the limited biliary excretion capacity was the general mechanism giving rise to higher plasma cholesterol levels in obese rats than in lean controls, and that the different levels in the obese rats apparently were dependent on the distribution of cholesterol over the two hypothesized pools, we supposed, because of the different initial values, that fasting might well give differences in the rates of reduction of the levels of plasma cholesterol, triglycerides, blood glucose and plasma insulin between the dietary groups.

We were curious to know, if the rate of clearance of triglycerides from the plasma was dependent on the type of diet that had been used previously. Barry & Bray (1969) and Bray et al. (1974) found a reduction of the hypertriglyceridaemia in obese Zucker rats, during fasting, with a half-life time of about two days, after the use of a stock diet.

In hyperinsulinaemic diabetes, contrasting to the non-diabetic state, a positive relationship may occur between plasma insulin and plasma triglyceride levels (Nikkilä 1969, Olefsky et al. 1974), presumably in connection with the inhibition of lipolysis with insulin in adipose tissue.

To make it possible also for the lean controls to participate in this experiment, a short fasting period of only four days was chosen. For obese Zucker rats much longer fasting periods, of up to 80 days, can be applied without deleterious effects of any serious kind (Zucker 1967, Bray et al. 1970a, Zucker & Antoniades 1972, York & Bray 1973a).

7.2 Methods

For this experiment the second halves (the younger series) of all groups of rats involved in the investigations were used. They were fasted for a period of four days, in two series, with a time space between two series of one week. They were weighed before and after the fasting period. The age of all the rats was approximately 200 days.

On days 1, 2, 3 and 4 blood was drawn (by orbit puncture, under slight ether anaesthesia) for determination of plasma cholesterol and triglycerides, and on days 2 and 4 blood glucose and plasma insulin levels were determined. The first blood sample was taken approximately 3 h after the last meal and the second, third and fourth sample 24, 48 and 72 h later.

7.3 Results

7.3.1 *Body weights*

The mean body weight of the obese rats at the start of this experiment was nearly 500 g (standard error of the mean 5 g, n = 45), and their weight loss during the period of four days fasting was, on average, 34 ± 0.8 g, n = 45, without any significant difference between the groups of obese rats. This implies a loss of body weight of approximately 7%. The lean rats, with a mean initial body weight of 330 ± 10 g (s.e.m., n = 5), lost on average 32 ± 0.6 g (n = 5), which makes approximately 10% of their body weight. Thus, although the absolute weight loss of the lean rats was similar, they lost proportionally more weight than did the obese rats.

7.3.2 *Plasma cholesterol concentrations*

The plasma cholesterol concentrations, measured on the first day of the experiment after a night's fasting, showed some differences with respect to the findings presented in Chapter 5 (Table 9), regarding their absolute concentrations as well as the ranking order

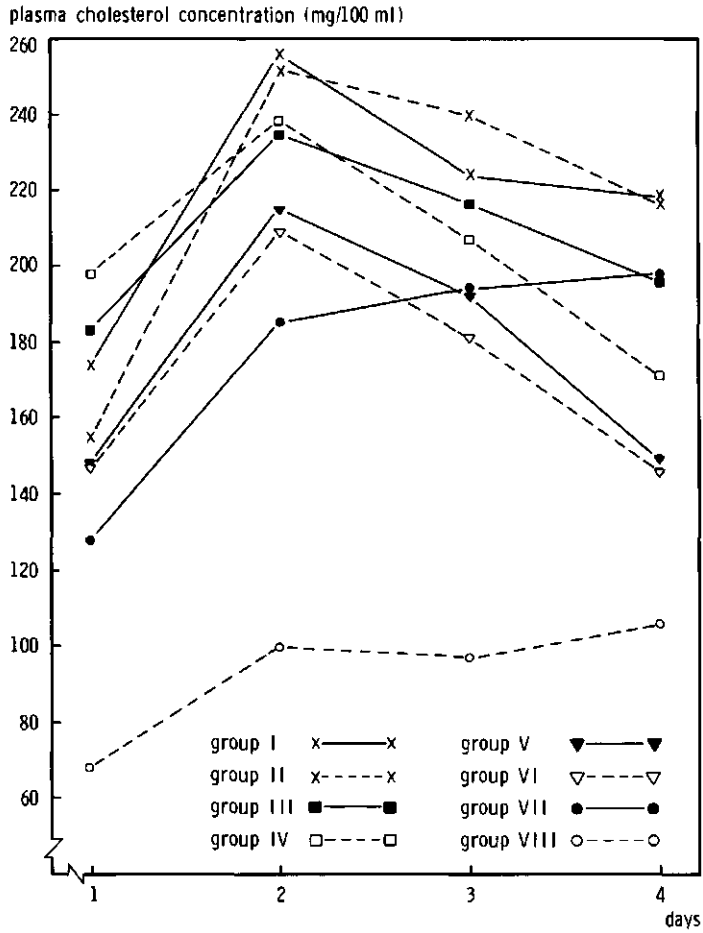

Figure 12. Mean plasma cholesterol concentrations of the groups of rats during 4 days of fasting (cf. Table 22).

of the groups. As was also observed in the other subgroups used for the turnover study (Chapter 6, Table 17), these concentrations were now higher in the groups fed on the high-saturated-fat diets (groups III and IV) than in those fed on the low-fat diets (groups I and II). It must be kept in mind that the Huang procedure as used in Chapter 6 gives approximately 10% higher values for cholesterol than does the enzymatic method used in Chapters 5 and 7.

On the second day of fasting a significant rise of the plasma cholesterol levels of approximately 45% was found in all groups of obese and also in the lean rats. During the following days, however, a gradual decrease was observed, which in some of the groups led to plasma cholesterol levels near to those measured on the first day of fasting, as shown in Table 22. These findings are also indicated in Figure 12.

Table 22. Mean plasma cholesterol concentrations (mg/100 ml, with standard errors of the mean) of the groups of Zucker rats, on four consecutive days of fasting.

group	number	day 1	day 2	proportional increase with respect to day 1 (%)	day 3	proportional decrease with respect to day 2 (%)	day 4
I	6	174 ± 22	256 ± 24	51 ± 10	224 ± 16	11 ± 3	218 ± 15
II	6	155 ± 8	252 ± 12	64 ± 7	239 ± 10	5 ± 5	217 ± 13
III	6	183 ± 8	235 ± 16	29 ± 8	216 ± 16	8 ± 2	196 ± 17
IV	6	198 ± 25	238 ± 28	21 ± 7	207 ± 20	12 ± 3	171 ± 12
V	7	148 ± 10	215 ± 14	46 ± 4	192 ± 11	10 ± 2	149 ± 8
VI	6	147 ± 14	209 ± 8	46 ± 10	181 ± 7	13 ± 2	146 ± 7
VI	7	128 ± 12	185 ± 18	44 ± 6	194 ± 22	–	198 ± 13
VIII	5	68 ± 1	100 ± 5	47 ± 5	97 ± 5	–	106 ± 5

The rise in plasma cholesterol concentration on the second day appeared to differ significantly between the groups of obese rats fed on the semi-synthetic diets ($p<0.01$); this was caused by a significant difference between the groups fed on the low-fat diets (groups I and II) and those fed on the high-saturated-fat diets (groups III and IV).

The plasma cholesterol levels on day 1 (three hours after the last meal before the fasting period had been provided) were found to be somewhat lower than those observed in the first part of the investigation (Chapter 5). On day 2, on the other hand, the cholesterol values were higher than those measured in part one. It seems that the levels observed during fasting overnight, as had been the case in part I, had already somewhat increased in comparison to those found in these rats when in the fed state.

7.3.3 *Plasma triglyceride concentrations*

The plasma triglyceride concentrations behaved differently. In this part of the study they were significantly higher ($p<0.05$) in the groups with saturated fat in their previous diets (groups III and IV), than in those with poly-unsaturated fat (groups V and VI). They diminished considerably already during the first day of fasting, and appeared to decrease more or less exponentially, that is to say more rapidly as the initial levels were higher (Nikkilä 1969). The figures for the plasma triglyceride concentrations measured during the four days of fasting are given in Table 23. This form of lipid clearing contrasts with that of plasma cholesterol. The inverse relationship may be connected with the metabolism of lipoprotein; chylomicrons and very-low-density lipoproteins, relatively poor in cholesterol, are transformed in cholesterol-rich low-density lipoproteins.

Nevertheless, the plasma triglyceride levels did not reach the low levels of the lean rats

Table 23. Mean plasma triglyceride concentrations (mg/100 ml, with standard errors of the mean) of the groups of Zucker rats after four consecutive days of fasting.

group	number	day 1	day 2	day 3	day 4
I	6	513 ± 172	226 ± 78	179 ± 42	188 ± 39
II	6	350 ± 58	108 ± 13	114 ± 17	92 ± 11
III	7	1459 ± 170	647 ± 199	413 ± 108	249 ± 57
IV	6	1448 ± 256	689 ± 197	360 ± 84	243 ± 53
V	7	442 ± 84	220 ± 45	128 ± 9	89 ± 6
VI	6	776 ± 170	279 ± 35	158 ± 17	113 ± 7
VII	7	527 ± 85	259 ± 38	239 ± 24	158 ± 19
VIII	5	44 ± 5	17 ± 3	11 ± 2	9 ± 2

within the short experimental period. In this group, a significant drop in triglyceride levels could also be observed. As a consequence of the exponential fall of the plasma triglycerides, on the last day of the experiment the highest levels were found in groups III and IV (previously fed on the high-saturated-fat diets), which at the start of the experiment had by far the highest levels. The data of all the groups are given graphically in Figure 13. In this experiment no significant differences were found between the effects of sucrose and starch, neither with respect to the absolute concentrations nor to the slopes of the decreasing plasma triglyceride levels.

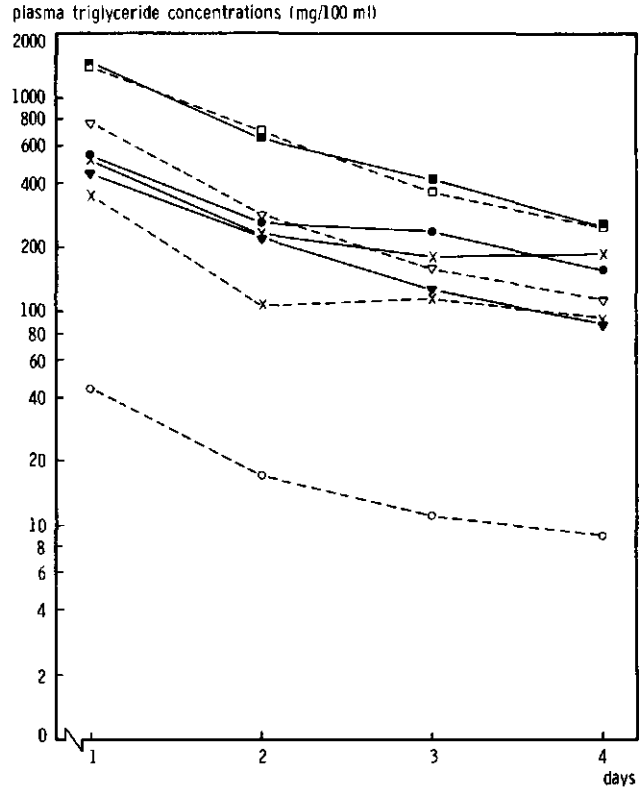

Figure 13. Mean plasma triglyceride concentrations, semi-logarithmically graphed, of the groups of rats during four days of fasting (cf. Table 23). For symbols see Fig. 12 (page 58).

7.3.4 *Blood glucose and plasma insulin concentrations*

A striking observation was the tendency of the blood glucose levels to increase significantly between the second and the fourth day of the experiment. This was found particularly in the groups which had been fed on the high-fat and on the control diets. The relevant figures are given in Table 24, and are presented graphically in Figure 14. There was no obvious influence related to the presence of sucrose in the diets provided prior to the period of fasting.

Table 24. Mean blood glucose concentrations (mg/100 ml, with standard errors of the mean) of the groups of Zucker rats after two and four days of fasting.

group	number	day 2	day 4	increase
I	6	84 ± 4	86 ± 4	2 ± 4
II	6	88 ± 2	88 ± 6	0 ± 6
III	7	87 ± 2	99 ± 4	12 ± 4
IV	5	87 ± 4	102 ± 3	15 ± 6
V	7	89 ± 4	95 ± 2	6 ± 3
VI	5	98 ± 5	107 ± 12	9 ± 9
VII	7	79 ± 6	96 ± 5	17 ± 7
VIII	5	74 ± 6	86 ± 9	12 ± 9

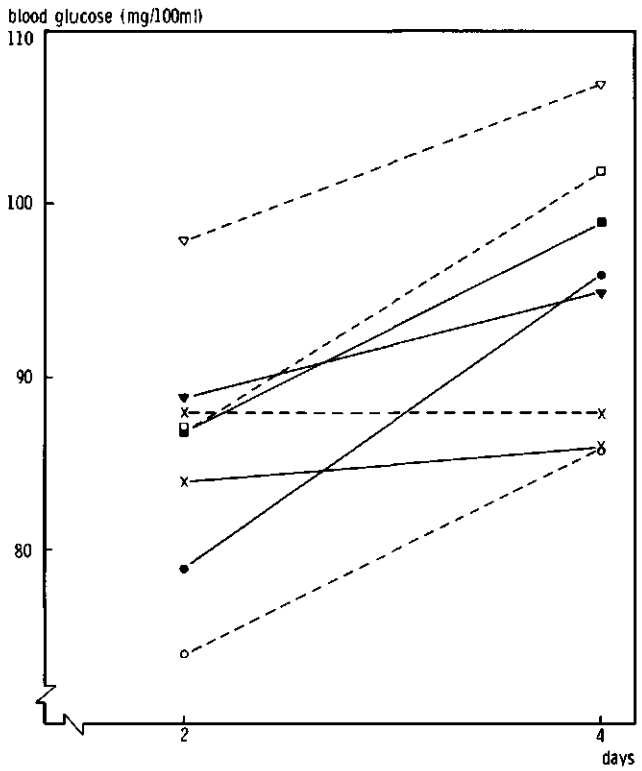

Figure 14. Mean blood glucose concentrations of the groups of rats during four days of fasting (cf. Table 24). For symbols see Fig 12 (page 58).

In no case did the group means show any decrease in the blood glucose concentration. Also in this part of the study the highest average values were observed in group VI. The lean rats had, on average, the lowest blood glucose levels, but they too showed an elevation during the experiment.

The plasma insulin levels were also assayed on the second and fourth day of the fasting experiment. These levels did not tend to decrease significantly in the course of a few days of fasting.

7.4 Discussion

7.4.1 *Plasma cholesterol concentrations*

As was already indicated in short in Chapter 6, the plasma cholesterol concentrations of the subgroups used for the study of the cholesterol turnover (Table 17) and for that on fasting (Table 22) differed to a certain extent from those presented in Chapter 5 (Table 9). When the figures in Table 9 are compared with those in Tables 17 and 22, it can be seen that they differ not only in an absolute sense — which is partly due to the different method (Huang et al. 1961) used in the turnover study (Table 17) — but also with respect to their mutual ranking order. In Tables 17 and 22 higher (initial) values are presented in the groups fed on the saturated-fat diets than in those fed on the low-fat diets. As both subgroups participating in either the turnover study or in this experiment on fasting were formed from the total groups as used in part I of the study (Chapter 5, Table 9), this change in ranking order will not be due to a deviant randomisation, but will possibly represent a time effect and may even be related to an improvement of the fat absorption of the groups fed on the high-saturated-fat diets with age. The final outcome is in agreement with findings in other animal species with regard to effects of dietary fats on plasma cholesterol concentrations (Vles et al. 1964, Hermus 1975).

The increase of the plasma cholesterol concentration on the second day of fasting can be explained by the liberation of this compound from the decreasing fat stores during this period (Angel & Farkas 1974, Swaner & Connor 1975, Kovanen et al. 1975, Kudchodkar et al. 1977). The differences in the rise of these concentrations seem to be related to the mass of pool B, which was found to be significantly larger in group I than the other groups investigated in the turnover study (Table 18).

No information was obtained on an altered synthesis of cholesterol which may have occurred in the liver as a result of the deprivation of dietary cholesterol during the period of fasting (Grundy et al. 1969, Quintão et al. 1971). This supposition, however, cannot explain the high plasma cholesterol levels on the final days of this experiment (i.e. relative to those on the first day of fasting) in the control groups, which had only traces of cholesterol in their previous diet.

7.4.2 *Plasma triglyceride concentrations*

The more or less exponential decrease of the plasma triglyceride concentrations (Nikkilä 1969) was in accordance with previous data (Barry & Bray 1969, Bray et al. 1974). No significant differences were found with regard to the slope of the plasma triglyceride levels expressed logarithmically (Figure 13). The final value in this experiment, however, did not reach the normal levels that were observed in lean control rats. This is in line with former observations, according to which it takes longer periods than were applied in our experiment to reach normality in triglyceride levels (Zucker 1967, Barry & Bray 1969). It may also be concluded that over-production rather than impaired removal is likely to be the cause of the hypertriglyceridaemia in these rats. The decrease in plasma triglyceride levels during fasting probably results from a diminishing supply of substrates, now that the alimentary route was eliminated.

As a consequence of the exponential character of the reduction of the triglyceride levels, the high initial values in groups III and IV decreased more than those in the other groups. Apart from the difference between obese and lean rats and that between the groups fed on the high-saturated-fat diets (groups III and IV) and the other groups of obese rats no significant differences were found, probably because of the large variability of the triglyceride levels. Nevertheless, there is some tendency of the rats fed on the low-fat diet with starch to show slower decreases of the triglyceride levels.

Kerpel et al. (1971) observed, in addition to a fall in triglycerides during fasting, no significant change in total cholesterol concentrations of the plasma. This may be due to their making use of normal (lean) rats without large lipid depots as compared with those of obese rats. In man an initial rise of plasma cholesterol concentrations during rigid dieting may also occur, probably as the result of the emptying of fat depots. This would be in accordance with the findings of Angel & Farkas (1974), who observed cholesterol mobilization during starvation in mice; they reported the proportionality of the adipose cholesterol pool and the degree of adiposity.

7.4.3 *Blood glucose concentrations*

The increase of the blood glucose concentrations on the fourth day, relative to that on the second day of the fasting period, is difficult to explain. However, it is known that there are animal species, such as dogs (Steele et al. 1968), pigs (Swiatek et al. 1968) and spiny mice (Wise 1977), which do not show a decrease in blood glucose during fasting. The supply of glucose must have been derived from gluconeogenesis. It might be that because of the large supply from the fat stores there was a preference for fatty acid utilization above glucose utilization. Free-fatty-acid concentrations were measured by Zucker & Antoniades (1972), who found an increase of free fatty acids in the blood of both obese and lean rats after four or five days of fasting and in both cases similarly decreasing plasma insulin levels. They observed, however, a decrease in blood glucose levels. On the other hand, their values for blood glucose of lean rats were, as in our experiment, slightly lower than those of fatties. They used rats of approximately fifteen weeks of age at the start of the fasting period, whereas our rats were nearly twice as old at that time.

7.5 Summary

1. During four days of fasting the weight losses in all groups of Zucker rats studied were almost identical, which means that the proportional weight loss in the lean rats was approximately 1.5 times as high as in the obese rats.
2. At the start of the experiment on fasting the ranking order of the plasma cholesterol concentrations of the rats was different from that observed in the first part of the study (Chapter 5, Table 9). These concentrations in the subgroups used for the present experiment were now higher — as they were also in the other subgroups used for the turnover study (Chapter 6) — in the groups fed on the high-saturated-fat diets than in those fed on the low-fat diets, which is in line with earlier findings in other animal species.
3. Also the initial plasma cholesterol concentrations already differed somewhat from those presented in Table 9 (Chapter 5). Now they were generally lower than in part I of the study, presumably due to the shorter time lag between food deprivation and the first blood sampling in the present experiment.

   A clear rise in the plasma cholesterol concentrations occurred on the second day of fasting in the obese as well as in the lean rats, with a particularly significant increase ($p<0.01$) in the groups fed on the low-fat diets, which will probably be re-

lated to the higher mass of pool B in group I (see Chapter 6). After day 2 the concentrations decreased gradually. Neither the type of fat nor the presence of sucrose in the previous diet influenced significantly the course of the plasma cholesterol levels during fasting.

4. Plasma triglyceride levels decreased more or less exponentially from the first day of fasting. From their different initial levels, the values found in the obese rats did not yet reach, after four days, those normally found in lean rats.
5. Blood glucose concentrations tended to rise between the second and the fourth day of fasting in lean as well as in obese rats without a statistically significant effect of the diet consumed previously. The plasma insulin levels, in general, did not change significantly between these times.

Chapter 8

Activity of the enzyme lecithin : cholesterol acyl-transferase (LCAT) in blood plasma

8.1 Introduction

Another way to obtain more knowledge of the dynamic balance of lipid metabolism was thought to be the assessment of the enzymatic activity of lecithin: cholesterol acyl-transferase (LCAT) in the blood of the rats in the various dietary groups.

Cholesterol as a polar substance is metabolically active in its free form in cellular processes, e.g. in cell membranes, on the borderline of the fat and the water phase. In the blood it is circulating and transported, as part of the lipoproteins, in predominantly an esterified form. In man, and also in rats, approximately two-thirds of the cholesterol in the blood is present in the form of its esters, and one-third, consequently, in the ,,free'' form (Goodman 1965).

8.1.1 *The role of LCAT*

The esterification of cholesterol in blood plasma takes place under the influence of the enzyme LCAT. According to Glomset (1968) almost all the cholesterol esters in the blood are formed with the help of LCAT, by transposing a fatty acid from the 2-position of the glycerol moiety of lecithin (liberated from lipoproteins) which is then left as lyso-lecithin.

On this place we will go into the lipoprotein pattern of the blood plasma somewhat deeper, because of the significance of the division of the lipoproteins with regards to atherogenesis (see Chapter 10, p. 86). The lipoproteins can be roughly divided, on the basis of their properties in the ultracentrifuge, in high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). This subdivision covers to a large extent their behaviour in electrophoresis, which distinguishes them in alpha-, beta- and ,,pre''-beta-lipoproteins respectively.

Additionally, chylomicrons, the very large lipid particles with a still lower density, can be present in the blood where they arrive via the lymph, after having been formed in the intestinal wall subsequent to the intake of the dietary fat*). These particles mostly are the ones which give the plasma its cloudy appearance for several hours after a meal. In cases of hyperlipoproteinaemia as exist in obese Zucker rats, the continuously very high levels of very low-density lipoproteins are responsible for this cloudy or even creamy or milky appearance of the plasma.

With respect to the distribution of free and esterified cholesterol in the blood it is of importance to know the lipoprotein pattern of the plasma. There appears to be a considerable difference between man and rats with regard to their lipoprotein patterns, which we want to make clear by presenting the overall patterns in Tables 25 and 26 and in Figures 15 and 16 respectively.

However, this distribution of lipoproteins in humans differs from that in several other

*) Theoretically, the free fatty acid (FFA)-albumen junctions with a still higher density must also be reckoned among the lipoproteins.

Table 25. General composition of plasma lipoprotein particles in man (%).

particles	triglycerides	cholesterol	phospho-lipids	proteins
chylomicrons	93	2	3	2
VLDL (d<1.006)	55	15 (esterified 5)	20	10
LDL (d 1.006-(1.019-)1.063)	10	45 (esterified 30)	25	20
HDL (d 1.063-1.210)	2	20 (esterified 15)	45	33

Table 26. Composition of plasma lipoproteins in rats (%), on a chow diet and, in parentheses, with addition of 1% cholesterol.

	triglycerides	cholesterol	phospho-lipids	proteins
VLDL (d < 1.006)	57 (31)	11 (49)	24 (8)	8 (12)
LDL (d 1.006-(1.030-)1.055)	8 (4)	34 (52)	32 (27)	26 (17)
HDL (d 1.070-1.210)	5 (4)	28 (44)	35 (23)	32 (29)
contents in whole serum (mg/100 ml) (According to Lasser et al. 1973)	49 (77)	49 (160)	52 (117)	

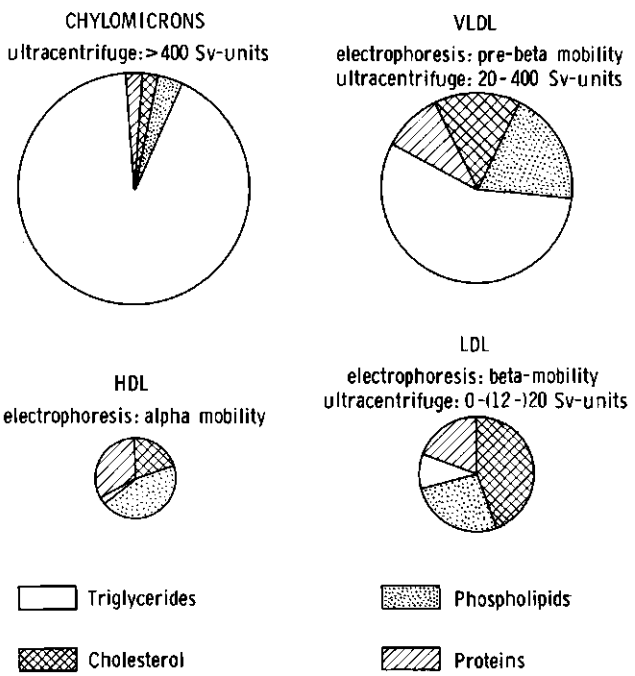

Figure 15. Proportional composition of lipoprotein particles in blood serum of man, with some physico-chemical characteristics.

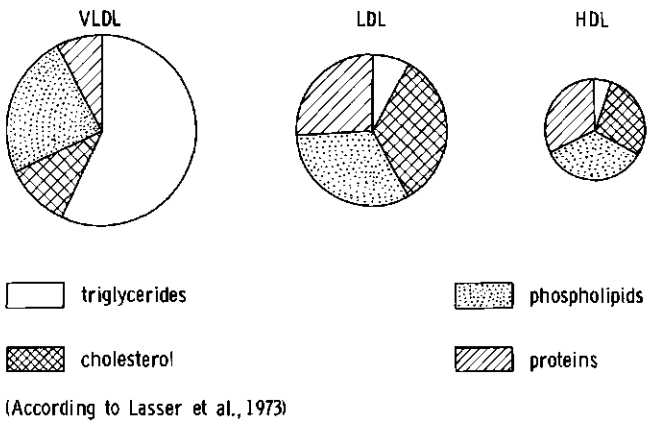

Figure 16. Proportional composition of lipoprotein particles in blood serum of normal (Sprague-Dawley) rats fed on a pelleted diet containing 0.05% cholesterol.

animal species. In Table 26 and in Figure 16 the values are given, as obtained by Lasser et al. (1973), of the composition of lipoproteins in rats, without and (between brackets) with 1% cholesterol added to a chow diet. As is seen in Table 26, there is a marked effect of dietary cholesterol on this composition.

To the significance of the lipoprotein pattern will be reverted to in Chapter 10 (Aortic atheromatosis).

Most of the cholesterol in plasma lipoproteins is derived from the liver and the intestinal mucosa (Dietschy & Wilson 1970), but the evaluation of the respective contributions of these tissues is difficult. The intestinal wall contributes the cholesterol of chylomicrons and, during fasting, that of VLDL in the blood (via the lymph), but the free cholesterol in the chylomicrons and VLDL can be derived from several sources: the diet, bile, desquamated mucosal cells, intra-cellular biosynthesis and the blood itself (Glomset & Norum 1973).

Unlike free cholesterol, the cholesterol esters from the intestinal wall are mainly present in the inner „core" of secreted chylomicrons and VLDL (Zilversmit 1965, Zilversmit et al. 1967). In some cases this contribution of cholesterol esters increases in response to dietary cholesterol (Zilversmit et al. 1967, Zilversmit 1968).

It is also difficult to evaluate the contribution of the liver to the cholesterol in the plasma lipoproteins; VLDL from the liver also contain „core" triglyceride surrounded by „surface" free cholesterol and lecithin (Sata et al. 1972) as well as cholesterol esters formed by the hepatic enzymes cholesteryl ester hydrolase (lysosomal fraction) or the microsomal enzyme acyl CoA : cholesterol acyl-transferase (ACAT), at least in the rat (Gidez et al. 1967). As this latter enzyme seems to be lacking in man (Stokke 1972a and b), all cholesterol esters in human plasma are resulting from the activity of LCAT (Stokke 1974), and no evidence has yet been furnished that cholesterol esters are formed by human liver and secreted in hepatic VLDL (Glomset & Norum 1973).

In the liver, and also in other tissues which are rich in cholesterol, the quantity of free cholesterol is fairly constant and apparently regulated, whereas the amount of cholesterol esters is strongly correlated with the plasma cholesterol level.

In liver diseases the percentage of esterified cholesterol in the plasma is decreased, depending on the severity of the disorder. This percentage of esterification is not

influenced under normal health conditions by dietary measures alone, but can in experimental animals be lowered by feeding cholesterol (Glomset & Norum 1973).

Lipoprotein lipase may play a role in removing triglycerides from chylomicrons and VLDL, leaving „remnants" rich in cholesterol esters, which can be hydrolyzed in the liver; their further fate, however, is not clarified. LDL are probably formed from VLDL in the blood stream, HDL is at least partly directly secreted by the liver.

Norum (1974) suggested that the liberation of lecithin from lipoproteins is facilitated by the continuous impingement of the small HDL particles on the preponderantly larger VLDL, which are abundantly present in the plasma of the obese Zucker rats. Indirect exchange of HDL cholesterol esters for triglycerides from other lipoproteins is also promoted in this way (Glomset 1970, Glomset & Norum 1973).

8.1.2 *LCAT deficiency*

A hereditary disease is described in which, in addition to anaemia, proteinuria and corneal opacity, a hyperlipidaemia occurs with a very low cholesterol esterification percentage (and abnormally low HDL levels) of the blood, caused by a deficiency of LCAT (Norum & Gjone 1967, Norum et al. 1970). The patients have foamy cells in their kidney parenchym and bone marrow, and show a strong tendency to atherosclerosis, but commonly die after adolescence probably from renal involvement in the disease (Glomset et al. 1973).

8.1.3 *LCAT and nutrition*

There is a number of reports on the influence of the diet on the LCAT activity. Gjone et al. (1972) made a study on two groups of five human subjects, who consumed diets with either a saturated or a poly-unsaturated type of fat (in both cases fat contributing 40% of the energy intake) for 21 days. In the persons on the diet with poly-unsaturated fat they observed a decrease in LCAT activity from 47 μmol free cholesterol esterified per litre per hour to 35 μmol. The plasma cholesterol and phospholipid values decreased from 219 to 183 mg/100 ml and from 215 to 156 mg/100 ml respectively, whereas these values on the saturated-fat diet did not change significantly.

Another study was published by D'Alessandro et al. (1975), who reported total as well as free cholesterol levels and LCAT values in the plasma of 61 normal, hypercholesterolaemic or clofibrate-treated subjects. Untreated hypercholesterolaemic patients showed higher LCAT activities than did normal and treated subjects, despite a lower net esterification percentage. An increased LCAT activity could be regarded as a defence mechanism against high levels of free cholesterol.

In their comparative study on the esterification rate of cholesterol, Lacko et al. (1974) pointed to the occurrence of a negative relationship between the susceptibility to atherosclerosis of animal species and the cholesterol esterification in the blood serum. Rats and man were found to be the extreme representatives on either side of the balance with regard to the fractional rate of this esterification as well as to their proneness to atherosclerosis: humans are very susceptible to this vascular process and rats are almost resistant to it. In a recent paper by Wallentin & Vikrot (1976), on the influence of fat ingestion on the LCAT activity in the plasma of normal subjects, it is suggested that an excess of phospholipids stimulates the LCAT activity.

8.1.4 *Design of the present study on LCAT*

The LCAT activity of the plasma is thus established to be a very intriguing and important factor in lipid metabolism and is regarded, in view of the transformation and exchange of VLDL into LDL and HDL, to be a link between triglyceride and cholesterol metabolism

(Wallentin & Vikrot 1976). The determination of the LCAT activity in obese Zucker rats might, therefore, offer valuable information to help explain the differences in plasma cholesterol concentrations that we observed in the present groups of Zucker rats.

It might be possible in this way to establish whether the differences in lipid metabolism observed were linked to the rate of the LCAT activities of the lean and the obese Zucker rats on their various diets.

8.2 Methods

8.2.1 *The* in vitro *assay of LCAT; problems on substrate availability*

In the LCAT assay of the plasma the quantities of the substrates for the biochemical reaction: fatty acids and cholesterol, differ in the various blood samples. Therefore, in the past problems have been sometimes arisen with regard to the availability of these substrates, leading to unreliable results, because the outcome will in several cases have reflected this substrate availability rather than the initial rate of the LCAT reaction, about which we want to be informed (Glomset 1968).

The cholesterol to be esterified will be derived from the lipoprotein particles present in the plasma, mainly from the smaller HDL, which on their turn obtain some of their constituents, particularly lecithin, from VLDL and LDL by exchange (Norum 1974). So, the lipoprotein pattern in the blood may also be of importance for the outcome of the LCAT assay.

The use of pool serum for the *in vitro* incubation has equally led to conflicting results in hyperlipidaemic states; sometimes a decrease and sometimes an increase of the LCAT activity has been reported under this condition (Rose 1972). The specificity of the LCAT enzyme in various species may be due to either the cholesterol/lecithin ratio or to the composition of the apoproteins in the lipoproteins, or to a preference for the fatty acid and the 2-position of the glycerides. In both rats and man the rate of acyl-transfer takes place for approximately 40% via linoleic acid, with palmitoleic, oleic, arachidonic, palmitic and stearic acids further in deceasing order (Glomset et al. 1973).

Stokke & Norum (1971) improved the LCAT determination considerably, a) by using the animal's own plasma for incubation; b) by using a shorter incubation time of sixty minutes for the *in vitro* reaction; c) by applying temporary inhibition by 2-nitrobenzoic acid of the LCAT enzyme for exchange of labelled cholesterol during pre-incubation with the endogenous lipoproteins, with reactivation by mercapto-ethanol; d) by also applying thin layer chromatography for rapid recovery of labelled cholesterol to be counted in a liquid-scintillation counter.

We have followed their method in order to avoid the above-mentioned problems. The only deviation of this method was the use in our case of a different cholesterol isotope, namely $1\alpha, 2\alpha$-^{3}H-cholesterol*).

8.2.2 *Execution of the* in vitro *assay of LCAT*

Approximately 20 μC ^{3}H-cholesterol dissolved in benzene (sufficient for about 50 samples to measure) is evaporated under nitrogen. Immediately after that the cholesterol is dissolved in 0.5 ml acetone. This solution is added, under slow stirring, to a solution of 250 mg albumin in 5 ml phosphate buffer 0.2 M of pH 7.1; the acetone then is evaporated under nitrogen. This cholesterol emulsion can be stored for two weeks at 0 °C.

The determination is carried out *in duplo* and with every sample a blanc is measured. In stoppered glass tubes 100 μl plasma, 30 μl ^{3}H-cholesterol-albumin emulsion and 20 μl

*) Radiochemical Centre Amersham, Buckinghamshire, England; see the footnote on page 45.

of Ellman's reagent (4 mg 2-nitrobenzoic acid in 1 ml phosphate buffer 0.2 M at pH 7.1) are pipetted. After 4 h of incubation in a shaking water bath at 37°C 20 μl mercaptoethanol is added to reactivate the enzyme. To the blanks no activator is added.

After 60 min the reaction is stopped by the addition of 4 ml Folch mixture I (chloroform-methanol 2 : 1). The tubes are then heated for 5 min in a heating block of 60-70°C. The tubes are left for 30 min, during which time they are shaken three times. Then 0.9 ml saline is added and the tubes are vigorously shaken. After centrifugation for 5 min at 4000 routes per minute the content of each tube is transferred to another tube leaving behind the protein membrane. These tubes are also centrifugated and the top layer is sucked off. After addition of 2 ml Folch mixture II (methanol-water-chloroform 43 : 47 : 3) the tubes are again shaken and centrifugated. The top layer is sucked off and the tubes are placed into a water bath of 40-50 °C and the remaining chloroform phase is evaporated under nitrogen. Finally, 0.2 ml hexane is added.

The lipid extract now is transferred to a plate for thin-layer chromatography (5 × 20 cm; layer thickness 0.5 mm silicagel H, Merck, without binding agent). The plates are developed in a mixture of petroleum ether : diethyl ether : acetic acid 85 : 15 : 3 (at 40-60 °C) and the bands are made visible by placing the plates into a developing tank with some iodine pellets. A standard solution of cholesterol and cholesterol esters is used to identify the bands. These bands of cholesterol and cholesterol esters are transferred quantitatively in glass counting flasks. To each flask 3 to 5 ml distilled water and 10 ml instagel (Packard) are added. Counts per minute are determined with a liquid-scintillation counter.

The percentage of cholesterol which is esterified per hour by the LCAT is calculated as follows:

$$\left(\left(\frac{\text{c.p.m.}_{CE}}{\text{c.p.m.}_{CE} + \text{c.p.m.}_{C}} \right)_{\text{sample}} - \left(\frac{\text{c.p.m.}_{CE}}{\text{c.p.m.}_{CE} + \text{c.p.m.}_{C}} \right)_{\text{blanc}} \right) \times 100\%$$

8.3 Results

The results of the determinations of the LCAT activity in the plasma of the rats in the different groups is given in Table 27, together with the corresponding levels of free and total cholesterol.

The plasma cholesterol concentration of the lean control group was again very much lower than that of the obese control group. The latter group had a lower plasma

Table 27. Free and total cholesterol concentrations, cholesterol esterification and LCAT activities in the blood plasma of the groups of Zucker rats fed on the different diets (means and standard errors of the mean).

group	number	free cholesterol (mg/100 ml)	total cholesterol (mg/100 ml)	esterification (%)	LCAT activity (cholesterol esterified/h) (%)	LCAT activity (μmol cholesterol esterified/l/h)
I	6	70.0 ± 3.0	252 ± 18	71.7 ± 1.6	5.16 ± 0.60	93.5 ± 11.1
II	6	66.8 ± 4.4	241 ± 12	72.4 ± 0.9	4.33 ± 0.47	73.1 ± 5.1
III	7	57.3 ± 11.3	231 ± 25	76.5 ± 2.2	5.05 ± 0.63	66.3 ± 8.6
IV	6	63.2 ± 13.6	256 ± 26	76.7 ± 2.8	3.81 ± 0.51	56.7 ± 8.2
V	7	63.7 ± 5.6	214 ± 20	69.5 ± 1.1	5.05 ± 0.53	82.2 ± 10.8
VI	5	49.6 ± 4.0	182 ± 13	72.9 ± 0.6	5.36 ± 0.60	68.6 ± 9.6
VII	7	55.1 ± 2.9	165 ± 14	65.4 ± 3.3	5.14 ± 0.39	74.8 ± 8.9
VIII	5	25.0 ± 3.4	85 ± 7	71.1 ± 1.9	11.80 ± 1.52	72.2 ± 4.0

cholesterol level than the groups fed on the high-poly-unsaturated diets. These on their turn had lower levels than the groups I-IV, which appeared to have more or less similar levels. The variability was largest in the groups III and IV fed on the high-saturated-fat diets, because of some hyperresponders in these groups with respect to the free and total cholesterol levels. These groups also showed the highest proportions of cholesterol esterification.

As can be seen from the table, there are clear variations in the LCAT activities measured. The results obtained for the obese and lean control groups are similar when expressed in absolute amounts of cholesterol esterified, but — because of the nearly two times higher plasma cholesterol concentrations of the obese rats — the relative esterification of cholesterol is approximately twice as high in the lean as in the obese rats.

By two-way analysis of variance there appeared to be statistically significant differences between the groups fed on the semi-synthetic diets, with regard to the dietary fat ($p<0.05$) as well as to the type of carbohydrate used ($p<0.05$), without an interaction between these two factors.

The low-fat diets (I and II), gave higher LCAT activities (in relative as well as in absolute sense) than did the high-saturated-fat diets (III and IV) ($p<0.02$, two-sided), which result can partly be explained by higher levels of supply of free cholesterol. There was no significant difference between the groups fed on the high-poly-unsaturated-fat diets (V and VI) and those fed on the low-fat diets (I and II).

The feeding with poly-unsaturated fat apparently had an enhancing effect on the LCAT activity compared to that with saturated fat ($p<0.05$, two-sided).

The sucrose containing diets gave a decrease of the LCAT activity in the plasma. In groups II and IV this activity was lower in both absolute and relative sense, in group VI the decrease in absolute activity contrasts with an increase in relative activity. This may be related to the lower plasma cholesterol concentration in this group with possibly a lower ratio between the plasma cholesterol and phospholipid concentrations.

8.4 Discussion

In general, a sufficiently high LCAT activity is needed for the lowering of free cholesterol to normal levels in the plasma. The groups III and IV (fed on the high-saturated-fat diets) showed the largest variations in free and total plasma cholesterol values. The most obvious hyperresponding individual rats were found in these groups; possibly the balance between nutritional loading and individual sensitivity is the most critical, notably in these groups.

It may be that obese Zucker rats usually have a relative LCAT deficiency, as their LCAT activities are scarcely higher than those measured in their lean litter-mates, despite a two-fold increase in the plasma cholesterol levels of the obese rats. This deficiency, then, is somewhat less marked when they are fed on low-fat or on high-poly-unsaturated-fat diets. The latter feature is in disagreement with the results of Gjone et al. (1972), who reported lower LCAT values — and also lower plasma cholesterol concentrations — with poly-unsaturated than with saturated fat. In our case this latter effect seems to be the result of a higher LCAT activity, possibly due to some sort of adaptation.

On the other hand, sucrose in the diet gave lower LCAT activities. This result may be related to the blood lipid levels being higher than those attained with the starch-containing diets, as established in the first part of our investigation (Chapter 5, Tables 9 to 12). The tendency of the increased plasma lipid levels in rats fed on the sucrose-containing diets, which will bear some relationship to changes in the lipoprotein composition, to decrease after some weeks, was already discussed. The return of these changes in the

lipoproteins (Nestel & Goldrick 1976) may be connected with the LCAT activities measured in this part of the study.

The plasma cholesterol concentrations, for the rest, had again changed somewhat in the course of time, compared with those contained in Tables 9 (Chapter 5), 17 (Chapter 6) and 22 (Chapter 7). The differences in these plasma cholesterol levels between the groups of Zucker rats may well be due to different LCAT activities in the plasma.

8.5 Summary

1. Obese *versus* lean control rats had similar lecithin : cholesterol acyl-transferase (LCAT) activities when expressed in absolute amounts, but the LCAT activities when expressed relatively to the plasma cholesterol concentrations were approximately twice as high in the lean rats. Obese rats may therefore suffer from a relative LCAT deficiency.
2. There were some statistically significant differences in LCAT activity related to dietary treatment: the low-fat diets gave higher LCAT activities than the high-saturated-fat diets; these latter diets gave also lower values than did the high-poly-unsaturated-fat diets.

   Sucrose appeared to give lower LCAT activities in comparison with starch. No interaction was found between the effects of dietary fat and sugar.

Chapter 9

Lipid content and fatty acid composition of liver and adipose tissue

9.1 Introduction

As one of the final parts of the study, the lipid content and fatty acid composition of the liver and adipose tissue of the rats in the various groups were determined.

The lipid content of the liver, subdivided into the lipid classes, as well as the cholesterol content of adipose tissue and the fatty acid composition of both tissues, could be measured after the rats had been sacrificed. The fatty acid composition of body fat is known to be, in the long run, very susceptible to dietary treatment. An interesting point could also be the difference in the cholesterol content of the large fat stores of the obese Zucker rats.

9.2 Methods

For this part of the study the same rats were used as those which had been participating in the study described in Chapters 7 and 8. They were sacrificed, by bleeding, at least ten days after they had been re-fed on their respective experimental diets, at an age of approximately eight months, after overnight fasting.

The livers were removed and weighed. As the site of adipose tissue to investigate, perirenal fat was chosen. Approximately 1 g of this fat was taken out for the lipid determination.

9.2.1 *Adipose tissue preparation*

This tissue was weighed, homogenized in a top-drive homogenizer (Silverson) after having been mixed with a quantity of chloroform-methanol (2 : 1), and further extracted by the method of Folch et al. (1957).

The cholesterol content was measured by the enzymatic method as mentioned in Chapter 5.

The fatty acid composition was determined by means of gas-liquid chromatography (Badings et al. 1975) of the methyl esters (Metcalfe et al. 1966) of the fatty acids in the fat.*)

9.2.2 *Liver preparation*

The livers were homogenized vigorously in a Waring blender, and the fat of exactly weighed portions of liver tissue was extracted according to Folch et al. (1957).

For the measurement of total fat, the extract then was dried and weighed. Total cholesterol and triglycerides were assayed enzymatically (see Chapter 5). Free cholesterol and phospholipids were determined by means of thin-layer chromatography of the fat extract from the livers (Van Gent 1968). The procedure for the determination of the fatty acid composition was identical with that for the determination of the perirenal fat.

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*) We thank our colleague Dr. Ir. H. T. Badings for providing the facilities for carrying out the fatty acid determinations.

9.3 Results

9.3.1 *Cholesterol content of adipose tissue*

The cholesterol content of the perirenal adipose tissue of the groups of rats fed on the different diets is given in Table 28.

Table 28. Cholesterol content of perirenal adipose tissue of the groups of rats fed on different diets (means and standard errors of the mean).

group	number	mg cholesterol per g adipose tissue	mg cholesterol per g fat
I	6	1.02 ± 0.08	1.16 ± 0.09
II	6	0.92 ± 0.05	1.06 ± 0.07
III	7	0.92 ± 0.07	1.01 ± 0.06
IV	6	0.84 ± 0.06	0.99 ± 0.08
V	7	0.97 ± 0.08	1.13 ± 0.07
VI	5	0.94 ± 0.11	1.13 ± 0.14
VII	7	0.90 ± 0.11	0.98 ± 0.10
VIII	5	0.51 ± 0.11	0.61 ± 0.14

In the obese rats of all groups the cholesterol values were significantly higher than those in the lean controls ($p<0.001$). This is in agreement with the results obtained in the cholesterol turnover study (Chapter 6), in which all groups of fatty rats, in contrast to group VIII, were found to have reached their maximal cholesterol excretion.

Because of the enhanced fat mass of obese rats, the actual cholesterol stores in their adipose tissue were calculated to be more than twelve times higher than those of lean rats.

The group means of the obese rats did not differ significantly.

9.3.2 *Liver weights*

The mean liver weights of the rats are given, per group, in Table 29, together with the corresponding percentages of their body weights.

Table 29. Liver weights and their percentages of the body weight for the groups of rats fed on different diets (means and standard errors of the mean).

group	number	body weight (g)	liver weight (g)	percentage of body weight (%)
I	6	470 ± 14	21.4 ± 1.8	4.54 ± 0.33
II	6	439 ± 18	24.0 ± 2.0	5.44 ± 0.29
III	7	491 ± 9	19.4 ± 0.8	3.96 ± 0.15
IV	6	471 ± 20	18.3 ± 1.1	3.93 ± 0.30
V	7	494 ± 15	17.6 ± 0.9	3.56 ± 0.15
VI	5	515 ± 12	17.6 ± 0.7	3.43 ± 0.16
VII	7	447 ± 10	18.4 ± 1.4	4.16 ± 0.35
VIII	5	315 ± 14	8.6 ± 0.4	2.74 ± 0.08

The differences in absolute and relative liver weights between the control rats in groups VII and VIII were highly significant ($p<0.001$). The lean rats weighed approximately 1.5 times less than did the fatties, but the liver weights of the latter were more than twice as heavy as those of their lean litter-mates.

The liver weights and their percentages of the body weight were statistically significantly different: they were higher in the groups fed on the low-fat diets than in those fed

on the saturated-fat diets ($p<0.02$, two-sided) and than in those fed on the poly-unsaturated-fat diets ($p<0.01$, two-sided).

The poly-unsaturated-fat diets gave slightly lower values than did the saturated-fat ones, but the difference was not significant.

Sucrose caused a statistically significant difference exclusively in the rats fed on the low-fat diets (groups I and II, of which the latter had a very much higher percentage of sucrose than groups IV and VI). This difference between group I and II only concerned the relative liver weight ($p<0.01$, one-sided) as a consequence of the lower body weights in group II (see Table 29).

With respect to the liver weights there was no statistically significant interaction found between the effects of dietary fat and sucrose.

9.3.3 *Lipid contents of the liver*

A high percentage of the livers of the obese rats clearly showed a fatty appearance. This was particularly the case in the rats fed on the low-fat diets, and most extremely so in group II, to which 60% of sucrose had been given in the diet. The results of the determinations on the hepatic lipid fractions of the groups of rats are given in Table 30.

Table 30. Lipid fractions of the livers of the groups of rats fed on different diets.

group	number	total fat (mg/g)	phospho-lipids (mg/g)	triglycerides (mg/g)	total cholesterol (mg/g)	free cholesterol (mg/g)	esterification (%)
I	6	133.3* ± 18.9	17**	75.2* ± 10.7	10.4* ± 1.1	2.8**	73
II	6	244.8 ± 19.7	22	170.8 ± 11.5	15.9 ± 1.5	2.6	84
III	7	103.0 ± 13.7	23	67.1 ± 10.9	6.1 ± 0.9	1.6	74
IV	6	71.3 ± 8.6	15	42.7 ± 5.7	3.8 ± 0.9	1.3	67
V	7	66.7 ± 5.0	21	36.6 ± 2.7	4.5 ± 0.5	1.7	63
VI	5	44.6 ± 2.4	12	26.4 ± 2.5	3.1 ± 0.5	1.5	53
VII	7	87.6 ± 16.8	17	65.7 ± 11.9	3.2 ± 0.5	1.3	59
VIII	5	18.0 ± 2.0	12-15	1.8 ± 0.2	0.9 ± 0.2	0.7-0.9	20 ***

*) Means and standard errors of the mean.
**) Pooled samples, after thin-layer chromatography.
***) The quantity of esterified cholesterol was so low that a very accurate figure for this could not be given.

There are large differences between the groups with respect to the hepatic lipid contents.

The figures for total fat, triglycerides and total cholesterol of the obese control group are very significantly higher than those of the lean control group ($p<0.001$).

The highest figures for total fat, triglycerides and cholesterol are observed in the groups fed on the low-fat diets (groups I and II). These figures differ significantly from those found in the other groups of obese rats ($p<0.001$).

Regarding the effect of the type of dietary fat, group III (high-saturated fat) did not differ significantly from group I (low-fat), but group V (high-poly-unsaturated fat) did ($p<0.001$, two-sided); this was true for total fat as well as for triglycerides. In the case of total cholesterol it was not only group V which differed significantly from group I ($p<0.002$, two-sided) but also group III ($p<0.01$, two-sided). Group V differed significantly from group III only with respect to total fat and triglycerides ($p< 0.05$, two-sided).

The livers of the rats in group II with sucrose in the diet had significantly higher lipid contents even than those of the animals in group I with starch ($p<0.01$, one-sided). The

Table 31. Levels of significance (probabilities)*) from corrected orthogonal polynomials in two-way analysis of variance, followed by „Student's" *t*-test, for contrasts between liver weights and between hepatic lipid contents.

		F-values									
treatment	dimension	liver weight	P	percentage liver weight	P	total fat	P	trigly-cerides	P	chole-sterol	P
dietary fat (A)	2	0.27	n.s.	1.93	n.s.	4.07	= 0.05	61.97	<0.001	0.73	n.s.
sucrose *vs* starch (B)	1	8.98	<0.005	22.26	<0.001	68.90	<0.001	8.94	<0.005	62.01	<0.001
A × B (interaction)	2	1.13	n.s.	2.96	n.s.	22.37	<0.001	28.80	<0.001	10.53	<0.001
variance	34	9.60		32.19		850.23		436.39		50.93	
		t-values									
low *vs* high-saturated fat	t_{23}	3.08	<0.005	4.70	<0.001	8.23	<0.001	8.28	<0.001	9.23	<0.001
low *vs* high-poly-unsaturated fat	t_{22}	4.05	<0.005	6.60	<0.001	11.51	<0.001	10.94	<0.001	10.35	<0.001
saturated *vs* poly-unsaturated fat	t_{23}	1.04	n.s.	2.02	= 0.05	2.77	<0.02	2.85	<0.02	1.30	n.s.
sucrose *vs* starch (groups): group I *vs* group II	t_{10}	1.51	n.s.	2.81	<0.05	6.76	<0.001	8.09	<0.001	4.35	<0.002
groups III and V *vs* groups IV and VI	t_{23}	0.37	n.s.	0.34	n.s.	2.31	= 0.05	2.07	= 0.05	2.05	= 0.05

*) two-sided
n.s.: not significant

same was found for triglycerides and total cholesterol. On the other hand, the hepatic lipid values in the groups fed on the high-fat diets containing sucrose compared to starch were lower, but in neither case significantly so.

There was a very significant interaction between the effects of dietary fat and sucrose on total fat, triglycerides and cholesterol of the liver ($p<0.001$).

The various levels of significance of the dietary effects on the hepatic lipid contents as well as on the liver weights are shown in Table 31, in a similar way as was done for the plasma cholesterol and triglycerides in Chapter 5 (Tables 10 and 12).

The differences in free cholesterol and phospholipid content between the groups appeared to be fairly small, in good agreement with what is already known e.g. about free cholesterol, namely that any excess of cholesterol in the liver occurs in the esterified form (Glomset 1968).

9.3.4 *Fatty acid composition of liver and perirenal fat*

The figures determined for the fatty acid composition of the liver and the perirenal tissue are presented in Tables 32 and 33. Only fatty acids representing more than 1% are mentioned, but those given in these tables make up over 95% of the total in both tissues.

Table 32. Fatty acid composition of the liver fat from the groups of rats fed on different diets, as percentages of the total fat (pooled samples).

group	palmitic acid ($C_{16:0}$)	palmitoleic acid ($C_{16:1}$)	stearic acid ($C_{18:0}$)	oleic acid ($C_{18:1}$)	linoleic acid ($C_{18:2}$)	arachidonic acid ($C_{20:4}$)	$C_{16:1}$ / $C_{16:0}$ ratio	$C_{18:1}$ / $C_{18:0}$ ratio	$C_{18:2}$ / $C_{18:1}$ ratio
I	36	12	5	40	2	1	0.33	7.8	0.06
II	36	13	4	42	1	1	0.37	11.0	0.03
III	35	7	7	42	2	4	0.19	5.9	0.06
IV	31	7	9	41	2	4	0.22	4.6	0.06
V	29	5	11	26	16	7	0.17	2.4	0.64
VI	31	4	13	25	15	7	0.14	2.0	0.60
VII	34	8	6	36	7	3	0.23	5.8	0.20
VIII	29	3	24	16	13	9	0.10	0.7	0.82

Table 33. Fatty acid composition of the perirenal fat from the groups of rats fed on different diets, as percentages of the total fat (pooled samples).

group	palmitic acid ($C_{16:0}$)	palmitoleic acid ($C_{16:1}$)	stearic acid ($C_{18:0}$)	oleic acid ($C_{18:1}$)	linoleic acid ($C_{18:2}$)	$C_{16:1}$ / $C_{16:0}$ ratio	$C_{18:1}$ / $C_{18:0}$ ratio	$C_{18:2}$ / $C_{18:1}$ ratio
I	26	10	3	54	5	0.37	17.5	0.09
II	26	11	3	53	5	0.44	20.4	0.09
III	28	6	4	57	4	0.20	13.2	0.07
IV	26	6	4	59	4	0.22	15.2	0.07
V	25	5	3	35	30	0.18	12.7	0.86
VI	24	4	3	37	29	0.17	10.5	0.79
VII	29	8	3	44	12	0.27	13.0	0.27
VIII	27	5	6	36	18	0.18	5.5	0.52

Marked differences were found in the fatty acid composition of liver and perirenal fat. All values for oleic and linoleic acid were significantly higher in perirenal than in liver fat, in contrast to those for arachidonic acid, which was scarcely present in perirenal fat. Palmitic and stearic acid occurred in higher percentages in liver than in perirenal fat.

Regarding the differences between the two phenotypes, the lean rats had, in both liver and perirenal fat, lower proportions of oleic, palmitic and palmitoleic acid, but higher proportions of linoleic acid than the obese rats on the control diet. This is in agreement with the findings of Wahle (1974); the arachidonic acid content of liver fat was also higher in lean than in obese rats.

Also, the lean rats had, in liver and perirenal fat, lower ratios of mono-desaturation (by microsomal Δ9-acyl-CoA desaturase) for palmitoleic and palmitic acids ($C_{16:1}/C_{16:0}$) and for oleic and stearic acids ($C_{18:1}/C_{18:0}$) than the obese rats on the same diet. A higher ratio as concerned here in the obese rats would be indicative not merely of an increased Δ9-desaturation, but also of an enhanced lipid synthesis, either solely in the liver (see Figure 17) or in general, as stated originally by Winand et al. (1968), from studies in obese mice, and confirmed by Wahle (1974) and York (1975b). On the other hand, the ratio between linoleic acid and oleic acid ($C_{18:2}/C_{18:1}$) was higher in the lean than in the obese rats fed on the control diet.

With regard to the dietary effects, the rats fed on the low-fat diets showed similarities as well as differences with those fed on the high-fat diets. They had, in liver and perirenal fat, similar proportions of oleic and linoleic acid when compared to those fed on the high-saturated-fat diets, but higher proportions for oleic and lower proportions for linoleic acid when compared to those fed on the high-poly-unsaturated-fat diets, and a lower proportion of arachidonic acid in the liver fat than the rats fed on the high-fat diets. Further, they had higher ratios of mono-desaturation, in liver and perirenal fat, than the rats fed on the high-fat diets (and the commercial ration).

The corresponding conclusions from these ratios of the fatty acids and the figures for hepatic lipid contents given in Table 30 justify the conclusion that the high fat contents in the livers are the result of an increased lipogenesis in the obese rats.

The high-poly-unsaturated-fat diets gave very much higher values for the linoleic acid content of liver and perirenal fat and a higher proportion of arachidonic acid in the liver fat than did the high-saturated-fat diets — mainly at the costs of oleic acid —, as was expected from the very high levels of the groups V and VI. The ratios of mono-desaturation were somewhat lower on the high-poly-unsaturated-fat than on the high-saturated-fat diets. Nevertheless, we observed that saturated fat in the diet of obese Zucker rats reduced lipid synthesis, although to a somewhat lesser extent than does poly-unsaturated fat. This latter effect was found already by Wahle & Radcliffe (1975), who established this also for lean Zucker rats. The $C_{18:2}/C_{18:1}$ ratios were very much higher on the poly-unsaturated-fat diets compared with those of the saturated-fat diets.

Sucrose compared to starch exerted, in the low-fat diets (groups I and II) only minor effects on the fatty acid composition of both liver and perirenal fat, despite the large differences in hepatic lipid contents between these groups; the ratios for mono-desaturation in liver and perirenal fat were somewhat higher in group II (with sucrose) than in group I (with starch). In the high-fat diets, the presence of sucrose did not have any significant effect as to the fatty acid composition and perirenal fat.

When viewing the Tables 32 and 33 on the whole, it appears that the linoleic/oleic acid ratios of the groups fed on the high-poly-unsaturated-fat diets are more or less similar to those of the lean control rats. The obese rats fed on the commercial ration — being also a low-fat diet — had ratios for this which were intermediate between those of the rats in the groups I to IV and in the groups V, VI and VIII. From the scale of the ratios

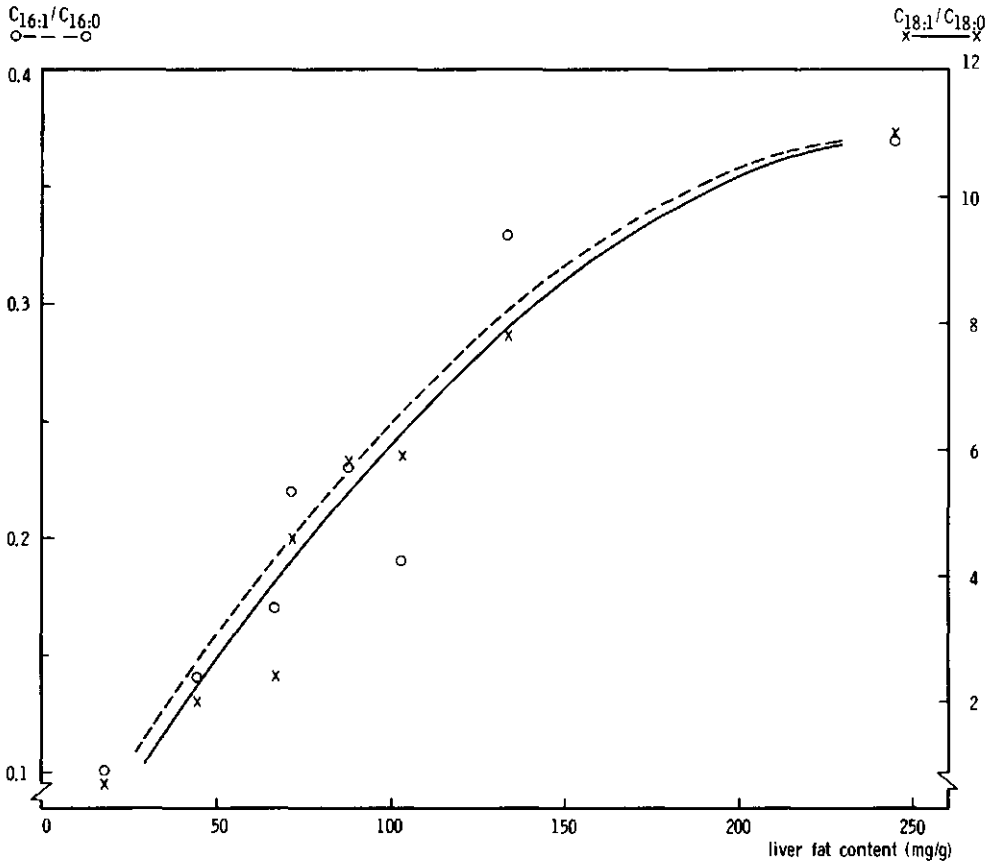

Figure 17. Relationship between total liver fat and the mono-desaturation ratios of the fatty acids from liver fat (cf. Tables 30 and 32).

of mono-desaturation we presented, because of the importance as to their relationship with lipid synthesis, a graph in which these ratios for liver fat were plotted against the total hepatic lipid contents of the respective groups as given in Table 30 (Figure 17).

9.4 Discussion

9.4.1 *Cholesterol content of adipose tissue*

The results of our study differ to some extent from those obtained by Angel & Farkas (1970) and Farkas et al. (1973), who found, in normal rats, a range of 0.6 to 1.6 mg cholesterol per g of adipose tissue (wet weight), approximately 90% of which was, as part of cell membranes, in the form of free cholesterol and made up approximately 0.1% of the triglyceride content. We found levels in this range, making up also approximately 0.1% of the fat, for all obese rats (which we regarded as to store cholesterol because of a limited capacity for cholesterol excretion) but, for the lean rats, lower levels than the minimum indicated by the above-mentioned authors.

Our finding of a clear difference in the cholesterol content of adipose tissue fat between obese and lean Zucker rats, expressed in mg per g of fat, was in disagreement with what was reported, for obese and lean Bar Harbor mice, by Angel & Farkas (1974),

who quoted almost similar figures in this respect. The larger storage of body cholesterol occurring in absolute amounts in obese subjects, however, has been generally agreed already for a long time (Nestel et al. 1969).

Angel & Farkas (1974), in a study on male Wistar rats using 0.05, 0.1, 0.5 and 5% cholesterol in their diets, concluded to a dependency of cholesterol storage on dietary cholesterol. These workers, furthermore, observed (without any change in membrane lipid composition) a linear relationship between the quantity of adipose tissue cholesterol and body weight (Farkas et al. 1973). Björntorp & Sjöström (1972) established a positive relationship between adipose tissue cholesterol and fat cell size in man. Nestel et al. (1969 and 1973) confirmed such a relationship of the total adipose tissue mass of humans with the quantity of cholesterol therein. Schreibman & Dell (1975) found, in addition, that cholesterol synthesis is increased in obesity.

The lack of any significant dietary effect on the cholesterol content of the adipose tissue in obese Zucker rats will be related to their similar cholesterol excretion capacities; as put forward in Chapter 6, these were regarded as to be fixed to a maximum. It must be kept in mind, however, that notably in the group fed on the low-fat diet in that part of the study the mass of the slowly exchangeable pool B (being the average between its maximal and minimal mass calculated) was found to be significantly higher than in the other groups. The cholesterol content of the perirenal fat from the various groups did not provide a reflection of the different distribution of cholesterol over the hypothesized, imaginary tissue pool masses. There will be, however, other sites for cholesterol storage (Crouse et al. 1972, Green et al. 1976), for instance the liver (Tables 28-30).

In an obese rat of 500 g with 40% body fat, adipose tissue containing 1 mg cholesterol per g can store about 200 mg of this compound, whereas a liver of 20 g, which can store an additional quantity of 10 mg cholesterol per g, will then contain a similar quantity thereof; it looks that the liver can store a slightly larger quantity of cholesterol than the adipose organ of an obese rat (Green et al. 1976). In lean rats not more than only 10 mg cholesterol will be present in their livers and approximately 15 mg in their adipose tissues.

9.4.2 *Liver weights and lipid contents of the liver*

The higher liver weights of the groups of rats fed on the low-fat diets are related to the increased hepatic lipid contents. This is already clear when the data in Tables 29 and 30 are compared. The low-fat diet containing starch as provided to group I did not cause growth depression (see Chapter 4, Table 7), although it showed a clear tendency to induce fatty livers (Table 28).

Whereas the liver fat content was higher in the groups fed on the low-fat diets than in the groups fed on the high-fat diets, the situation with regard to the blood was different. In the plasma, the triglyceride levels (but not the cholesterol concentrations) of the groups I and II were lower than those of the groups on the high-fat diets. Apparently, there is a shift in groups I and II of triglycerides from the blood to the liver. This may indicate an inability of the livers in these groups (and to some extent also in group VII) to synthesize lipoproteins, possible as a result of an impaired protein metabolism (Martin 1976).

Of the groups fed on the high-poly-unsaturated-fat diets, the figures in Table 29 and those for total fat and triglycerides in Table 30 were all lower than those of the groups fed on the high-saturated-fat diets, but the differences were not statistically significant.

As confirmed by the calculated statistical interaction, the extremely high values of hepatic lipids in group II are apparently the result of the combination of sucrose with a

low-fat diet (interaction). The poorer weight gain of the rats in this group may have been caused by an impaired liver function. This adverse influence of sucrose in a low-fat diet has already been observed in obese mice by Winand & Christophe (1977). The extremely fatty livers of these rats may be related to the low plasma triglyceride levels of these animals on the fourth day of the fasting period as compared with that of the rats in group I (Chapter 7, Table 22), as a result of a lowered fat mobilization from the liver.

That similar effects were not observed in groups IV and VI may be caused by the level of sucrose in the diet, which in high-fat diets can never reach a maximum as high as in low-fat diets. Aoyama & Ashida (1973), reporting a similar result, found that sucrose-induced lipogenesis was prevented even by feeding large proportions of lard, in studies with conventional rats, fed on repletion diets for three days, after protein depletion for fourteen days; later these authors stressed the role of linoleic acid in this respect (Aoyama et al. 1974). The linoleic acid content of the diets fed to groups I, II, III and IV, however, was similar. Therefore it may be that the proportion of sucrose in the diets is critical, rather than the linoleic acid content. The level of approximately 39 energy % (see Table 2, Chapter 3) in a high-fat diet was, anyway, apparently not sufficient to produce such fatty livers as to cause impaired growth.

As there is, on the one hand, a combined effect of dietary fat and sugar (interaction, see above), on the other hand, there is a difference between the effects of either dietary fat or sucrose, in that sense that already partial replacement of sucrose by any type of fat in the diet apparently exerts a more beneficial effect on the condition of the liver than does total replacement of sucrose by starch in a low-fat high-sucrose diet.

This conclusion drawn from our study in obese animals may not be valid for man, because the genetically obese rodents (Zucker rats as well as Bar Harbor mice) are known to have a shift of triglycerides from the plasma to the liver compared to normolipidaemic humans. In contrast to man, these rodents have, when fed on high-fat diets, very much higher levels of triglycerides in the plasma — which are mainly of dietary origin — than on low-fat diets, as stated by Schonfeld & Pfleger (1971), Lemonnier et al. (1974) and Hunt et al. (1976). Humans normally have higher plasma triglyceride levels on low-fat than on high-fat diets, so that well-established differences exist between man and our animals with regard to fat metabolism.

Winand (1968) as well as Olefsky et al. (1974b) and York (1975a) suggested that insulin levels contribute to the increased fatty acid synthesis when low-fat diets are provided. This could be then brought about by the action of acetyl CoA-carboxylase, the enzyme involved in the first steps of lipogenesis (Maragoudakis et al. 1974).

9.4.3 *Fatty acid composition*

The differences between the fatty acid composition of hepatic and perirenal fat correspond with differences in lipid composition between these tissues. Liver fat consists for approximately two-thirds of phospholipids with relatively high quantities of arachidonic, stearic and palmitic acid, and for approximately one-third of triglycerides, relatively rich in palmitoleic, oleic and sometimes linoleic acid. Adipose tissue contains almost exclusively triglycerides.

A fair agreement seems to exist between the results mentioned in Tables 32 and 33 for obese and lean rats fed on the commercial ration (groups VII and VIII) and those reported by Wahle (1974).

A number of abilities with respect to fat synthesis of liver tissue from obese Zucker rats, in comparison with Wistar rats, was recently described by Chanussot & Debry (1977a and b) and is mentioned broadly in Chapter 2 (liver metabolism) of our study.

The proportion of dietary fat as well as its composition influence the fatty acid

composition of body fat. The similar tendency of both ratios for mono-desaturation of fatty acids ($C_{16:1}/C_{16:0}$ and $C_{18:1}/C_{18:0}$) given in Figure 17 can be explained by the relationship between these ratios and lipid synthesis, which latter increases as the diet is lower in fat content. This could be due to the influence of insulin (Wahle 1974); this was also suggested by York (1975b) from studies with genetically obese mice. It was already well-established in normal mice and rats that lipogenesis can be decreased by dietary treatment in the following order: fat-free, palmitate, oleate, linoleate (Bartley & Abraham 1972). Therefore, we are allowed to regard the high fat contents in the livers of the obese rats (see Table 30), in particular those in group I and still more so in group II, as to be clearly the result of increased hepatic lipogenesis.

Wahle & Radcliffe (1975) stated that supplementary linoleic acid, in the form of sunflower oil added to the diet, decreased the ratios of mono-desaturation $C_{16:1}/C_{16:0}$ and $C_{18:1}/C_{18:0}$ in liver lipids from obese as well as from lean Zucker rats, indicating a decreased Δ9-desaturase activity. We were able to confirm their observations in obese rats, but can also conclude that the same features, although to a somewhat lesser extent, can be brought about by addition of a saturated type of fat to the diet. This may be due to the presence of a fair amount of oleic acid in the dietary fat (Triscari et al. 1978). These authors reported a lower lipogenesis in the livers of female Charles River rats when fed on a diet with 20% corn oil containing, besides 46% linoleic acid, 32% oleic acid than when fed on a diet with 20% hydrogenated (soybean) oil with 87% stearic and 11% palmitic acid.

9.5 Summary

1. Perirenal fat of obese Zucker rats was found to contain almost twice as much cholesterol per g adipose tissue as that of lean controls, which will imply an approximately 12 times higher storage of cholesterol in the total body of obese rats.

   There was no dietary effect as to the cholesterol content of the adipose tissue in the obese rats.

2. Obese rats compared to lean controls have very significantly higher liver weights and hepatic lipid contents ($p<0.001$).

   They also have a different fatty acid composition of liver and perirenal fat, with larger proportions of palmitic, palmitoleic and oleic acid, and lower proportions of stearic, linoleic and arachidonic acid.

   The ratios of mono-desaturation ($C_{16:1}/C_{16:0}$ and $C_{18:1}/C_{18:0}$) indicative of lipogenesis, were very much higher for the obese than for the lean rats.

3. The livers of the groups fed on the low-fat diets were very significantly heavier ($p<0.001$) with very significantly higher contents of total fat, triglycerides and total cholesterol than those of the other groups ($p<0.001$), particularly and significantly so with regard to all these aspects ($p<0.01$), when the diet also contained sucrose.

4. High-poly-unsaturated-fat diets compared to high-saturated-fat diets gave a significant difference, with regard to the hepatic contents, only for the triglyceride levels ($p<0.005$).

5. The presence or absence of sucrose in the high-fat diets, given for a period of approximately six months, did not have any significant effect, neither on the liver weights nor on the hepatic contents of total fat, triglycerides and total cholesterol.

6. The quantity as well as the type of dietary fat had significant effects on the fatty acid composition of liver and perirenal fat.

   Rats fed on low-fat diets, when compared with rats fed on high-saturated-fat diets, showed higher proportions of palmitoleic, but lower proportions of stearic and

arachidonic acid were found; the proportions of palmitic, oleic and linoleic acid were similar.

Rats fed on the high-poly-unsaturated-fat, when compared with rats fed on the high-saturated-fat diets, had higher proportions of linoleic and arachidonic acid, mainly at the cost of oleic acid, whereas the proportion of palmitoleic acid was slightly lower.

7. The ratios of mono-desaturation were very high for the low-fat diets, in particular when combined with sucrose, with lower ratios for the high-saturated-fat diets, and slightly more so for the high-poly-unsaturated-fat diets.

   These findings permit the high lipid contents of the liver to be regarded as being the result of increased hepatic lipogenesis.

   Hepatic lipid synthesis, thus, can be diminished, not only by the addition of poly-unsaturated fat but, to a large extent, also by saturated fat.

8. Sucrose in the diet, in comparison with starch, had no effects on the fatty acid composition of liver and perirenal fat.

9. A very significant interaction was found between the effects of dietary fat and carbohydrate on the total fat, triglyceride and cholesterol contents of the liver, in the sense that sucrose had an enhancing lipogenetic effect, leading to hepatic steatosis and growth depression, only when provided in a low-fat diet.

   Even partial replacement of dietary sucrose by fat seems to have a more beneficial effect on the condition of the liver, in obese animals, than does the total replacement of sucrose by starch.

Chapter 10

Aortic atheromatosis

„A general lesson to be learned . . . is the importance of looking out for a good experimental material when trying to tackle a specific biological problem."

Hans A. Krebs (1975),
J. Exp. Zool, 194, *221-226*

10.1 Introduction

The oldest rats from groups II and IV, which had been excluded from the cholesterol turnover study (Chapter 6) were kept alive for several months and were finally sacrificed at the age of approximately nine months for an investigation of their aortas and main arterial branches.

It was attempted to establish whether any degree of atherosclerosis had occurred in these groups, which on the basis of their increased plasma cholesterol levels offered the best chance of having developed vascular abnormalities of this kind. This concerned the groups fed on the low-fat and the high-saturated-fat diets, both with sucrose.

10.2 Method

Immediately after the rats had been sacrificed, their aortas were taken out. They were stored in formalin 4%, and were subsequently stained with 3% Sudan III/IV 1 : 1 in equal amounts of acetone/ethanol, and finally rinsed in dilution with distilled water and with 50% ethanol (Kloeze et al. 1969). An evaluation was made by eye, and the preparations obtained were photographed. These photographs are given in Figure 18.

Any final positive indication of fat deposition or of more advanced stages of atherosclerosis was intended to be represented by a figure ranging from 0 to 4 (Hermus 1975).

10.3 Results

Neither in group II nor in group IV (final body weights 482 ± 18 (s.e.m.) g (n = 6) and 554 ± 29 g (n = 5) respectively) could any sign be observed of an atherosclerotic process in the aortas. The photographs that were made after the preparations had been stained, do not show any vascular lesion which can be identified as being of an atherosclerotic nature.

10.4 Discussion

Neither atherosclerosis nor fatty infiltrations of the large arterial vessels was observed in the two groups of obese Zucker rats. Atherogenesis is not related to high-tissue cholesterol of the organs but to its plasma concentrations, particularly to the cholesterol in low-density lipoproteins. The hyperlipoproteinaemia in Zucker rats is mainly the result of the increase of very low-density lipoproteins (Schonfeld & Pfleger 1971). Therefore, our observation is not surprising.

It was already known that the conventional rat is not a suitable model for atherosclerosis research in general, although it may be a good experimental animal for studies on lipid metabolism of various kinds. This is not due only to its low plasma cholesterol concentration under normal conditions, as even after dietary manipulation to induce hypercholesterolaemia vascular involvement is a rare phenomenon.

Atherosclerotic deviations have been described in the Koletsky rat, which is also

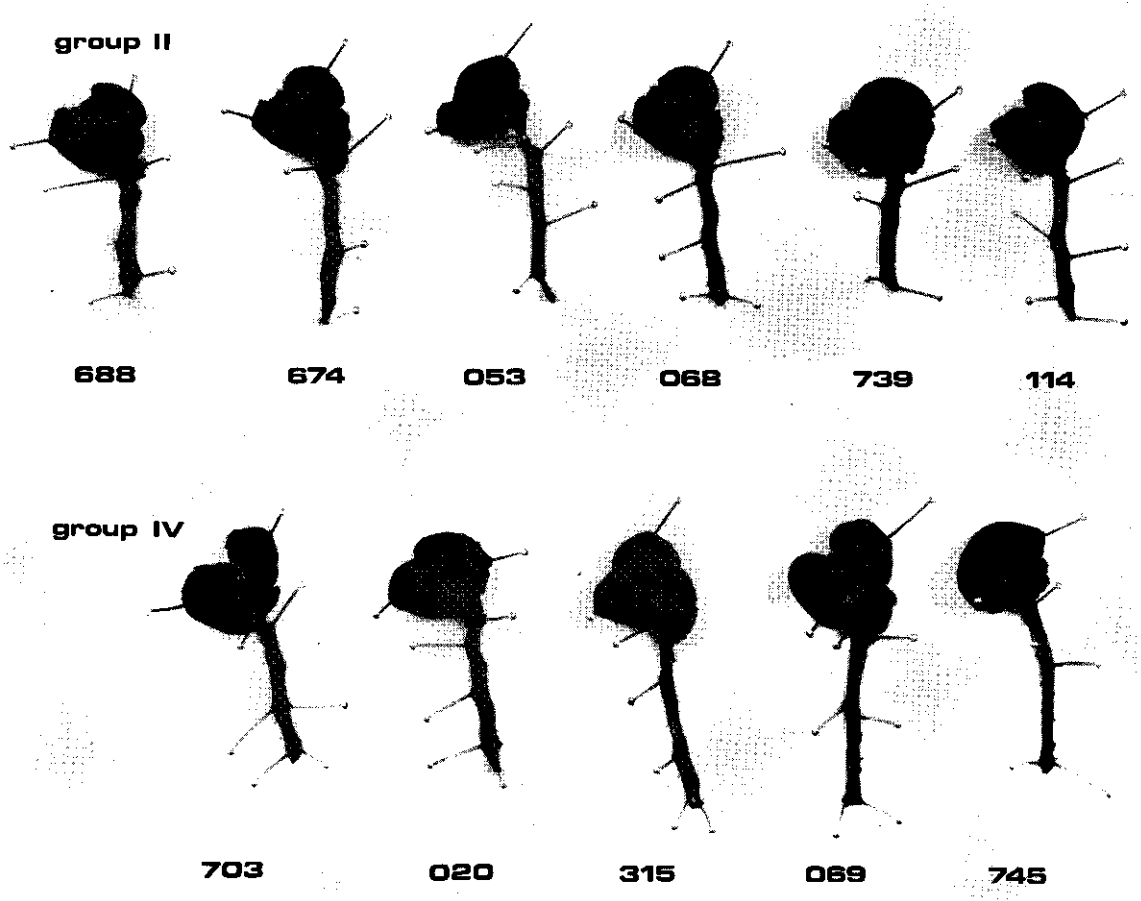

Figure 18.
Reproduction of the heart and aorta preparations of groups II and IV after approximately nine months of dieting.

hypertensive (Koletsky 1973 and 1975a and b*). In BHE rats (Berdanier 1974a) the frequency of the occurrence of atherosclerotic lesions is positively related to the carbohydrate content of the diet (Berdanier et al. 1974b). The diabetic rat fed on high sucrose diets, as it was used by Cohen et al. (1972), showed disturbances only of the small renal arterial vessels (Cohen et al. 1977).

The absence of atherosclerotic processes even under these circumstances may be the consequence of the mode of distribution of the lipid classes over the lipoproteins present in the blood plasma. In man, chylomicrons and very low-density lipoproteins (VLDL) contain mainly triglycerides, whereas cholesterol occurs predominantly in low-density lipoproteins (LDL), and phospholipids are typically the main representatives of the high-density lipoproteins (HDL), as reflected already in Figure 15 and in Table 25 (see Chapter 8).

Even animal species within the same genus, as are the New World monkeys, may show significant differences with respect to the above-mentioned metabolic balance: the squirrel monkey (*Saimiri sciureus*) appears to resemble the human „model" in its proneness to atherosclerosis at high plasma cholesterol levels, whereas the cebus monkey (*Cebus albifrons*) under these circumstances does not develop any vascular lesion of this kind. This is thought to be related to significant differences in their lipoprotein composition (Nicolosi et al. 1977).

This latter phenomenon observed in the cebus monkey also occurs in rats, even in the obese Zucker rat, as seems likely from our study which was made when our laboratory rats were at the approximate age of 9 months and had been on a diet for about 7½ months. This may be connected with the distribution of the plasma cholesterol over the various lipoprotein classes which was not investigated in our study.

The same conclusion was drawn by Zilversmit et al. (1977) from a study on mink showing plasma cholesterol concentrations similar to those in human subjects living in industrialized countries, when the animals were fed on a commercial diet moderately high in cholesterol or on a cholesterol-free semi-purified diet. In contrast to the situation in man, 80% of the plasma cholesterol in mink was in the HDL fraction.

Moreover, the esterification of cholesterol has some relation to the type of lipoprotein (see Table 26, approximating the situation in man).

In our opinion it is irrelevant to apply the ordinary system of phenotyping hyperlipoproteinaemias, which may have validity in human disease, in animals because of the differences in lipoprotein patterns between man and animal species, e.g. the Zucker rat. The composition of the food may, therefore, also have different effects between the latter and man, not so much with respect to blood lipid levels as to atherogenesis.

There are indications from the literature that cholesterol in the HDL fraction does not induce vascular lesions but that, on the contrary, HDL even has a protective function in this respect (Gordon et al. 1977, N. E. Miller et al. 1976 and 1977, Stanhope et al. 1977, Nicolosi et al. 1977 and Rösner et al. 1978), so that atherosclerotic processes do not develop at sufficiently high levels of HDL, although considerable plasma cholesterol concentrations may then be present. This effect may be related to the quantity of HDL in combination with the LCAT activity (Glomset 1970). The separate measurement of cholesterol in LDL (and possibly in the other lipoprotein fractions) may be of significance with regard to this, in particular when different species are subjected to investigation.

*) After our studies were finished we became aware of the findings of Koletsky & Puterman (1977), who established a reduction of the atherosclerotic lesions in their rats as the result of a restricted food intake. The effect of changes in food composition on these lesions was not investigated, nor was the lipoprotein pattern determined (Nutr. Reviews 1978).

10.5 Summary

The obese Zucker rat, just like the conventional rat, does not appear to be a good laboratory animal for atherosclerosis research, despite the fact that in the former some kind of hypercholesterolaemia can be easily induced. This is probably due to the lipoprotein pattern of rats, which differs from that of species with a greater tendency to vascular lesions.

Chapter 11

General discussion

From the point of view of epidemiology and, more particularly, genetics, the main lesson to be drawn from the existence of these animal models of adiposity is clearly related to this genetic multiplicity, including entirely different modes of transmission (dominant, recessive, dominant requiring one or more recessive modifiers, polygenic).
M. Berger, W. Muller & A. E. Renold, in: Diabetes, Obes., Vasc. Dis., Part 1 (H. M. Katzen & R. J. Mahler, Eds.), Hemisphere, Washington D. C. (1978) Adv. Mod. Nutr. 2, Ch. 6, 211-228.

11.1 Aim of the study

This study was undertaken in order to obtain more knowledge of the effects of certain of the main constituents of food, notably fat and sugar, on the lipid and the glucose metabolism of the genetically obese Zucker rat. This was regarded as an important subject because of the nutritional problems occurring in man, which are related to food composition, lipid metabolism, obesity and a tendency to diabetes mellitus. A better understanding of these relationships might be useful for the prevention of atherosclerosis, the process which has been held responsible for the increasing frequency of a number of vascular diseases during the past few decades.

From the broad description of the genetically obese Zucker rat it is clear that there are metabolic differences between this animal model and man, although it will be equally clear that nutritionally these rats with respect to their obesity and plasma cholesterol level show a better resemblance to man than do conventional rat strains. The obese rats have higher insulin levels than their lean controls, as well as higher blood lipid concentrations which are also quite sensitive to dietary variations.

This rat type, therefore, as an animal model, can simulate to some extent the nutritional situation in man, also with respect to its physical activity (Simonelli & Eaton 1978). However, with regard to atherosclerosis large differences exist: the rat, not being particularly liable to this condition, was found to be at the other end of the scale in this respect than man. Nevertheless, the detailed study on the obese rat, particularly with regard to its lipid metabolism as influenced by some of the important dietary factors, was thought valuable.

11.2 Dietary composition

In our study we were concerned with two types of dietary fat: saturated and poly-unsaturated. For the latter type sunflower oil was used, and for the former a mixture of fats was chosen which were also of vegetable origin, in order to avoid problems related to the presence of cholesterol in animal food products. The proportion of cholesterol absorbed from animal fats by the intestines may differ from the proportion absorbed from a mixture of cholesterol and vegetable fats, because of a different particle size distribution.

In addition to this qualitative approach a low-fat regimen was used, compounded on a semi-synthetic basis similar to that of the high-fat diets. Commercial rations for rodents

are normally low-fat diets, and in our case the control diet had a fat content similar to that of the low-fat diets. From the results of our study it was also clear that there were large differences in metabolic effects between the low-fat and the control diets. A great deal of attention to these differences has already been given by Kritchevsky (1976, 1977a and b). Also Hermus (1975) established that saturated fat added to a „chow" diet did not lead to any elevation of the plasma cholesterol concentration, whereas it did in a semi-synthetic diet.

The second dietary component studied was sucrose which in half of the semi-synthetic diets was substituted iso-energetically for starch. These two types of carbohydrate are known, in the human diet, to be the most important from a quantitative viewpoint, and sucrose is under suspicion of exerting hyperlipidaemic and other adverse metabolic effects (see e.g. Macdonald & Braithwaite 1964), which are not attributed to starch. The substitution of sugar for starch in the three types of diet described above also gave a quantitative difference in sucrose content, since low-fat diets (when prepared on an iso-energetic and iso-nitrogenous basis) intrinsically contain a higher carbohydrate content than do high-fat diets.

The differences in the degree of purity between both types of carbohydrate were met by the addition of cellulose. Although a type of cellulose was used which had been found in preliminary work not to affect plasma cholesterol concentrations, it may well have affected cholesterol metabolism in general, as became apparent when we studied the intestinal absorption of cholesterol (Chapter 6). It must be remembered that a small amount of cholesterol was added to the semi-synthetic diets, at a level of 0.1% by weight in the low-fat diets.

The problems relating to the consumption of quantities that were iso-energetic to the best possible extent and those relating to differences in apparent digestibility, as well as the food restrictions applied only to the groups fed on the high-poly-unsaturated-fat diets, have already been discussed (Chapter 4).

11.3 Dietary effects

Demarne et al. (1975) mentioned that no differences were observed in growth, body composition, food efficiency and lipid and protein synthesis between rats fed on either saturated or poly-unsaturated fat containing diets. In our studies on obese rats clear differences were found as regards plasma cholesterol, triglyceride and blood glucose concentrations as the result of dietary treatment (Chapter 5), although these differences were considerably smaller than those between obese and lean rats. The only difference in insulin levels observed was that these values of the lean rats were significantly lower than those of the obese rats, apart from a tendency for these levels to be lower in the obese rats of the control group than those in the rats fed on the semi-synthetic diets.

The differences in plasma lipid concentrations were first of all dependent on the type and the quantity of the fat consumed (see Tables 9-12, Chapter 5). It may be mentioned that the plasma cholesterol concentrations of the rats fed on the low-fat diets (groups I and II), as reported in Chapter 5 but not in the subgroups as reported in Chapters 6-8, exceeded those of the rats fed on the high-saturated-fat diets (groups III and IV), which will have been the result of the impaired intestinal absorption of the fat by the latter groups. The rats fed on the high-poly-unsaturated-fat diets (groups V and VI) had lower plasma cholesterol levels than those in groups I and II — which is in line with what was stated by Vergroesen (1972) — , but the obese rats on the (low-fat) control diet had even lower levels. This was also found by others (Kritchevsky 1976 and 1977a and b); the reasons for these results are not completely clear.

Secondly, the plasma lipid levels were affected by the type of carbohydrate used. No

interaction was found between the effects of sucrose and dietary fat on plasma lipid, insulin and blood glucose concentrations. The effect of sucrose on plasma lipid levels was established in the beginning in the triglyceride part and later during the experiment in the cholesterol fraction, whereas the differences in plasma triglyceride levels arising from sucrose consumption had meanwhile disappeared.

This time effect is in full agreement with the known facts about the transformation of chylomicrons and very low-density lipoproteins, being rich in triglycerides, into low-density lipoproteins, which contain a larger proportion of cholesterol (Schonfeld et al. 1974). We confine this reference to Zucker rats, but it has been found in all animal species studied, and a further exchange to high-density lipoproteins is also accepted as occurring. It would have been very interesting also for us to study the lipoprotein patterns of our rats, but this could not be undertaken in this investigation. However, from preliminary studies we know that these patterns deviate considerably from those obtained from human plasma (see Tables 25 and 26) and, in addition, are age-dependent.

The occurrence of a dependency of the lipoprotein lipase activity on the composition of the diet is suggested from the studies described by Schonfeld et al. (1974), but De Gasquet & Péquignot (1974) did not find this in twelve month old obese Zucker rats, using either a high-fat diet for seven months or a low-fat diet. A possible effect in this respect, however, would probably be secondary to the increased lipogenesis found in these rats (Bray et al. 1970b and 1974, Martin 1974).

11.4 Cholesterol balance

Very intriguing results were obtained from the cholesterol turnover study on six groups of Zucker rats. Lean rats were shown to have a significantly larger excretory capacity of cholesterol, whereas all dietary groups of obese rats participating had similar production rates of cholesterol (PR_A, Table 18, Chapter 6), indicating — under steady state conditions — a similar synthesis as well as a similar metabolic excretion of cholesterol.

These results are in line with those obtained by Corey et al. (1976), who studied three species of monkeys and used either safflower or coconut oil in their diets (all containing 0.1% of cholesterol). The authors attribute this finding, just as we put forward from our study, to a different distribution of cholesterol over the tissue pools (*cf.* Bieberdorf & Wilson 1965, and Grundy & Ahrens 1970). In their studies a difference was found in the intestinal absorption of cholesterol between *e.g.* squirrel monkeys and cebus monkeys, but no such difference as a result of the dietary fat used. In our study, the difference measured in this respect (Table 19, Chapter 6, groups III and V) must probably be ascribed to the steatorrhoea occurring in group III (high-saturated fat with starch), contrasting to group V (high-poly-unsaturated fat with starch).

On the basis of the principles of cholesterol balance and its metabolic regulation (Dietschy & Wilson 1970), confirmed in studies of, among others, Quintão et al. (1971), the adaptive synthesis of cholesterol may be taken to have been the factor that was responsible for the nearly identical PR_A-values of the obese rats (see Chapter 6). These animals may have, according to their excessive lipogenic properties (Bray et al. 1970b and 1974, Martin 1974), a large capacity for cholesterol synthesis but a restricted one for its metabolic excretion, in comparison with that of lean rats. This may, generally, be valid for the development of hypercholesterolaemia in obesity, also in man (Grundy et al. 1969).

The cholesterol balance was also studied by Green et al. (1976) in guinea-pigs, which were given lard or sunflower oil with or without 0.1% of additional cholesterol in their diets. These authors mention the storage of cholesterol in the liver as another compensatory mechanism in the regulation of cholesterol metabolism.

11.5 Metabolic stress and reactivity

We did not study to any extent the discrepancy between the amazing tolerance of the obese Zucker rats to fasting and their great sensitivity to cold exposure, but only made some measurements during a few days when they were fasting. Despite the considerable decreases in the plasma triglyceride levels of the obese rats (Table 23, Chapter 7) these values were still several times higher than those of the lean rats (even when the latter were being fed), which is in agreement with the findings of Zucker & Antoniades (1972). This would imply — in combination with the losses in body weight which were similar to those of the lean rats — that the large lipid stores of the obese animals allow metabolic energy conversion for a very long period at a low thermogenetic capacity. The decrease in the plasma triglyceride levels from the start of the period of fasting disagrees with the initial rise found by Stout et al. (1976) in man. These authors established, on the other hand, a clear decrease in free fatty acid concentrations in the plasma, which decrease would reflect utilization of these substances for metabolic purposes.

However, we observed an initial rise in the plasma cholesterol concentrations after the rats had fasted for two days, which would indicate the utilization of lipid stores of the rats. This finding is in line with the view held by Angel & Farkas (1974). Our results with regard to glucose and insulin metabolism did not provide us with a clear picture which we could discuss on any broad basis.

11.6 Fatty livers: the role of low-fat diets and sucrose

Finally we want to come back once more to the finding of fatty livers in the rats fed on the low-fat diets (Table 30, Chapter 9). It can be postulated than an increase in the fat content of the diet (and somewhat more so in high-poly-unsaturated- than in high-saturated-fat diets) protects obese rats from the development of hepatic steatosis to a certain extent. However, the control ration, being also a low-fat diet, exerts this protective function too for reasons which are not completely understood but which should be related to some food component(s) or other (Kritchevsky 1976 and 1977a and b). The hepatic steatosis, however, was much more pronounced in the animals fed on the high-sucrose diet provided to group II, which is in agreement with the findings of Winand et al. (1977) and of Tuovinen & Bender (1975). From the figures in Table 30 (Chapter 9) it can be concluded that the sucrose content of the high-fat diets did not attain a level sufficiently high to give rise to fatty livers to a degree higher than that found in the rats of the corresponding groups which had been fed on the starch-containing diets. Also in this respect a high fat content of the diet seems to exert a protective effect on the rats as regards the development of fatty livers. A sucrose-containing „chow" diet was not used in our studies.

The development of a fatty liver was deliberately attempted, for the purpose of experimentation on hepatic steatosis, by Novikoff (1977), who described a dietary regimen, being a high-sucrose, low-fat diet, for obese Zucker rats. This diet, however, also contained 1% of orotic acid, which is known to cause, under certain conditions, fatty livers within 3 days even in rats fed on a chow diet (Creasey et al. 1961). We showed that, also without the addition of orotic acid, a low-fat high-sucrose semi-synthetic diet leads to the development of fatty livers in obese Zucker rats to a very pronounced degree, although perhaps less rapidly.

11.7 Possible implications regarding human nutrition

The study described was concerned with a number of metabolic events taking place in obese Zucker rats under the influence of some dietary factors, and with the comparison of these events with those in lean rats of the same strain. The results are, anyhow,

valuable whether they confirm or not the data already known from studies which had been carried out on various animal species, but not yet on Zucker rats. Most of the data point to similarities between these rats and other animals, although quantitative differences did occur. Our results dealt mainly with the lipid metabolism of obese Zucker rats as influenced by their diets. These differences, however, appeared to be fairly small when compared with those observed between obese and lean Zucker rats. They are discussed broadly in this Thesis, and are also summarised.

Despite confining our attention to Zucker rats in these studies, it might be worthwhile to answer some questions as to what extent our findings — in connection with what was already known — would be applicable to man. Of course, our suggestions in this respect are given with great caution, as extrapolation of such findings to the development of human atherosclerosis is always fraught with errors (Lacko et al. 1974). The nutritional situation of man has for that matter, from the beginning of our study formed the background of our concern with this subject.

11.7.1 *Dietary fat and the type of diet*

It can be stated that, in purified diets, the presence of a large amount of poly-unsaturated fat results in a lower plasma cholesterol concentration than that resulting from similar diets with saturated fat or from a low-fat diet. Although this is presumably not due to a higher excretion of cholesterol (at least not in the long run) but to a redistribution of cholesterol over the tissue pools (as can also be concluded from our study), the lowering of the cholesterol concentration will be of interest from the viewpoint of atherogenesis as long as the infiltration theory on the origin of the cholesterol in the fatty streaks in the vascular wall is held. According to this theory, the lipid compound of the atheromas is essentially derived from the blood circulating in the vessels concerned, to an extent which will depend on the cholesterol concentration and, in addition, on the lipoprotein pattern of the plasma.

The control diet, however, results in a plasma cholesterol level that is still lower, in spite of the fact that the content of poly-unsaturated fat in this diet is lower. (The weight gain on this type of diet was also somewhat less.) Even additional saturated fat in a ,,chow'' diet does not lead to any increase of plasma cholesterol concentrations (Hermus 1975). The biochemical mechanism of this effect has not been sufficiently elucidated and would repay considerable attention in the near future.

The tendency to a lower insulin level that we found in rats fed on a chow diet in comparison with the insulin level resulting from the intake of purified diets, may give an indication of the nutritional significance of this type of feeding. Even the smaller capacity for the digestion of protein, as observed in the rats fed on the control diet, may be related to this effect.

Interest may also be paid to the intestinal absorption of dietary cholesterol, which has been suggested not to exceed a level of about 400 mg per day in man (Kaplan et al. 1963, Wilson & Lindsey 1965, Connor & Connor 1972). Also in 1972, Lutton & Chevallier already came to the conclusion, from their studies on cholesterol turnover rates measured by an isotopic equilibrium method, that dietary cholesterol, and certainly not when provided at a high level, is not a main determinant of the plasma cholesterol concentration, as this concentration is more dependent on variations in the synthesis, biliary flow and intestinal absorption of cholesterol. (see also Chevallier 1977 and Glueck & Connor 1978, mentioned in section 6.4.3, p. 54).

11.7.2 *Sucrose in the diet*

The effect of sucrose on plasma lipids is apparently only a temporary one. Most of the

information from the literature is based on short-term studies, and some other workers have already mentioned its temporary nature.

However, sucrose may cause or aggravate the development of fatty livers, particularly in obese subjects, when it is consumed in large amounts in low-fat diets such as may be used in order to avoid problems of elevated blood levels. In those circumstances some limitation of the sucrose consumption (or a lower level of purity of the carbohydrate in the diet) would be preferable. We do not know precisely to what extent the sucrose intake should be restricted (we did not try out a ,,chow'' or control-type diet with sucrose), but some indication can be given from our study: in the high-fat diets some 40 energy % of sucrose did not aggravate the development of fatty livers, in contrast to the 70 energy % of this sugar added to the low-fat diet.

11.7.3 *The significance of obesity*

Obesity is a separate factor in the various abnormalities which are related to lipid and glucose metabolism. The question whether obesity should be regarded as a ,,disease'' may be answered as follows: obesity is a ,,state'' in which regulatory mechanisms of lipid metabolism are still functioning, although to a different level of metabolic equilibrium, with regard to adipose tissue (Winand et al. 1977) as well as to hepatic lipid metabolism (Jeanrenaud et al. 1977); however, it may lead to diseases, among other things *via* hypercholesterolaemia. Olefsky et al. (1975) experimentally found, in man, that an additional intake of 2000 kcalories (= 8.3 Mega-Joules) influenced lipid and carbohydrate metabolism. Blood glucose and plasma cholesterol concentrations increased to a lesser extent than did plasma triglyceride and insulin levels. In our study the provision of ,,slimming diets'' was not attempted. Also in a study on human obesity, a tendency to steatosis of the liver has been reported (Kral et al. 1977). The hereditary type of obesity as present in the obese Zucker rat transfers the balance of food intake to another level. A restricted food intake in fatties will decrease body weight as well as plasma lipids and insulin levels, but it may interfere with the protein balance and may fail to override the tendency to obesity. Under circumstances of abundant food supply, therefore, the prevention or the treatment of obesity and its adverse effects, at least of genetic forms of this condition, will remain a difficult matter.

Summary

The nutritional problem with regard to fat and sugar consumption in relation to lipid and glucose metabolism, and the ultimate goal of the study are generally outlined in Chapter 1. The obese Zucker rat was chosen as being likely a suitable animal model for a study like this. Chapter 2 is a review of the literature on the Zucker rat strain, of restricted size but aimed to be complete.

In Chapter 3 the design of the study is provided with regard to the grouping and dieting of the rats involved. To six groups of at least 12 obese rats per group, of approximately 6 weeks of age, semi-synthetic diets were given, being either low-fat or high-fat diets; the groups fed on the high-fat diets were provided with either a saturated (consisting of two parts of cocoa butter and one part of palm oil) or a poly-unsaturated (sunflower oil) type of fat. Each of the three types of dietary fat (low-fat, high-saturated- and high-poly-unsaturated-fat) was combined with either sucrose or starch. One group of obese and one group of lean Zucker rats fed on a commercial ration served as control groups. Only male rats were used.

Chapter 4 deals with the results of the body weight gains, food consumption and digestion as obtained during the first part of the experiment, at an age of the rats of approximately 6 to 22 weeks. There was a large similarity in body weight gain of the group of obese rats. On the high-saturated-fat diets a steatorrhoea was observed; the food intake of the rats fed on the poly-unsaturated-fat diets had to be somewhat restricted. In the rats fed on the commercial ration a decreased apparent digestibility of protein was found.

In Chapter 5 the results are given for the plasma levels of cholesterol, triglycerides, insulin and the blood glucose, as measured after 2, 4, 9 and 15 weeks of the experiment. The lean rats showed, as expected, by far the lowest plasma cholesterol concentrations. The low-fat diets gave the highest plasma cholesterol levels, even higher than did the high-saturated-fat diets (which latter, however, gave rise to steatorrhoea). The high-poly-unsaturated-fat diets gave lower levels, but the lowest values for obese rats were seen on the commercial ration. The plasma triglyceride concentrations were significantly higher on all high-fat diets than on the low-fat diets. Again, the values observed in the lean controls were by far the lowest. Sucrose in the diets had an elevating effect particularly during the first weeks of dieting, firstly on the triglyceride and somewhat later on the cholesterol levels of the plasma, but this effect gradually disappeared. There was no interaction found between the effects of dietary fats and sugar on the plasma lipid levels.

The high-poly-unsaturated-fat diets gave significantly higher blood glucose concentrations (after the rats had fasted overnight) than did the other diets ($p<0.001$). The presence of sucrose in the diets had no significant effect on the blood glucose levels. The only tendency to a significant difference between the plasma insulin concentrations of the obese rats was a slightly lower level in the rats of the obese control group; this level, however, was significantly higher than that of the lean controls ($p<0.05$).

Chapter 6 gives a description of the cholesterol turnover study carried out on half of the rats from most dietary groups, with the help of two cholesterol isotopes provided. This turnover was higher in obese than in lean rats. The obese rats had larger pool masses and higher values for the transfer from the rapidly to the slowly exchangeable pool. The most striking result was the observation that all groups of obese rats, in contrast to the lean rats, appeared to have reached their maximal capacity for excretion of cholesterol. This leads us to the conclusion that the differences in plasma cholesterol concentrations observed between the groups of obese rats are the result of a different distribution of the cholesterol over the tissues.

Further, a positive correlation was found between the mass of the slowly exchangeable pool and the intestinal absorption of cholesterol. Of the significant differences found in the intestinal absorption of cholesterol between the dietary groups ($p<0.05$), the higher value for this on the low-fat *vs.* the high-saturated-fat diet and that for the starch *vs.* the sucrose containing diet must supposedly be ascribed to the concomiting lower cellulose content of the former respective diets (*cf.* Table 19 with Table 2 in Chapter 3). The lower cholesterol absorption from the high-saturated-fat diet than from the comparable high-poly-unsaturated-fat diet will be related to the impaired fat absorption from this former diet.

The excretion of 3-β-OH-sterols in the faeces (pooled samples) was somewhat higher in the obese control group than in that of the lean rats. It was also higher than in the groups fed on the semi-synthetic diets, with the exception of the groups fed on the high-saturated-fat diets which gave the highest values for this, probably connected to the steatorrhoea observed in these latter groups. Since the rats fed on this type of fat had shown to have almost identical figures for the cholesterol turnover (= the production rate) as the obese rats in the other groups, a different synthesis of cholesterol will presumably have compensated for this difference in sterol excretion with the faeces.

Chapter 7 reveals the data obtained on the blood parameters mentioned (*vide supra*) during a period of four days of fasting of the second half of the rats from all groups participating in the study. Body weights decreased similarly in all groups, plasma triglycerides fell almost exponentially, whereas the plasma cholesterol levels showed an initial rise, with highest figures on the second day, and the most pronounced in the groups fed on the low-fat diets, to be followed by a gradual decrease. The blood glucose concentrations tended to increase between the second and the fourth day of fasting, whereas the plasma insulin levels did not change significantly.

Chapter 8 is devoted to the measurements, in the blood plasma of half of the rats from all groups, of the enzyme lecithin : cholesterol acyl-transferase (LCAT). A relative LCAT deficiency might be involved in the development of the increased plasma cholesterol concentrations of obese Zucker rats. Significant differences ($p<0.05$) were found as to a decreasing effect on the LCAT activity of the high-saturated-fat compared to the low-fat and the high-poly-unsaturated-fat diets, and a decreasing effect of sucrose compared to starch. There was no interaction found between dietary fat and sugar regarding the LCAT activities.

In Chapter 9 the results are presented of the lipid determinations performed in liver and perirenal fat of the same rats as were used in the preceeding latter two chapters, after their sacrifice. The perirenal fat of obese rats contained twice as much cholesterol as that of lean rats, without a dietary effect measured. The livers of the obese rats were very much fatter than those of the lean rats and had a different fatty acid composition. Very pronounced fatty livers were found in the groups fed on the low-fat diets, particularly in the combination with sucrose. There was a statistically very significant interaction ($p<0.001$) between dietary fat and sugar with regard to the total fat, triglyceride and

cholesterol content of the livers. The hepatic fat content of the obese rats was lowest in the groups fed on the high-poly-unsaturated-fat diets. In the high-fat diets, the presence of a proportion of approximately 39 energy % of sucrose had no increasing effect on the liver lipids, contrasting to the steatotic effect on the liver with approximately 70 energy % of sucrose in the low-fat diets. Lipogenesis appeared to correlate negatively with the degree of mono-desaturation of the fatty acids present in the tissue fats.

Chapter 10 on the aortic atheromatosis points to the lack of atherosclerotic processes occurring in our Zucker rats despite a dietary induced hyperlipidaemia during a period regarded as sufficiently extended, of more than seven months. This negative result will be related to the different lipoprotein composition of rats in general, making them not particularly prone to a development of atherosclerosis in their large arteries.

In Chapter 11 the combined aspects of the fat and sugar metabolism as arising from the respective parts of the total investigation are discussed. Although the differences between obese and lean Zucker rats exceeded by far those observed between the dietary groups of obese rats, a number of significant differences was found between these groups, which resulted from dietary treatment. These differences concern plasma cholesterol and triglyceride levels as well as blood glucose concentrations, cholesterol pool masses, LCAT activities of the plasma, hepatic lipid metabolism and fatty acid composition of both liver and perirenal fat. Further, these differences are related to the quantity and the type of dietary fat as well as to the type of carbohydrate used and, in addition, to the degree of purity of the diet.

The differences in dietary composition, however, apparently did not affect the hepatic excretion of cholesterol, which was found to be similar, and nearly maximal, in all groups of obese rats studied.

In the end an investigation was made to determine the significance of these results for human nutrition.

Samenvatting

In het eerste hoofdstuk worden enige voedingskundige problemen uiteengezet met betrekking tot de consumptie van vet en suiker, en wordt de doelstelling van het onderzoek in het algemeen weergegeven. Als mogelijk geschikt proefdier voor een onderzoek van deze aard viel de keuze op de vetzuchtige Zucker-rat.

Hoofdstuk 2 geeft een overzicht van de literatuur over de Zucker-rat, dat, hoewel het beknopt is gehouden, aanspraak maakt op volledigheid.

In hoofdstuk 3 wordt de opzet van het onderzoek vermeld wat betreft de indeling van de ratten in groepen en hun voedering. Zes groepen van tenminste 12 vetzuchtige ratten van ongeveer zes weken oud kregen semi-synthetische voeders verstrekt die òf weinig vet, òf veel vet bevatten; de groepen die met vetrijke voeders werden gevoerd kregen òf een verzadigd vettype (bestaande uit 2 delen cacaoboter en 1 deel palmolie) òf meervoudig onverzadigd vet (zonnebloemolie). Elk van de gebruikte typen voedingsvet (vetarm of vetrijk verzadigd of vetrijk meervoudig onverzadigd) was gecombineerd met sucrose of zetmeel. Er waren twee controlegroepen: één bestaande uit vetzuchtige en één bestaande uit magere Zucker-ratten, die beide een handelsvoer kregen toegediend. Er werden uitsluitend mannelijke ratten gebruikt.

Hoofdstuk 4 behandelt de resultaten van het onderzoek, wat betreft lichaamsgewicht, voedselconsumptie en verteerbaarheid, die werden verkregen in het eerste gedeelte van het onderzoek bij ratten die een leeftijd hadden tussen ongeveer 6 en 22 weken. Er bestond grote overeenstemming tussen de groepen vetzuchtige ratten wat de toeneming in gewicht betreft. Bij de ratten waaraan voeder met veel verzadigd vet was verstrekt werd vetontlasting waargenomen, terwijl de vetzuchtige ratten die het controlevoer kregen een verminderde schijnbare verteerbaarheid van het eiwit vertoonden.

In hoofdstuk 5 zijn de resultaten weergegeven van de bepalingen van de plasmaconcentraties van cholesterol, triglyceriden en insuline en het bloedglucose-gehalte, die werden gemeten na 2, 4, 9 en 15 weken van het onderzoek. Zoals werd verwacht, bleken de magere ratten verreweg de laagste plasmacholesterolspiegels te hebben. De vetarme semi-synthetische voeders veroorzaakten de hoogste plasmacholesterolconcentraties; deze waren zelfs hoger dan die welke werden geconstateerd als gevolg van de consumptie van verzadigd vet (dit vettype gaf echter aanleiding tot vetdiarree). De voeders met veel meervoudig onverzadigd vet hadden lagere gehalten ten gevolge, maar de laagste waarden bij vetzuchtige ratten werden gevonden bij de dieren die het controlevoer kregen. Het plasmatriglyceridegehalte van de ratten die een vetrijk voer gebruikten was significant hoger dan van die welke een vetarm voer kregen. Ook hier werden verreweg de laagste waarden gevonden bij magere ratten. Suiker in de voeding had een verhogend effect, vooral gedurende de eerste weken van de voederperiode, allereerst op het triglyceridegehalte en korte tijd later op het cholesterolgehalte van het plasma. In het verloop van het onderzoek verdween dit effect geleidelijk. Er werd geen interactie gevonden tussen de invloed van vet en suiker in de voeding op de plasmalipidengehalten.

De vetrijke voeders met meervoudig onverzadigd vet gaven significant hogere bloedglucoseconcentraties (nadat de ratten 's nachts hadden gevast) dan de andere voeders ($p < 0{,}001$). De aanwezigheid van sucrose in het voer had geen significante invloed op de bloedglucosespiegels. De enige neiging tot een significant verschil in plasma-insulineconcentratie tussen de groepen vetzuchtige ratten was een wat lager gehalte bij de vetzuchtige controleratten; dit gehalte was echter significant hoger dan dat van de magere ratten ($p < 0{,}05$).

In hoofdstuk 6 is een studie over de cholesterol-turnover beschreven, die werd uitgevoerd met de helft van de ratten uit de meeste groepen, en waarbij gebruik werd gemaakt van twee radioactieve cholesterol-isotopen. Deze turnover was hoger in vetzuchtige dan in magere ratten. De vetzuchtige ratten hadden een grotere massa van de „pools" en hogere waarden voor de overdracht van de snel uitwisselbare naar de langzaam uitwisselbare „pool". Het meest opmerkelijke resultaat was de waarneming dat alle groepen vetzuchtige ratten hun maximale capaciteit voor de uitscheiding van cholesterol bleken te hebben bereikt, in tegenstelling tot de magere ratten. Dit leidt ons tot de conclusie dat de waargenomen verschillen in plasmacholesterolconcentratie tussen de groepen vetzuchtige ratten het gevolg zijn van een verschil in de verdeling van het cholesterol over de weefsels.

Verder werd er een positieve correlatie gevonden tussen de grootte van „pool" B (de langzaam uitwisselbare „pool") en de absorptie van cholesterol uit de darm. Van de significante verschillen die werden geconstateerd in de absorptie uit de darm van cholesterol tussen de voedergroepen ($p < 0{,}05$), moet de hogere waarde die hiervoor werd gevonden bij gebruik van het vetarme voer ten opzichte van het voer dat rijk was aan verzadigd vet, en die bij gebruik van zetmeel ten opzichte van sucrose, vermoedelijk worden toegeschreven aan het lagere cellulosegehalte dat voorkwam in het bij deze vergelijkingen telkens het eerst genoemde voeder (vergelijk hiervoor tabel 19 en tabel 2 in hoofdstuk 3). De lagere absorptie van cholesterol uit het voer dat rijk was aan verzadigd vet dan uit het overeenkomstige voer met veel meervoudig onverzadigd vet hangt waarschijnlijk samen met de gestoorde vetabsorptie uit eerstgenoemd voeder.

De uitscheiding met de faeces van 3-β-OH-sterolen (gemeten in samengevoegde monsters) was iets hoger in de groep vetzuchtige dan in de groep magere controleratten. Deze uitscheiding was tevens hoger dan in de groepen die met de semi-synthetische voeders werden gevoerd, met uitzondering van de groepen die de voeders kregen met veel verzadigd vet; bij deze groepen werden de hoogste waarden gevonden, waarschijnlijk verband houdend met de vetdiarree die in deze groepen was geconstateerd. Uit het feit dat de ratten die met dit vettype waren gevoerd een vrijwel even hoge turnover van cholesterol (= de produktiesnelheid) bleken te hebben als de vetzuchtige ratten uit de andere groepen, kan worden geconcludeerd dat het verschil in uitscheiding met de faeces van sterolen moet zijn gecompenseerd door een verschil in synthese van cholesterol.

Hoofdstuk 7 bevat de uitkomsten van experimenten waarbij de bovengenoemde bloedparameters werden bepaald na een periode van voedselonthouding van vier dagen bij de tweede helft van de ratten van alle aan het onderzoek deelnemende groepen. Het lichaamsgewicht nam in alle groepen gelijkelijk af, het plasmatriglyceridegehalte daalde welhaast exponentieel, terwijl het plasmacholesterolgehalte aanvankelijk een stijging vertoonde (waarbij de hoogste waarden zich op de tweede dag voordeden) die het meest uitgesproken was in de groepen die een vetarm (semi-synthetisch) voer gebruikten, en die werd gevolgd door een geleidelijke daling. De bloedglucosegehalten vertoonden een stijgende tendens tussen de tweede en vierde dag van de periode van vasten, terwijl de insulinespiegels niet significant veranderden.

Hoofdstuk 8 is gewijd aan de bepaling van het enzym lecithine : cholesterol-acyltransferase (LCAT) in het bloedplasma van de helft van de ratten uit alle groepen. Een relatief tekort aan LCAT zou betrokken kunnen zijn bij de ontwikkeling van de verhoogde plasmacholesterolgehalten van Zucker-ratten. Er werden met betrekking tot de LCAT-activiteit significante verschillen gevonden ($p<0,05$), en wel een verlagende werking als gevolg van het gebruik van de voeders die veel verzadigd vet bevatten vergeleken met voeders die vetarm waren of rijk aan meervoudig onverzadigd vet, en een verlagende werking als gevolg van het gebruik van de voeders met sucrose vergeleken met zetmeel. Er werd geen interactie geconstateerd tussen voedingsvet en suiker met betrekking tot de LCAT-activiteit.

In hoofdstuk 9 worden de resultaten weergegeven van de vetbepalingen die werden uitgevoerd met leverweefsel en perirenaal vet van dezelfde ratten als die welke werden gebruikt in de proeven beschreven in de twee vorige hoofdstukken, nadat ze waren opgeofferd. Het perirenale vet van vetzuchtige ratten bevatte tweemaal zoveel cholesterol als dat van magere ratten, waarbij geen invloed van de voeding kon worden vastgesteld. De levers van de vetzuchtige ratten bevatten veel meer vet dan die van de magere ratten en hadden een andere vetzuursamenstelling. Zeer uitgesproken vetlevers werden waargenomen in de groepen die werden gevoed met vetarm voeder, speciaal wanneer dit bovendien sucrose bevatte. Er was een statistisch zeer significante interactie ($p<0,001$) tussen voedingsvet en suiker ten aanzien van het totale vet-, het triglyceride- en het cholesterolgehalte van de levers. Het gehalte aan levervet van de vetzuchtige ratten was het laagst in de groepen die voer kregen dat rijk was aan meervoudig onverzadigd vet. In de vetrijke voeders had de aanwezigheid van circa 39 energie % aan suiker geen verhoging van de leverlipiden tot gevolg, in tegenstelling tot het vervettende effect op de lever met circa 70 energie % aan suiker in de vetarme voeders. Er bleek een negatieve correlatie te bestaan tussen de vetsynthese en de verzadigingsgraad (mono-desaturatie) van de in het weefselvet voorkomende vetzuren.

Hoofdstuk 10 gaat over atherosclerose van de lichaamsslagader en wijst op het ontbreken van atherosclerotische processen bij de Zucker-rat, ondanks een verhoogd lipidengehalte van het bloed als gevolg van de voeding welke werd gegeven gedurende een periode die als voldoende lang kan worden beschouwd, nl. van meer dan zeven maanden. Dit negatieve resultaat hangt samen met de samenstelling van de lipoproteïnen bij ratten in het algemeen, welke verschilt van die bij de mens, en die hen weinig gevoelig doet zijn voor het ontstaan van atherosclerose in de grote bloedvaten.

In hoofdstuk 11 worden de verschillende aspecten van de vet- en suikerstofwisseling besproken zoals die in de diverse hoofdstukken aan de orde komen. Hoewel de verschillen tussen vetzuchtige en magere ratten veel groter waren dan die tussen de diverse voedergroepen van vetzuchtige ratten, zijn er toch tussen de laatstgenoemde groepen enige duidelijke verschillen geconstateerd die terug te brengen zijn tot hun voeding. Deze verschillen betreffen zowel het plasmacholesterol- en het plasmatriglyceridegehalte als het bloedsuikergehalte, de grootte van de cholesterol-"pools", de LCAT-activiteit van het plasma, de vetstofwisseling van de lever en de vetzuursamenstelling van het levervet en het perirenale vet. Overigens hangen de verschillen niet alleen samen met de hoeveelheid en het type voedingsvet maar ook met de gebruikte soort koolhydraat, en bovendien nog met de graad van zuiverheid van het voer.

De verschillen in samenstelling van de voeders oefenden echter geen invloed uit op de uitscheiding van cholesterol door de lever, welke bij alle onderzochte groepen vetzuchtige ratten nagenoeg even groot en ook vrijwel maximaal was.

Tenslotte wordt een poging ondernomen om aan te geven wat deze gegevens betekenen voor de voeding van de mens.

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Curriculum vitae

H. de Waard was born in Utrecht on August 22, 1929. After completing the course of the municipal grammar-school (Gymnasium) in this town he studied medicine at the Utrecht University from 1947 to 1956. During his military service he was attached to the Military School of Hygiene and Preventive Medicine.

From 1958 he specialized in pediatrics at Deventer and Utrecht; in 1962 he studied haematology in the latter town for one year. Thereafter he worked as chef-de-clinique of the Pediatric Departments at Deventer for more than four years, and then became head of one of these, which position he fulfilled for another three years.

In October 1970 he was appointed head of the newly formed section of Nutrition at the Netherlands Institute for Dairy Research (NIZO). After having visited Cambridge for three terms to obtain the Diploma in Nutrition, which he gained in 1972, he performed, among other things, the present study. He is a full member of the Dutch Nutrition Council.

He is particularly interested in comparative physiology and also in sports medicine. He loves classical music and, in his leisure time, is an enthusiastic flute player and musician.