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**Physiological regulation of energy balance**

A review of the literature

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## Abstract

### **Physiological regulation of energy balance. A review of the literature.**

World-wide, the prevalence of overweight and obesity is increasing tremendously. Clues to the prevention of overweight may well be found in a better understanding of the physiological processes involved in the regulation of energy balance and factors that influence these processes, such as dietary factors and smoking.

A review of available literature presented here showed the central nervous system to play an important role in the regulation of energy balance through effects on feeding behaviour and energy expenditure. The physiological response to weight loss seems to be more vigorous than to weight gain and may explain why it is so difficult to lose weight. The physiological regulatory mechanisms do not differ at low levels of energy expenditure from those at high levels, but it is easier to overeat at low levels of energy expenditure. Therefore, increasing physical activity or maintaining it at high levels is important.

Based on physiological mechanisms, diets low in energy density, low in fat, high in fibre and void of energy-containing liquids between meals constitute an effective strategy for preventing a positive energy balance or maintaining the weight reached after weight loss.

The dynamic nature of research on the mechanisms involved in regulation of energy balance, however, may make it necessary to update this review in several years' time.

**Keywords:** obesity, energy balance, regulation, diet, smoking.

## Rapport in het kort

### **Fysiologische regulatie van de energiebalans. Een literatuuroverzicht.**

Overgewicht en obesitas (ernstig overgewicht) komen wereldwijd en ook in Nederland steeds vaker voor. Overgewicht en obesitas zijn het gevolg van een langdurige positieve energiebalans. Kennis van de fysiologische mechanismen die de energiebalans reguleren en van factoren die deze regulatiemechanismen beïnvloeden, zoals voedingsfactoren en roken, is noodzakelijk en levert mogelijk aanknopingspunten op die gebruikt kunnen worden ter voorkóming van overgewicht.

Dit rapport geeft een overzicht van de beschikbare literatuur. Hieruit blijkt dat het centrale zenuwstelsel een belangrijke rol speelt bij de fysiologische regulatie van de energiebalans. Het beïnvloedt zowel voedingsgedrag als energieverbruik. De fysiologische repons bij gewichtsverlies lijkt veel sterker te zijn dan de respons op gewichtstoename. Dit verklaart mogelijk waarom het zo moeilijk is gewicht te verliezen. Er is geen bewijs voor een veranderde fysiologische regulatie van de energiebalans bij een lage lichamelijke activiteit. Echter, bij een laag niveau van lichamelijke activiteit is het makkelijker te “overeten”. Daarom is het belangrijk de lichamelijke activiteit op een hoog niveau te houden of te brengen. Gebaseerd op het effect op fysiologische regulatiemechanismen lijkt een voeding laag in energiedichtheid, laag in vet, rijk aan vezel en met een lage consumptie van energierijke dranken tussen de maaltijden door een effectieve strategie om een positieve energiebalans te voorkómen of om gewichtsverlies te handhaven. Het onderzoeksgebied dat de regulatie van de energiebalans bestudeert is een zeer dynamisch veld. Dagelijks komt er nieuwe informatie bij. Daarom is het aan te bevelen om dit rapport over enkele jaren bij te werken.

Trefwoorden: obesitas, energiebalans, regulatie, voeding, roken.



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## List of abbreviations

|               |  |
|---------------|--|
| ADP           | Adenosine diphosphate                      |
| ADRB          | $\beta$ -adrenergic receptors              |
| AMP           | Adenosine monophosphate                    |
| AgRP          | Agouti related protein                     |
| AMPK          | AMP-activated protein kinase               |
| ATP           | Adenosine triphosphate                     |
| $\alpha$ -MSH | $\alpha$ -Melanocyte stimulating hormone   |
| CART          | Cocaine-and-amphetamine-related transcript |
| CCK           | Cholecystokinin                            |
| CRH           | Corticotropin-releasing hormone            |
| GLP1          | Glucagon-like peptide 1                    |
| LHA           | Lateral hypothalamic area                  |
| MC4R          | Melanocortin 4 receptor                    |
| MC3R          | Melanocortin 3 receptor                    |
| MCH           | Melanin-concentrating hormone              |
| nAChR         | Nicotinic acetylcholine receptor           |
| NEAT          | Non exercise activity thermogenesis        |
| NPY           | Neuropeptide Y                             |
| POMC          | Proopiomelanocortin                        |
| PVN           | Paraventricular hypothalamic nucleus       |
| PYY           | Fragment peptide YY 3-36                   |
| RMR           | Resting metabolic rate                     |
| SCD-1         | Stearoyl-CoA desaturase 1                  |
| SNS           | Sympathetic nervous system                 |
| TRH           | Thyrotropin-releasing hormone              |
| UCP           | Uncoupling protein                         |

## Summary

World-wide the prevalence of overweight and obesity is increasing dramatically. When energy intake exceeds energy expenditure a positive energy balance develops. This will on the long-term result in overweight and obesity. Knowledge about the mechanisms involved in the regulation of energy balance is important and may well offer clues to its prevention. Therefore, the aim of this report was to describe the physiological mechanisms involved in the regulation of energy balance and to identify factors influencing these mechanisms, such as physical activity, dietary factors and smoking.

Key components of the energy balance system are energy intake, energy expenditure and energy storage. Carbohydrates and fats provide most of the dietary energy intake; proteins provide only a fraction. As the body maintains a nearly constant protein content by adjusting amino acid oxidation to protein intake, intake and utilisation of fat and carbohydrates primarily determine body weight regulation. Daily energy expenditure can be divided in three main components. The largest component (60-70%) is the resting metabolic rate, i.e. the energy expended to maintain basic physiological bodily functions. An additional 10% of daily energy expenditure comes from the production of heat induced by food intake and cold (thermogenesis). The remaining and most variable component of daily energy expenditure is physical activity. When energy intake exceeds energy expenditure, energy is stored primarily as fat in adipose tissue. Therefore, obesity is characterised by an increase in the amount of white adipose tissue.

The regulation of energy balance is complex. The central nervous system, especially the hypothalamus, plays an important role in this process.

The regulation of food intake can be divided into a short-term and a long-term system.

The short-term regulation of food intake involves meal initiation, meal termination and determination of the interval between meals through signals that are released from the gastrointestinal tract, such as ghrelin and peptide YY. These gut peptides stimulate (ghrelin) or inhibit (peptide YY) appetite. Also food intake itself determines the onset of satiety by gut distension.

The long-term regulation of food intake balances energy intake with energy expenditure and is mainly regulated by the melanocortin pathway. Leptin (excreted by adipose tissue) and insulin (secreted by the pancreas) circulate in the body at levels proportional to the body fat content, and cross the blood-brain barrier to activate this pathway. Both hormones reduce the expression of neuropeptides that increase appetite and reduce energy expenditure. Otherwise, they induce the expression of neuropeptides that have opposite effects, i.e. inhibition of eating and stimulation of energy expenditure. These neuropeptides, in turn, affect the melanocortin-4-receptor within a specific part of the hypothalamus (the arcuate nucleus), which is central to this pathway. As a result, the expression of other neuropeptides in specific parts of the hypothalamus and thereby energy intake is affected.

Obese subjects have relative high levels of the appetite suppressant leptin and it seems that most cases of obesity are associated with insensitivity to leptin. However, the exact mechanisms explaining this leptin resistance are still unknown. Less is known about the association between the other signalling factors and obesity.

From the three components of daily energy expenditure at least adaptive thermogenesis is under physiological control. It has been proposed that excessive energy intake is sensed by the brain, which then triggers an increase in energy expenditure. Adaptive thermogenesis is



regulated by the sympathetic nervous system via  $\beta$ -adrenergic receptors. Lower sympathetic nervous system activity and/or lower sensitivity to a certain level of sympathetic activity may play a role in the aetiology of obesity. Additionally, uncoupling proteins may influence the efficiency of energy expenditure and so affect energy balance. Uncoupling proteins prevent the conversion of energy to adenosine triphosphate, an important energy delivering substrate, by making the energy disappear as heat.

Evidence suggests that the melanocortin pathway not only affects food intake, but also energy expenditure. Leptin may influence energy expenditure by affecting enzymes involved in the synthesis of fatty acids. Additionally, several neuropeptides of the melanocortin pathway also influence the activity of the sympathetic nervous system.

At low levels of physical activity it is difficult to maintain body weight, but there is no evidence that physiological regulatory mechanisms differ in this situation from those at high energy expenditure. However, at low levels of energy expenditure it is easier to overeat and the physiological response to weight loss (even when already overweight) seems more vigorous than the response to weight gain. This underlines the urgent need to prevent the development of overweight by among others increasing physical activity in addition to “treating” subjects who are overweight already.

Within our “obesigenic” environment there are individuals who do and individuals who do not become obese. Strong evidence suggests that susceptibility to obesity is determined by genetic factors. Until now, several rare single mutations in human genes causing monogenetic obesity have been identified. Remarkably, all these mutations are in genes that are part of the melanocortin pathway. This implies that this pathway may indeed be the primary pathway in the regulation of energy balance. Most human obesity is, however, the result of multiple genes (polygenic), each with modest effects, which interact with each other and with environmental factors. Many genetic factors are studied, but only for a limited number of specific genetic variants an effect on obesity has been consistently found.

Several factors may influence the physiological mechanisms that regulate energy balance. Physical activity can create an energy deficit. However, on the short-term, the body seems not to possess a rapidly acting physiological mechanism that automatically matches energy intake to this deficit. Therefore, physical activity is important to induce weight loss and avoid weight gain. On the long-term, leptin may be involved in the establishment of a new steady state in which energy intake matches energy expenditure.

Based on the results of scientific research, diets low in energy density, fat and high in dietary fibre as well as low consumption of energy-containing beverages between meals may well be effective strategies to prevent a positive energy balance or maintain weight loss. Fat, water and fibre content predominantly influence the energy density of foods. Mainly because of their greater volume, foods with a low energy density reduce gastric emptying rate (in kJ/min) and delay the return of hunger. Due to the absence of chewing and an increased rate of gastric emptying, liquids may be less satiating when not consumed with or close to a meal. Therefore, the consumption of energy-rich beverages between meals may lead to caloric over-consumption.

It remains unclear whether macronutrient composition (e.g. fat versus carbohydrates) influences body weight independently of total energy intake. Consequently, there is no sound scientific evidence to date for the Atkins theory (low-carbohydrate diet for weight loss).

Furthermore, long-term information about the efficacy and safety of low-carbohydrate diets is lacking. Conclusive evidence for an association between breastfeeding and overweight is also lacking to date.

Besides dietary factors and physical activity, smoking also affects the regulation of energy balance. Smoking causes a decrease in body weight of about 3 to 4 kg, which is regained after smoking cessation. These alterations in weight may be explained by acute changes in energy intake. Nicotine influences the melanocortin pathway and partly causes the changes in energy intake. Additionally psychological factors may well be involved. Furthermore, nicotine causes an acute increase in energy expenditure, by affecting the sympathetic nervous system. Whether this results in a chronic effect on basal metabolic rate is unclear.

The research field that studies the regulation of energy balance is a very dynamic area. New information is becoming available daily. Most knowledge comes from animal studies. In the near future, our knowledge about the regulation of energy balance in humans will expand. This may make it necessary to update this review in several years' time.

## Samenvatting

Overgewicht en obesitas (ernstig overgewicht) komen wereldwijd en ook in Nederland steeds vaker voor. Zij zijn het gevolg van een langdurige positieve energiebalans. Vanwege de toename in het vóórkomen van overgewicht en obesitas is kennis van de fysiologische mechanismen die de energiebalans reguleren noodzakelijk. Deze kennis levert mogelijk aanknopingspunten op die gebruikt kunnen worden ter voorkóming van overgewicht. Het doel van het rapport is daarom op basis van de beschikbare literatuur een beschrijving te geven van deze regulatiemechanismen en van factoren die deze regulatiemechanismen beïnvloeden, zoals voedingsfactoren, lichamelijke activiteit en roken.

De energiebalans wordt bepaald door energie-inname, energieverbruik en energieopslag. Het grootste deel van de energieinname wordt bepaald door de hoeveelheid koolhydraten en vetten in de voeding. Slecht een beperkt deel van de energieinname komt van eiwitten. Het lichaam houdt het gehalte aan eiwitten constant door de oxidatie van aminozuren aan te passen aan de hoeveelheid eiwit die wordt geconsumeerd. Daarom bepalen met name koolhydraten en vetten de regulatie van het lichaamsgewicht.

Het dagelijkse energieverbruik kan in drie componenten worden onderverdeeld. De hoeveelheid energie die gebruikt wordt voor fundamentele fysiologische lichaamsfuncties (rustmetabolisme) levert de grootste bijdrage aan het dagelijkse energieverbruik (60-70%). Het energieverbruik voor het verteren, metaboliseren en opslaan van voedsel en het op peil houden van de lichaamstemperatuur (de adaptieve thermogenese) is verantwoordelijk voor ongeveer 10% van het totale dagelijkse energieverbruik. Lichamelijke activiteit is de derde en meest variabele component (15-30%).

Wanneer de energieinname het energieverbruik overschrijdt, wordt het teveel aan energie opgeslagen als vet. Overgewicht en obesitas worden dan ook gekarakteriseerd door een toename in wit vetweefsel.

De regulatie van de energiebalans is complex. Het centrale zenuwstelsel, en met name de hypothalamus, speelt een belangrijke rol in dit proces. De mechanismen die de voedselinname reguleren kunnen worden onderverdeeld in een korte- en een lange-termijn regulatiesysteem.

Het korte-termijn systeem regelt het beginnen en beëindigen van een maaltijd en de periode tot de volgende maaltijd door afgifte van signalen vanuit het maagdstelsel, zoals ghreline en peptide YY. Deze darmeiwitten stimuleren (ghreline) of remmen (peptide YY) de eetlust. De inname van voedsel bepaalt zelf ook de mate van verzadiging, door het darmkanaal uit te rekken.

Het lange-termijn systeem balanceert de voedselinname en het energieverbruik en wordt voornamelijk gereguleerd door de melanocortine route. Leptine (afgegeven door de vetcel) en insuline (afgegeven door de alvleesklier) circuleren in verhouding tot de hoeveelheid lichaamsvet en passeren de bloed-hersen-barrière om deze route te activeren. Beide hormonen werken in op de melanocortine-4-receptor in een bepaald deel van de hypothalamus (de arcuate nucleus). Uiteindelijk veranderen ze de expressie van neuropeptiden in andere delen van de hypothalamus en beïnvloeden zo de voedselinname. Obese mensen hebben over het algemeen relatief hoge niveaus van de eetlustremmer leptine en zijn dus blijkbaar minder gevoelig voor de werking ervan. Het exacte mechanisme dat verantwoordelijk is voor deze leptineresistentie is nog onduidelijk. Over het verband tussen andere signaalstoffen en obesitas is minder bekend.

Van de drie componenten van het dagelijkse energieverbruik staat er tenminste één onder fysiologische controle, namelijk de adaptieve thermogenese. Er wordt wel verondersteld dat overmatige energie-inname gedetecteerd wordt door de hersenen, waardoor vervolgens een toename in het energieverbruik teweeg wordt gebracht. De adaptieve thermogenese wordt waarschijnlijk gereguleerd door het sympathisch zenuwstelsel via  $\beta$ -adrenerge receptoren. Een lagere sympathische activiteit en/of een verminderde gevoeligheid voor een bepaald niveau van sympathische activiteit speelt mogelijk een rol bij het ontstaan van obesitas. Ontkoppelingseiwitten zijn mogelijk ook betrokken door hun invloed op de efficiëntie van het energiegebruik. Ontkoppelingseiwitten voorkómen de omzetting van energie naar adenosine trifosfaat, een belangrijk energieleverend substraat, door de energie als warmte te laten verdwijnen.

Leptine beïnvloedt mogelijk niet alleen de voedselinname, maar ook het energieverbruik. Het beïnvloedt mogelijk enzymen die betrokken zijn bij de vetzuursynthese. Tevens beïnvloeden verschillende andere neuropeptiden van de melanocortine route de activiteit van het sympathische zenuwstelsel.

Ondanks de nauwe regulatie van de energiebalans, blijkt het moeilijk te zijn om het lichaamsgewicht te handhaven bij een laag energieverbruik. Er is geen bewijs dat de fysiologische mechanismen die de energiebalans reguleren verschillen in situaties waarin de lichamelijke activiteit laag is, vergeleken met situaties waarin de lichamelijke activiteit hoog is. Het is echter makkelijker om te overeten als het energieverbruik laag is. Ook zijn er aanwijzingen dat de fysiologische respons op gewichtsverlies (zelfs bij aanwezigheid van overgewicht) sterker is dan de respons op gewichtstoename. Tezamen onderstreept dit het belang van het voorkómen van overgewicht in plaats van het behandelen van mensen die reeds overgewicht hebben.

Ondanks de “obesogene” omgeving waarin we leven, worden sommige mensen wel en andere mensen niet obees. De gevoeligheid om overgewicht te ontwikkelen wordt deels door genetische factoren bepaald. Een aantal zeldzame mutaties met monogenetische obesitas als gevolg zijn geïdentificeerd. Opvallend is dat al deze mutaties zich bevinden in genen die deel uitmaken van de melanocortine route. Dit impliceert dat dit inderdaad de primaire route is, die de energiebalans reguleert. De meeste gevallen van obesitas zijn echter het gevolg van variaties in meerdere genen (polygenetisch), elk met een bescheiden effect. Zij interacteren met elkaar en met omgevingsfactoren. Veel van deze genetische factoren zijn bestudeerd, maar voor slechts een beperkt aantal specifieke genvarianten is een consistent verband met obesitas aangetoond.

Diverse leefstijlfactoren kunnen mogelijk de fysiologische regulatie van de energiebalans beïnvloeden.

Lichamelijke activiteit kan een tekort aan energie creëren. Op korte termijn lijkt het lichaam geen acuut werkend mechanisme te bezitten dat automatisch de energie-inname aanpast aan het energieverbruik. Daarom is lichamelijke activiteit belangrijk om gewichtsverlies te induceren en gewichtstoename te voorkómen. Op de lange termijn is leptine mogelijk betrokken bij het weer in evenwicht brengen van energieinname en energieverbruik.

Gebaseerd op het effect op de fysiologische regulatie van de energiebalans is een voeding laag in energiedichtheid, laag in vet, rijk aan vezel en met een lage consumptie van energierijke dranken tussen de maaltijden door een effectieve strategie om een positieve energiebalans te voorkómen en om gewichtsverlies te handhaven. Vet, water en vezelgehalte

bepalen in belangrijke mate de energiedichtheid van een voedingsmiddel. Vooral vanwege hun grote volume, verminderen voedingsmiddelen met een lage energiedichtheid de maaglediging (in kJ/min) en vertragen ze de terugkeer van de eetlust. Vloeistoffen hebben een verminderd verzadigend vermogen als ze niet tijdens of vlak bij een maaltijd worden geconsumeerd. Daarom kan de consumptie van energierijke dranken tussen de maaltijden door leiden tot overconsumptie van calorieën.

Vandaag de dag blijft het onduidelijk of de macronutriëntensamenstelling (vet versus koolhydraten) het lichaamsgewicht beïnvloedt onafhankelijk van de totale energieinname. Er is dan ook geen overtuigend wetenschappelijk bewijs voor de theorie van Atkins (een dieet laag in koolhydraten). Ook is er nog weinig bekend over de effectiviteit en veiligheid van laag-koolhydraat diëten op de lange termijn. Tevens is er geen sluitend bewijs voor een verband tussen borstvoeding en de latere ontwikkeling van overgewicht.

Naast voedingsfactoren is ook roken van invloed op de regulatiemechanismen van de energiebalans. Roken veroorzaakt een afname in het lichaamsgewicht van 3 tot 4 kg, terwijl stoppen met roken een toename veroorzaakt. Deze effecten kunnen mede worden verklaard door acute veranderingen in de energie-inname, welke gedeeltelijk veroorzaakt worden door het effect van nicotine op de melanocortine route. Echter, psychologische factoren spelen waarschijnlijk ook een rol. Nicotine geeft ook een acute stijging in energieverbruik, maar in hoeverre er sprake is van een chronisch effect op het rustmetabolisme is onduidelijk.

Het onderzoeksgebied dat de regulatie van de energiebalans bestudeerd is een zeer dynamisch veld. Dagelijks komt er nieuwe informatie bij. De meeste kennis is afkomstig van dierstudies. In de nabije toekomst zal de kennis over hoe de energiebalans in mensen gereguleerd wordt toenemen. Daarom is het aan te bevelen om dit rapport over enkele jaren bij te werken.



# 1. Introduction

World-wide there is a tremendous increase in the prevalence of overweight and obesity. In the Netherlands the number of people with obesity has almost doubled between 1987 and 2001. Fifty-five percent and 45% of respectively men and women are overweight and about 10% of Dutch adults are obese (1).

The rapid increase in the prevalence of obesity may suggest a dramatic change in food and physical activity habits in a population. However, during a long period, relatively small changes in energy intake and energy expenditure have large consequences for body weight. For example, to increase the prevalence of obesity from 10 to 15% in 10 years time, the mean body mass index has to move from 25 to 26 kg/m<sup>2</sup>. At a mean height of 1.75 meter, this corresponds to an increase in mean weight of 3 kg (76.6 to 79.6 kg). Only a small positive energy balance of 6.5 kcal (27 kJ) per day is sufficient to obtain this increase and corresponds approximately with halve a cube of sugar or sweet per day (2).

Figure 1 was adapted from the report “overweight and obesity” of the Health Council of the Netherlands. It shows the relation between energy intake and energy expenditure. The straight line indicates the situation of balance between energy intake and energy expenditure over a period of time. The ellipse is a hypothetical reproduction of the variation in energy intake and expenditure of an individual. The position of this ellipse suggests that it is difficult to maintain energy balance at low levels of energy expenditure. In those circumstances, a conscious increase in physical activity or decrease in food intake is necessary to maintain energy balance (3).

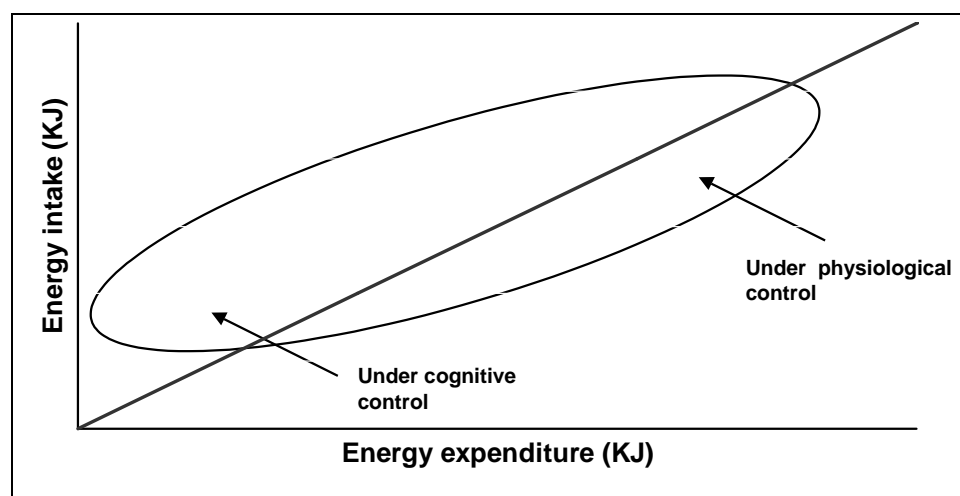


Figure 1. Relation between energy intake and energy expenditure (from (3))

The obesity epidemic is by many attributed to a mismatch between our environment now and our metabolism developed thousands of years ago. The human physiological system has developed in times when food was often scarce and a high level of physical activity was required for daily subsistence. People were stimulated to eat when food was available and to rest when physical activity was not required in order to have enough energy for healthy living. Moreover, our physiological system has probably evolved with an additional bias to conserve energy in times of rest (4).

Today, we live in a society where the chance of overeating is high. Food is omnipresent, served in large portions, relatively cheap, tasting good, high in fat and energy dense.

Conversely, the amount of daily energy expenditure has strongly decreased. Responsible for this reduction are among others, a decrease in employments that require strenuous physical activity, a decrease in energy expenditure at school and in daily life and an increase in the amount of time that is spend watching television, surfing the internet, and playing computer games. Also, the development of central heating may have contributed to the decline in energy expenditure, as maintenance of body temperature under cold circumstances is an energy demanding process (4).

A better understanding of the physiological processes involved in the regulation of energy balance may shed light on the question why those processes seem to fail in modern society. This information may well offer clues for the prevention of overweight. In this report we present a literature study to provide a “state of the art” overview about these regulatory mechanisms.

In chapter 2 the components of the energy balance system, i.e. energy intake, energy expenditure and energy storage are described. Chapter 3 describes the physiological regulation of energy balance and is divided in the physiological regulation of food intake, and the physiological regulation of energy expenditure. Differences in body weight regulation between individuals based on genetic factors are described in chapter 4. Chapter 5 describes the influences of several major dietary factors on the physiological processes involved in the regulation of energy balance. The influences of components of energy expenditure on the regulation of energy balance are described in chapter 6. In chapter 7 the influence of smoking (cessation) on the regulation of energy balance and body weight are described. Conclusions are drawn in chapter 8 and in the last chapter recommendations are given.



## 2. Components of the energy balance system

Key components of the energy balance system are energy intake and energy expenditure. When energy intake exceeds total body energy expenditure, the surplus of energy is stored as fat. The components of the energy balance system, i.e. energy intake, energy expenditure and energy storage will be described below.

### 2.1 Energy intake

Carbohydrates and fats provide most of the dietary energy intake, whereas the fraction of dietary energy that is provided by protein intake is relatively small. The total chemical energy content of nutrients can be measured by bomb calorimetry. The principle behind this method is that the food or nutrient is burned and the liberated heat is measured in calories or joules (one calorie corresponds with 4.2 joule). However, not all of the energy measured using this method is available to the human body, because not all foods ingested are digested and absorbed in the gastrointestinal tract. The quantity of alcohol, carbohydrates, fats and proteins that is absorbed is 100%, 98%, 95% and 92%, respectively. Proteins also contain nitrogen, which is not completely oxidised, but converted to urea and excreted in the urine and so not available as energy source. To estimate the energy of food components available for the body, energy loss through faeces and urine needs to be subtracted from the total chemical energy content. The available energy for alcohol, carbohydrates, fat and protein is 29.8, 17.3, 37.1 and 15.9 kJ/g respectively (5;6).

Amino acids from food proteins are used for the synthesis of body proteins or can be converted to glucose. The body maintains a nearly constant protein content by adjusting amino acid oxidation to protein intake. Therefore, intake and utilisation of fat and carbohydrates primarily determine body weight regulation (7). After consumption, carbohydrates are broken down into glucose molecules that are stored in the body as glycogen, predominantly in the liver and in muscles. Evolution has led to the development of metabolic and endocrine regulatory responses that gives high priority to adjustment of glucose oxidation to carbohydrate intake. This is necessary because glycogen stores are important for maintaining stable blood glucose concentrations, for muscular responses to sudden demands and to ensure sufficient supply of glucose to the brain. The body's capacity to store glycogen is limited to a few hundred grams and is not much larger than the amount of carbohydrate usually daily consumed (8).

In contrast to protein and carbohydrate balance, fat balance is not tightly regulated. Oxidation of fatty acids is not significantly increased shortly after the consumption of a high fat meal. Furthermore, oxidation of triglycerides is inhibited by carbohydrate intake through the effect of insulin on lipolysis and fat oxidation. Moreover, the capacity to store triglycerides in adipose tissue is much larger than the capacity to store glycogen. As a consequence, surplus energy intake is mainly stored as fat (see 2.3).

## 2.2 Energy expenditure

The daily energy expenditure of humans can be divided in three main components (figure 2). The largest component is the resting metabolic rate (RMR), which accounts for 60-70% of daily energy expenditure (9). Other components are adaptive thermogenesis and physical activity, which account approximately for 10% and 15-30% of daily energy expenditure, respectively (10).

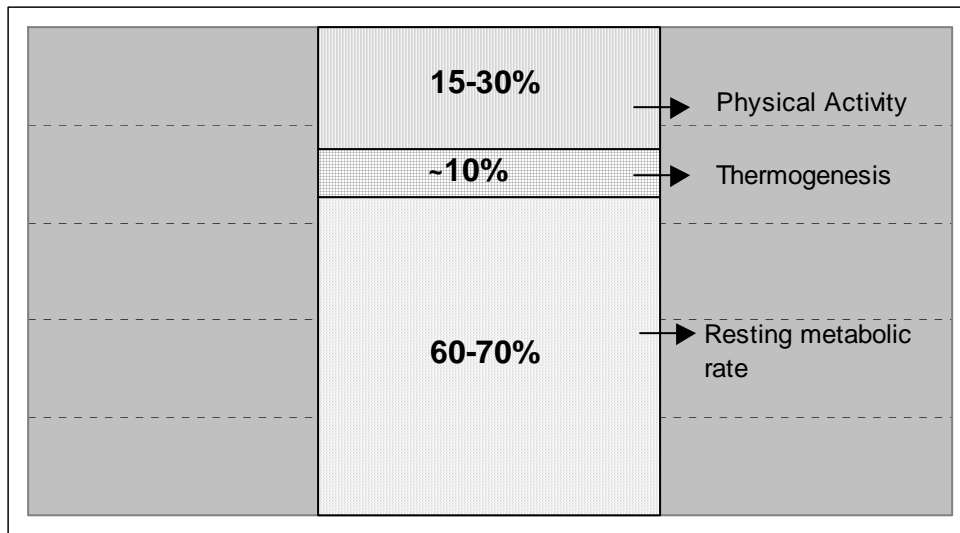


Figure 2. Components of energy expenditure and their contribution to the daily energy expenditure.

RMR can be defined as the energy expended to maintain basic physiological functions of the body, e.g. heartbeat, muscle function and respiration. RMR is determined primarily by fat free mass. The quantity of fat free mass explains 60-80% of the RMR. Fat mass also contributes to RMR by 10-13 kcal per kg fat mass. RMR declines with age and males have higher RMR values than women do, independently of differences in fat free mass. Subjects who are more physically active tend to have a higher RMR. Collectively, these factors explain 80-90% of the variance in RMR. The remaining part of variation in RMR has been ascribed to specific genetic factors (9;11).

Thermogenesis is the production of heat induced by food intake and cold. The intake of food causes an increase in metabolic rate, which is known as the thermic effect of a meal or diet-induced thermogenesis. The surplus of energy is expended in order to digest, metabolise and store ingested macronutrients. The increase in metabolic rate occurs over at least five hours after food intake and it is usually assumed to amount to around 10% of the energy ingested. Cold also induces a thermogenic effect which involves shivering and non-shivering effects to maintain body temperature (5;9).

The third component of energy expenditure is the increase in metabolic rate due to physical activity. The amount and type of activity and the intensity at which the activity is performed determine this component. Normally, physical activity is the most variable component of daily energy expenditure.

## 2.3 Energy storage

When energy intake exceeds energy expenditure, energy is stored primarily as fat in adipose tissue. The number and size of fat cells (adipocytes) are found to be greater in obese individuals compared with lean individuals. To date, convincing evidence has been provided that adipose cell acquisition and deletion (apoptosis) can occur in humans (12).

Mammals have two types of adipose tissue, brown and white, which have different functions. Brown adipose tissue has a very active metabolism that provides body heat. In human neonates, brown adipose tissue is clearly present, especially around the thymus gland, in the neck and between the shoulder blades. Later in life the expansion of white adipose tissue overgrows that of brown adipose fat, brown adipocytes become rare and lie scattered throughout white adipose tissue (10).

The primary function of white adipose tissue is to store energy, and obesity is characterised by an increase in the amount of white adipose tissue. In humans, about two-third of the adipose tissue is located under skin (subcutaneous) and one-third internally (visceral). Today, adipose tissue is recognised to be more than just an energy depot. Adipose tissue is a biologically active and dynamic tissue with major endocrine and possibly immunological roles. The endocrine role of adipose tissue in the regulation of energy balance will be described in chapter 3.



### 3. Physiological regulation of energy balance

The regulation of energy balance and consequently body weight is a complex process wherein many factors, such as neural, endocrine, metabolic, emotional and cognitive signals, are involved. The central nervous system plays an important role in this regulation. The central nervous system influences energy balance through effects on feeding behaviour and through effects on energy expenditure via effects on autonomic nervous system activity. Physiological systems that are involved in the regulation of food intake and energy expenditure will be described in more detail below.

#### 3.1 Physiological regulation of food intake

Food intake is a complex process involving cognitive, psychosocial and autonomous physiological components (13). Hunger is a feeling to eat because of physiological need for energy and is modulated by peripheral satiation and satiety signals (see figure 3). Satiation is the suppression of hunger within a meal that leads to meal termination and is predominantly caused by sensory properties. Sensory properties of food are taste, smell, temperature, texture and appearance. Satiety can be defined as the inhibition of hunger after food consumption that affects the interval between meals and therefore meal frequency as a response to cognitive, post-ingestive and post-absorptive processes. Post-ingestive mechanisms include release of gastric and gut hormones and gastric distension and emptying. Post-absorptive processes imply effects of blood glucose concentrations and hepatic fat oxidation (13;14). Although satiation and satiety can be considered as two independent processes there is also a considerable overlap in the processes involved (13). Therefore, in the remainder of this report the term satiety will be used for both.

The hypothalamus is a region of the brain that plays an important role in the regulation of energy balance. The arcuate nucleus, which is located within the hypothalamus, is a critical region for integrating the peripheral hormonal signals and sending efferent signals that result in behavioural, autonomic and endocrine responses to energy demands (15;16). The physiological mechanisms that regulate feeding behaviour in humans can be divided into a short- and a long-term control system.

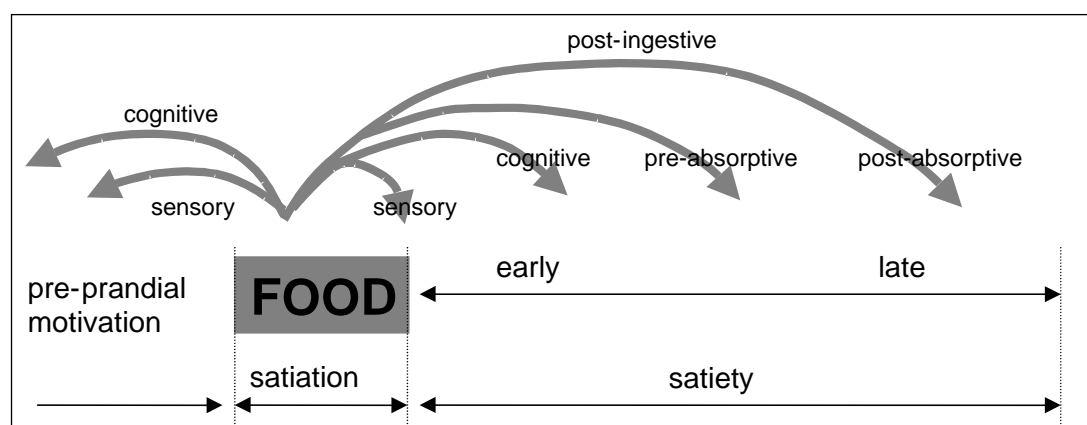


Figure 3. The satiety cascade (adapted from (14))

### 3.1.1 Short-term system

Short-term control of energy intake involves the initiation and termination of meals and feeding throughout the day (16). After food intake, gut peptides such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP1) are released from the gastrointestinal tract to mediate satiety. CCK and GLP1 influence satiety through inhibition of gastric emptying rate or through neural signals from the gut and may possibly have direct effects on central feeding centres in the brain (13;17). Recently, the gut hormone fragment peptide YY 3-36 (PYY) was identified as an appetite suppressant (18). PYY is released from the gastrointestinal tract after a meal in proportion to the caloric content of that meal. PYY reduces food intake through inhibition of gut motility, which causes a sense of satiety and through effect on appetite circuits in the hypothalamus.

Also, food intake itself is a determinant of the onset of satiety (13). The intake of food causes gut distension that induces neural signals (16;19;20). These neural and endocrine signals are transmitted via the vagus nerve, to the nucleus tractus solitarius. This is an area of the caudal brain stem that integrates sensory information from the gastrointestinal tract and abdominal organs, as well as taste information of the oral cavity (16;21). From this area, afferent neuronal signals are transmitted to the hypothalamus and other brain areas (13;21;22). However, the exact mechanisms underlying these signalling pathways are not completely understood yet.

In contrast to meal termination, the initiation of a meal tends to be a less biologically controlled process. However, in 1999 ghrelin was discovered. This peptide is produced by the stomach and duodenum and appears to be a potent appetite stimulator (23;24). Ghrelin levels rise an hour or two before a meal, rise with food restriction or starvation and fall shortly after every meal (15;25). Ghrelin stimulates food intake through action on the arcuate nucleus in the brain.

### 3.1.2 Long-term system

The long-term system balances food intake and energy expenditure and is thereby ultimately important for regulating body weight (16). The pancreatic hormone insulin was the first hormonal signal implicated in the control of body weight (21). In 1994, Friedman and co-workers discovered the hormone leptin (26). This hormone is excreted by adipocytes and reports information about a person's body fat store to the brain. Leptin and insulin are long-term signals that circulate at levels proportional to body fat content. They enter the central nervous system in proportion to their plasma levels where they act to reduce energy intake through the leptin-receptor (21;27). Leptin-receptors and insulin-receptors are among others expressed by brain neurones involved in energy intake.

Administration of leptin or insulin directly into the brain of respectively mice or baboons reduces food intake, whereas deficiency of leptin does the opposite (28;29). *Ob/ob* mice are obese and have no leptin as a result of a mutation in the leptin gene. Their brains therefore lack the signal that there are adequate fat stores and as a result the animals become hyperphagic and obese. Adding leptin reverses obesity in *ob/ob* mice and causes leanness in wild-type mice, but these effects may be temporary. *Db/db* mice are also obese but as a result of mutations in the leptin-receptor gene. Due to this disruption, these mice are unresponsive to endogenous or exogenous leptin (21;29).

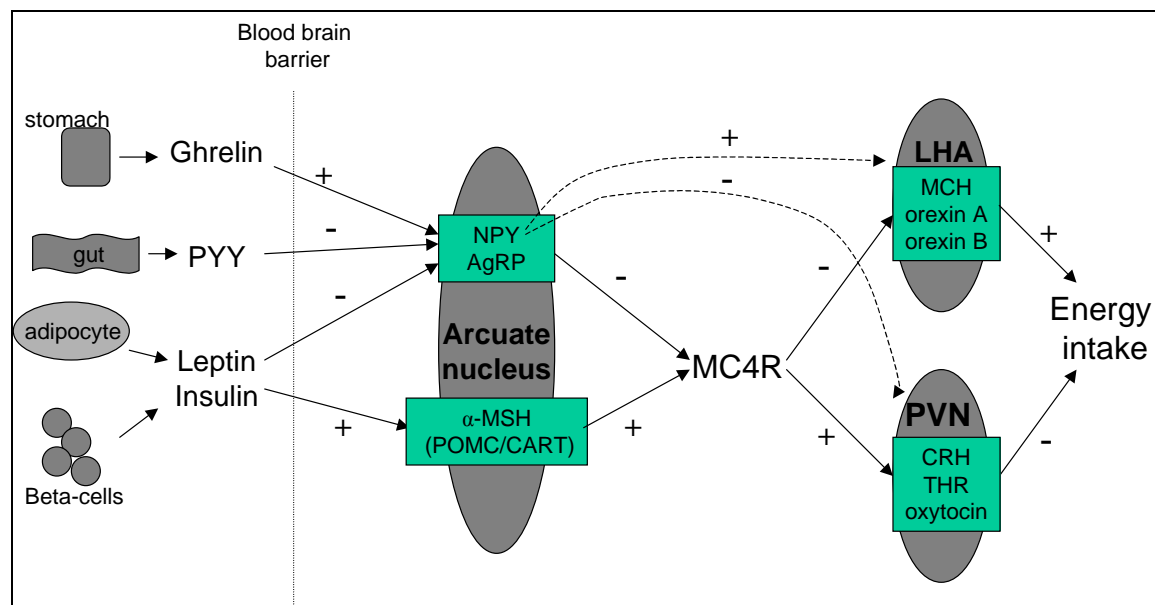
In contrast to leptin deficiency, insulin deficiency is not associated with obesity. In fact, insulin is required for fat deposition. Therefore, even when energy intake is very high, weight gain cannot occur in the presence of insulin deficiency. This is supported by findings in humans and mice that uncontrolled type I diabetes mellitus (lack of insulin) may be accompanied by hyperphagia, while body adiposity and leptin levels nevertheless remain

low. In a rat-model of uncontrolled Type I diabetes mellitus, replenishment of leptin to normal levels prevented the development of diabetic hyperphagia. This suggests that leptin has a more important role than insulin in the control of energy homeostasis (21).

The short- and long-term systems that regulate food intake seem to be interrelated. Leptin may exert its action on food intake in interaction with post-ingestive signals as CCK (see 3.1.1) and so decreases meal size with no effect on meal frequency (30). Additionally, ghrelin has also been implicated in the long-term regulation of body weight in humans (25). The pathway through which leptin and other signals work in the brain is described in more detail in the next paragraph.

### 3.1.3 The melanocortin pathway

Ghrelin, PYY, insulin and leptin all act through the arcuate nucleus in the hypothalamus on an important neuropeptide system called the melanocortin pathway (see figure 4).



**Figure 4. The Melanocortin Pathway**

LHA: lateral hypothalamic area, PVN: paraventricular hypothalamic nucleus, PYY: fragment peptide YY 3-36, AgRP: Agouti related protein, NPY: neuropeptide Y, α-MSH: α-melanocyte stimulating hormone (derived from proopiomelanocortin (POMC)), CART: cocaine-and-amphetamine-related-transcript, MC4R: melanocortin 4 receptor, MCH: melanin-concentrating hormone, CRH: corticotropin-releasing hormone, TRH: thyrotropin-releasing hormone.

Leptin and insulin cross the blood brain barrier and act through respectively leptin- or insulin-receptors on two distinct populations of neurones within the arcuate nucleus. Both hormones reduce the expression of specific genes in the neurones producing the neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY). These peptides increase appetite and reduce energy expenditure and are therefore called to be orexigenic. Otherwise, leptin and insulin induce the expression of neurones that cause the release of cocaine-and-amphetamine-related-transcript (CART) and (α-MSH), which is derived from proopiomelanocortin (POMC). These so called anorexigenic peptides inhibit eating and increase energy expenditure (15;21).

AgRP and α-MSH both act through the melanocortin 4 receptor (MC4R), which is central to the melanocortin pathway. α-MSH stimulates this receptor, while AgRP suppresses it. How the MC4R signals produce downstream effects on appetite and energy expenditure is yet

unclear. It has been hypothesised that these melanocortin signals act on the paraventricular hypothalamic nucleus and the lateral hypothalamic area. These hypothalamic areas synthesise anorexigenic and orexigenic signalling molecules. Neurones of the paraventricular hypothalamic nucleus synthesise corticotropin-releasing hormone, thyrotropin-releasing hormone and oxytocin, which reduce food intake and body weight. Corticotropin-releasing hormone also activates the sympathetic nervous system. Neurones of the lateral hypothalamic area synthesise melanin-concentrating hormone, orexin A and orexin B, which increase food intake (16;21).

NPY stimulates food intake and controls autonomic and endocrine actions aimed at sparing energy (31). When NPY is administered to the hypothalamus it increases feeding and suppresses energy expenditure (16). In contrast to AgRP, which acts through MC4R, NPY decreases the synthesis of thyrotropin-releasing hormone more directly, possibly via an inhibitory effect on NPY receptors of the paraventricular hypothalamic nucleus. NPY also increases the synthesis of melanin-concentrating hormone and orexin in the lateral hypothalamic area (27).

Similar to leptin, PYY may decrease food intake by inhibiting the expression of NPY/AgRP neurones and stimulation of the expression of the POMC neurones (27). Conversely, ghrelin increases food intake through stimulation of ghrelin receptors on NPY/AgRP neurones. There is also evidence that ghrelin may activate the synthesis of orexin in the lateral hypothalamic area independent of NPY stimulation (32).

Another melanocortin receptor, melanocortin 3 receptor (MC3R) which is highly expressed in the hypothalamus and is stimulated by  $\alpha$ -MSH, seems also to be involved in the regulation of energy balance (27;33). Mice lacking the MC3R have normal food intake and no disturbance in metabolic rate, but have increased body fat, decreased lean mass and are relatively inactive. This excessive adiposity is caused by increased feed efficiency, i.e. the amount of weight gained and fat deposited per calorie consumed (33). The mechanism and exact role of MC3R in the regulation of the energy balance and increased feed efficiency are, however, unclear.

### **3.1.4 Serotonin pathway**

It has long been known that serotonin affects energy balance. An increase in hypothalamic serotonin was found to reduce food intake and mice lacking a receptor for serotonin (the serotonin-2c receptor) spontaneously became fat (21;34). Because of its effect on energy balance, serotonergic receptors are used as primary targets for several anti-obesity drugs that suppress food intake (34). Nevertheless, the exact physiological mechanism to explain the effect of serotonin on food intake is unclear. Recently, it has been demonstrated that serotonin may act along with the melanocortin pathway to exert its effect on food intake and body weight (reviewed in (21)). Serotonin activates POMC neurones in the arcuate nucleus and subsequently food intake was reduced. This effect was attenuated when MC4R and MC3R are blocked. These results suggest that serotonin may affect food intake through serotonin-2c receptors expressed at POMC neurones. Furthermore, it seems that an intact serotonin pathway is required to maintain a normal energy homeostasis.

### **3.1.5 Ghrelin, PYY, Leptin and human obesity**

Overweight and obesity result from energy imbalance. Since ghrelin, PYY and leptin are important signals for the regulation of energy intake and expenditure, it may be that the levels of these signalling factors are associated with obesity.



*Ghrelin* seems to be part of the short-term appetite-control system and theoretically, overproduction may lead to obesity. The highest ghrelin levels ever measured in humans was in subjects with extreme obesity caused by the Prader-Willy syndrome (15). However, Prader-Willy syndrome is a rare disorder, and most obese humans tend to have lower ghrelin levels as compared to subjects with normal weight (15). Cummings and co-workers (25) investigated ghrelin levels in obese subjects before and after a six-month dietary weight loss program. After diet-induced weight loss the levels of circulating ghrelin over a 24-h period were increased as compared to levels before weight loss. This finding suggests a role for ghrelin in the long-term regulation of body weight in humans and in the adaptive responses that constrain weight loss. Obese subjects who underwent gastric bypass surgery causing loss of appetite and body weight, had plasma ghrelin levels that did not oscillate in relation to meals and were markedly lower compared to levels in normal-weight subjects (25). Ghrelin antagonists might reduce appetite and induce weight loss and are a possible candidate to be used in the treatment of obesity.

*PYY* is an appetite suppressant and may therefore also be involved in the pathogenesis of obesity. One study showed that *PYY* levels were negatively correlated with body mass index and significantly lower in obese subjects as compared to normal-weight subjects (35). However, on the basis of these results it remains unknown whether the low *PYY* level is the cause or the consequence of obesity. Infusion of *PYY* in obese subjects significantly decreased plasma ghrelin levels, 24-h caloric intake and appetite. These findings show that obese subjects respond to the anorectic effects of *PYY*. Therefore, *PYY* is a potential candidate to be used in the treatment of obesity. Long-term studies are needed to elucidate the possible role of *PYY* in body weight regulation.

The role of *leptin* in the pathogenesis of obesity has been studied extensively. Abnormalities in the production of leptin, its receptor, the cells that receive leptin's signal, or the efferent pathways that respond to leptin and affect changes in weight may be involved in alterations of body weight. When a person's adipose tissue mass declines so does leptin production, which in turn stimulates food intake and decreases metabolism. In addition, decreased levels of leptin activate a hormonal response that is characteristic for the starved state. Conversely, an increase in adipose tissue is associated with an increase in leptin levels, which acts to reduce food intake. However, as fat mass further increases, further rises in leptin have a limited ability to suppress food intake and prevent obesity (29;36). It seems that leptin's main role is to protect against weight loss in times of deprivation rather than protect against weight gain in times of abundance (15). The anti-obesity role of leptin might have been limited through evolutionary pressure to promote fat storage in times of plenty (16).

Based on the anorexic effects of leptin, one would expect that obese individuals have lower leptin levels than those with normal weight. Indeed, in pre-obese Pima Indians relatively low plasma leptin concentrations predispose to weight gain (26). Surprisingly, further research observed relatively low levels of leptin in five to ten percent of obese humans only. These findings suggest that in a fraction of the population obesity results from an abnormal secretion rate of leptin (29). Most obese humans have relatively high circulating levels of leptin, which suggests that most cases of obesity are associated with insensitivity to leptin (leptin resistance), rather than to leptin deficiency.

The mechanisms that contribute to the development of leptin resistance are still unknown. One proposed mechanism is that leptin transport to the brain may be dysfunctional and responsible for leptin resistance. This hypothesis is supported by the finding that obese subjects have relatively low leptin levels in cerebrospinal fluid as compared to levels in

plasma. Another potential mechanism involves a defect in obese subjects in leptin signal transduction within the hypothalamus (16;21;29). More research is needed to elucidate the mechanisms that contribute to leptin resistance.

## 3.2 Physiological regulation of energy expenditure

The strongest support for the hypothesis that defects in energy expenditure may lead to obesity comes from *ob/ob*, *db/db*, and the melanocortin-4-receptor gene knock-out mice, which do not have functional leptin, leptin receptors, or MC4R (see 3.1.2 and 3.1.3). These mutant mice are hyperphagic. However, when food intake of these mutant animals is restricted to normal levels they still develop obesity. Thus energy expenditure must be decreased (37).

At least one component of energy expenditure is under control of physiological regulatory mechanisms, namely adaptive thermogenesis. It is proposed that excessive caloric intake may be sensed by the brain, which then triggers an increase in energy expenditure to avoid excessive weight gain (37). Uncoupling proteins and/or the sympathetic nervous system are involved in the regulation of adaptive thermogenesis and will be described below.

### 3.2.1 Uncoupling proteins (UCP)

Many processes in the body require adenosine triphosphate (ATP) as energy delivering substrate. This molecule can release energy by donating one or two phosphate groups, leaving adenosine diphosphate (ADP) or adenosine monophosphate (AMP), respectively. However, ATP storage is limited and therefore, ATP has to be re-synthesised continuously from ADP in the mitochondria of a cell in a process called oxidative phosphorylation. To convert ADP into ATP, the enzyme ATP synthase uses energy that is released from protons (re-)entering the mitochondria. Protons may also re-enter (leak) through an uncoupling protein (UCP). As a result energy is not converted to ATP but lost as heat (10;37).

UCP1 is the first described uncoupling protein with gene expression almost exclusively in brown adipose tissue. An increase in protons leaking via UCP1 in brown adipocytes is responsible for non-shivering thermogenesis in new-born humans to maintain body temperature. However, in human adults the contribution of UCP1 mediated thermogenesis and the role of UCP1 in body weight regulation is controversial because brown adipose tissue is relatively scarce (16).

In humans, skeletal muscle is the most important tissue for adaptive thermogenesis (38). Since UCP1 is not present in skeletal muscle, other UCPs were expected to exist. Two UCP1 homologues, UCP2 and UCP3, were found and also have suspected uncoupling activities. However, their exact function remains largely unknown to date (37). UCP2 is expressed in most tissues, whereas UCP3 is expressed predominantly in skeletal muscle and brown adipose tissue (10). In contrast to what would be expected, fasting increases the expression of UCP2 in white adipose tissue and skeletal muscle as well as UCP3 expression in skeletal muscle in both lean and obese individuals (39). This increase in UCP2 and UCP3 expression suggests that these proteins may play a role in the metabolic adaptation to fasting. Because UCP2 and UCP3 are, like UCP1, able to uncouple ATP formation from energy substrate oxidation and are expressed in tissues that have an important role in energy expenditure it can be assumed that those UCPs are involved in regulation of energy balance.

Obesity in humans may partly be caused by abnormalities in energy expenditure. In Pima Indians low energy expenditure, relative to lean body mass, predicted future weight gain (37).

Differences in UCP expression between humans may be involved in these aberrations in energy expenditure and subsequently in the development of obesity.

The role of UCP1 in the development of human obesity is probably small, because brown adipocytes are rare in adult humans (10;37). Therefore, the role of UCP2 and UCP3 in human obesity and energy expenditure has been widely studied. It was found that higher UCP2 expression in white adipose tissue was associated with a higher body mass index. However, in skeletal muscle no differences in UCP2 and UCP3 expression were found between obese and lean Caucasians (39). In contrast, in Pima Indians, higher levels of skeletal muscle UCP3 expression were associated with a lower body mass index and with a higher resting metabolic rate (10). These data indicate that low skeletal muscle UCP3 expression may result in a reduced resting metabolic rate and possibly in an increased BMI at least in Pima Indians. In conclusion, the exact role of UCPs in humans and their potential role in human obesity are still unclear.

### **3.2.2 Sympathetic nervous system (SNS)**

Sympathetic nervous system activity is involved in the regulation of energy expenditure. It is thought to be the pathway by which the brain regulates adaptive thermogenesis (16;37;40). This idea is supported by the finding that exposure to cold and food increases sympathetic activity. Additionally, brown adipose tissue seems to be heavily innervated by sympathetic nerves (16). The SNS activates brown adipose tissue mediated thermogenesis via  $\beta$ -adrenergic receptors (ADRBs) (41). So far, in humans three types of ADRBs are known to exist ( $\beta$ 1-,  $\beta$ 2- and  $\beta$ 3-adrenergic receptors) that are found in various tissues (41). The catecholamines noradrenaline and adrenaline are ligands for the ADRBs and endogenous infusion of these significantly increases energy expenditure (42). This may partly be explained by the observation that stimulation of ADRBs on brown adipocytes causes an acute increase in UCP1 proton leakage (and therefore heat loss, see 3.2.1). However, less is known about the role of ADRBs in skeletal muscle. The different types of ADRBs differ in their affinity for catecholamines. Stimulation of human ADRB1 and ADRB2 causes an increase in thermogenesis, oxidation of fatty acids and lipolysis (41). ADRB3 also seems to be involved in these processes, but studies aimed at resolving this question were inconclusive (43). Evidence that SNS is also involved in diet-induced thermogenesis comes from mice lacking all three ADRBs. These mice develop massive obesity when fed a high-fat diet (37). This finding indicates that ADRBs seem to play a significant role in the defence against diet-induced obesity.

In humans, variations in 24-h energy expenditure may be caused by differences in SNS activity. However among the obese, SNS activity is generally normal or increased. Nevertheless, there is some evidence that the response of the SNS to various physiological stimuli as underfeeding or cold exposure may be blunted in obese subjects. The sensitivity to a certain level of SNS activity may also be reduced. Schiffelers and co-workers for example, observed that during  $\beta$ 2-adrenoceptor stimulation, the increase in energy expenditure was significantly lower in obese subjects as compared with normal weight subjects (44). These observed impairments in SNS responsiveness may play a role in the development of obesity, because they often remain after weight reduction (41). Further support for the involvement of SNS in the development of obesity comes from findings among Pima Indians. In this population lower SNS activity at baseline was associated with more weight gain during follow-up as reviewed by Spiegelman and Flier (41).

In summary, the role of ADRBs in human skeletal muscle needs to be further elucidated. Most evidence suggests that low SNS activity and/or low sensitivity may be involved in the aetiology of obesity.

### 3.3 Physiological regulation of the energy balance at low levels of energy expenditure

In the introduction it has been suggested that it is difficult to maintain energy balance at low levels of energy expenditure (see figure 1 in chapter 1). This suggests that the physiological regulation of energy balance differs at low levels of energy expenditure from that at high levels of energy expenditure. However, the literature does not seem to support such a hypothesis. Nevertheless it seems reasonable to propose that it is more difficult to maintain body weight at low levels of energy expenditure for the following reasons. Firstly, it is easier to overeat when energy expenditure is low. Additionally, when physical activity is low, the relative contribution of resting metabolic rate to daily energy expenditure is very high. The absolute value of the small positive energy balance of 6.5 kcal (which is sufficient to obtain an increase in body weight of 3 kg in 10 years time, see chapter 1), is equal at low and high levels of energy expenditure. However, relatively speaking 6.5 kcal is much more at low levels of energy expenditure. The opportunity to spend this excess in energy by increasing energy expenditure may be greater at high than at low levels of physical activity.

Schwartz and co-workers have described a model of energy balance to better understand its controlling pathways and hypothesised that this system may have a tendency towards weight gain (45). They proposed that the response to weight gain is less vigorous with regard to the changes in anorexigenic and orexigenic peptides relative to the basal state than the response to weight loss (see table 1). The basal state was defined as the steady state in which energy balance and fat storage remain constant in a given environment. The authors proposed that in this steady state peptides that inhibit appetite and stimulate energy expenditure (anorexigenic: POMC/CART) are activated. Conversely, the synthesis of peptides with opposite effects (orexigenic: NPY/AgRP) is inhibited. So, in the basal state energy intake is suppressed and metabolic rate is increased in order to maintain weight.

*Table 1. Model of energy balance of Schwartz et al. to explain a tendency towards weight gain (45).*

|              | Response of peptides involved in melanocortin pathway |                         |  |
|--------------|---|-------------------------|--|
|              | Anorexigenic <sup>a</sup>                             | Orexigenic <sup>b</sup> | Net effect (energy intake versus energy expenditure) |
| Steady state | ↑   | ↓                       | 0  |
| Weight gain  | ↑↑  | ↓                       | ↓  |
| Weight loss  | ↓   | ↑                       | ↑↑   |

a: inhibit appetite and stimulate energy expenditure, b: stimulate appetite and inhibit energy expenditure

According to their theory the peptides that inhibit energy intake and increase energy expenditure are further activated in response to weight gain, but there is little change in the already inhibited orexigenic peptides. Weight loss, on the contrary, in their view causes a more vigorous reaction. Peptides that stimulate appetite and decrease energy expenditure are activated (instead of inhibited), while the peptides that inhibit food intake and increase metabolic rate are inhibited instead of activated. Consequently, the energy balance system seems to be more prone to weight gain than to weight loss (45).

Several findings support this hypothesis. For example, destruction of the arcuate nucleus – and thus the destruction of both orexigenic and anorexigenic stimuli - causes obesity. Furthermore, disruption of the MC4R, which also disrupts both orexigenic and anorexigenic signalling causes hyperphagia and obesity. In contrast, mice lacking appetite inducing NPY, maintain normal body weight. This provides evidence for the hypothesis that under usual conditions peptides that inhibit appetite and stimulate energy expenditure predominate over peptides that stimulate food intake and decrease energy expenditure. The appetite inhibiting peptides, but not the appetite stimulating peptides are required for maintaining normal body weight.

### **3.4 The melanocortin pathway and energy expenditure**

The metabolic response that leptin elicits can not be explained by its anorectic effects alone. Additionally to its effect on energy intake, leptin may increase energy expenditure by altering the pathways through which fatty acids are metabolised. It was showed that leptin activates AMP-activated protein kinase (AMPK) in skeletal muscle (46). AMPK suppresses the activity of an enzyme that catalyses a key step in fatty acid synthesis, of which its product suppresses the import of fatty acids into the mitochondria for oxidation. As a final result the synthesis of fatty acids is suppressed and fatty acid oxidation is increased in skeletal muscle by leptin (46). Recently, in mice it was found that leptin inhibited AMPK activity in the hypothalamus (47). This inhibition seems to be necessary for leptin to exert its effect on food intake and body weight.

Furthermore, leptin may influence other pathways that are involved in fatty acid metabolism. For example, leptin represses the activity of stearoyl-CoA desaturase (SCD-1) in the liver. This enzyme catalyses the biosynthesis of monounsaturated fatty acids. A significant proportion of leptin's effect on energy expenditure may result from inhibition of SCD-1. Repression of SCD-1 may prevent fat accumulation due to an increase in oxidation of fatty acids (48). Indeed, mice lacking SCD1 have reduced body fat due to an increase in energy expenditure (49).

Additionally, several neuropeptides of the melanocortin pathway also influence SNS activity. In rats NPY was found to decrease SNS activity, whereas corticotropin-releasing hormone and orexin-A stimulates SNS activity. Furthermore, these neuropeptides are also associated with UCP expression. Infusion of NPY reduces UCP1 expression in brown adipose tissue, but increases UCP3 expression in muscle. CART as well as leptin were found to increase UCP1, UCP2 and UCP3 expression in respectively brown adipose tissue, white adipose tissue and in muscle (50).

In conclusion, there is evidence that the melanocortin pathway that controls food intake also affects energy expenditure



## 4. Interindividual differences in weight regulation

Twin studies, analyses of familial aggregation, and adoption studies all indicate that obesity is for a (large) part determined by genetic factors. However, since our genes have not changed substantially for centuries, genetic factors are not responsible for the dramatic rise in the prevalence of obesity over the past two decades. It seems that changes in our environment are mainly contributing to this obesity epidemic (51). However, within our “obesigenic” environment there are individuals who do and individuals who do not become obese. It is this variability in risk for developing obesity between individuals that is largely determined by genes. The susceptible-gene hypothesis is supported by findings from twin studies in which pairs of twins were exposed to long-term overfeeding (52). The amount of weight gained in response to overfeeding and the site where fat was deposited showed greater similarity within twin pairs than between twin pairs. These findings suggest that differences in genetic predisposition determine those who are most likely to become obese in any given set of environmental circumstances. The 2003 Update of the Human Obesity Gene Map shows that more than 430 genes, markers, and chromosomal regions have been associated or linked with human obesity phenotypes (53).

Several single mutations in human genes with severe effects on functionality of the gene product have been identified that cause monogenic obesity. Among these are the genes that code for leptin, the leptin receptor, POMC, MC4R and the enzyme proconvertase, which cleaves POMC (54;55). Mutations in the MC4R gene are involved in about 5% of extremely obese individuals. Mutations in the other four obesity genes are also rare in humans (16). Remarkably, all of these genes code for proteins that are part of the leptin (melanocortin) pathway. In contrast, no mutations in genes involved in other pathways potentially regulating energy balance are found so far. This suggests that the melanocortin pathway may indeed be the primary pathway that regulates energy balance (55).

Current evidence suggests that genetic susceptibility to most of human obesity is the result of multiple genes (polygenic), each with a modest effect, that interact with each other and with environmental factors such as nutrients, physical activity and smoking (55). To search for susceptibility genes underlying polygenic obesity, the candidate gene approach has often been used. Candidate obesity genes are selected based on their suspected physiological involvement in energy homeostasis. So far, the  $\beta$ 3-adrenergic receptor (ADRB3) gene is the most extensively studied candidate gene for human obesity. The ADRB3 may be involved in obesity through its effect on thermogenesis in adipose tissue (see 3.2.2). A polymorphism in the ADRB3 gene at codon 64 leads to the replacement of tryptophan by arginine (Trp64Arg) in the receptor protein. Until now, three meta-analysis of the association of Trp64Arg polymorphism of ADRB3 with body mass index are carried out. Each of the studies differed slightly by the criteria used for inclusion of studies and by differing statistical methods, but the absolute magnitude of the effect was very similar among the three studies. They showed that carriers of the arginine allele exhibited a higher BMI (0.19-0.30 kg/m<sup>2</sup>) than non-carriers (43;56;57).

In humans, the genes encoding leptin, leptin receptor,  $\beta$ 2-adrenergic receptor, UCP2 and UCP 3 are also frequently studied as candidate genes in relation to obesity-related phenotypes as BMI, body fat and WHR. The results were however inconclusive. Only for the leptin receptor a meta-analysis was carried out pooling the results of nine studies. No significant association was observed between three major polymorphisms in the leptin receptor and BMI or waist circumference (58). In conclusion, many genetic factors that may influence

susceptibility to obesity are studied, but only for a limited number of specific genetic variants positive results are found. This emphasises the importance to study multiple genes in interaction with each other and with environmental factors.



## 5. Influences of diet on energy balance

Several dietary components as energy density, macronutrients and dietary fibre as well as the influence of food texture potentially have effect on the regulation of energy balance. The energy density is the amount of metabolically available energy per unit of weight or volume of food (59). The effects of fat (macronutrients), dietary fibre and water (texture) on energy balance are discussed first. Thereafter, the influence of energy density on energy balance is described. Finally, the popular Atkins diet will be discussed as well as the source of infant feeding and its effect on energy balance and obesity later in life.

### 5.1 Effect of macronutrients on energy balance

Macronutrients (fat, protein and carbohydrate) may have different effects on energy balance because of differences in (biological) properties, such as energy density, satiety, thermic effect and absorption efficiency (see table 2).

*Table 2. Properties of nutrients that may affect energy balance (adapted from (60))*

|  | <b>Protein</b> | <b>Carbohydrate</b> | <b>Fat</b> |
|--|----------------|---------------------|------------|
| <b>Energy density</b>                  | 17 kJ/g        | 17 kJ/g             | 38 kJ/g    |
| <b>% absorbed</b>                      | 92%            | 95%                 | 98%        |
| <b>Metabolic priority of oxidation</b> | First          | Second              | Last       |
| <b>Satiating capacity; short term</b>  | High           | Medium              | Low        |
| <b>Satiating capacity; long term</b>   | -              | Low                 | High       |
| <b>Thermic effect</b>                  | High           | Medium              | Low        |

Fat is more energy-dense than carbohydrate or protein, so high-fat foods are often high-energy dense foods (see paragraph 5.4) (61). Furthermore, fat is more effectively absorbed from the intestine than proteins and carbohydrates (see 2.1). The oxidation of fat has the lowest metabolic priority, followed by carbohydrate and protein oxidation, which has the first priority (see 2.1) (14).

Fat has a smaller satiating effect within a meal and early after a meal followed by carbohydrate and protein (62). Its satiating effects may be too delayed to prevent excessive energy intake during a high-fat meal. On the other hand, fat appears to be more satiating than carbohydrate on the long term, because it has been shown that fat prolongs the intermeal interval (63). However, as the initiation of a meal tends to be less biologically controlled (see 3.1.1) it is likely that it is easier to overeat on a high-fat diet.

The effect of different types of fat and carbohydrate on satiety has also been studied. It has been suggested that in lean subjects diets high in polyunsaturated or saturated fat induce a higher level of satiety and a lower subsequent energy intake than diets high in monounsaturated fat (64). However, these results could not be confirmed in a recent study among overweight subjects (65). The influence of carbohydrates on satiety may also depend on the type of carbohydrate. Fructose, sucrose and glucose are metabolised through different pathways and therefore may have differential effects on satiety (66;67).

The different effects of macronutrients on satiety may be mediated by different effects on expression of hormones or neuropeptides involved in the regulation of energy balance. Intake of dietary fat causes the release of the potent satiety hormone CCK from the gut, which is involved in mediating short term satiety (14;61). Another peptide involved in short-term food intake regulation and which seems to be differently influenced by dietary factors is ghrelin.

Among women, this appetite stimulator and the feelings of hunger were found to decrease more after consumption of a high-carbohydrate meal than after a high-fat meal (68). Human leptin levels seemed not to be clearly influenced by dietary fat, but possibly by fatty acid composition (69). In humans, n-3 polyunsaturated fatty acids may decrease serum leptin levels (70).

Compared with rats that ingested a high-carbohydrate diet, rats that ingested a high-fat diet had lower expression of the orexigenic NPY gene in the arcuate nucleus (50). Highly saturated diets potentially decreases the expression of the appetite stimulating neuropeptides NPY and AgRP in the arcuate nucleus more than a high-polyunsaturated fat diet. In conclusion, there are indications for an effect of diet on the expression of neuropeptides involved in food intake regulation.

Macronutrients also differ in their thermic effect, which is the energy used to absorb, process and to store macronutrients. The thermic effects of protein, carbohydrate and fat - expressed as a percentage of their energy content - are respectively 25-30%, 6-8% and 2-3% (60). These differences mainly reflect the differences in energy cost to store macronutrients (60;61). Because of their different thermic effects, macronutrients may theoretically differ with respect to their effects on physiological components involved in the regulation of energy expenditure, such as uncoupling proteins or stearoyl-CoA desaturase. It has been suggested that high-carbohydrate diet may decrease the capacity for thermogenesis, because rats fed a high-carbohydrate diet show a marked suppression of the expression of UCP1 (50). Furthermore, it was found that sucrose feeding of rats increases UCP3 expression in muscle and decreases the efficiency of energy use (50).

Also, the expression of SCD1 seems to be influenced by dietary factors in several tissues in mice. A high-fat diet decreased hepatic SCD1 expression compared with a low-fat diet (4%) (71). Moreover, a high-carbohydrate may cause an increase in SCD1 expression in the liver and adipose tissue (72). These findings concerning SCD1 are in accordance with what would be expected due to the role of SCD1 in biosynthesis of monounsaturated fat. The exact relevance of these findings for body weight regulation in humans is, however, unknown.

Based on the above mentioned physiological differences between macronutrients in energy density and satiating capacity, one expects that a high consumption of fat may more easily lead to excess energy consumption. Additionally, because of its higher absorption rate and lower thermic effect consumption of fat may more easily lead to weight gain than carbohydrates or protein with equal energy content. Whether or not low-fat diets should be the first choice to prevent overweight is currently the subject of heavy debate (73;74).

According to Willett (73) the percentage of dietary energy from fat does not play an important role in weight maintenance, because long-term epidemiological studies and several long-term randomised trials show little, if any, effect of the percentage of energy from fat on body fatness. Furthermore, in a lot of affluent countries, a decline in percentage of energy from fat was observed while in the same time the prevalence of obesity increased massively. Astrup (74) claims that dietary fat is an important issue with regard to obesity, as high fat diets more easily lead to overconsumption. Therefore, in his opinion the percentage of energy from fat is an important determinant of weight, at least when people are allowed to eat as much as they like. In the literature these two aspects, i.e. the possible effect of dietary composition on weight *independent from* total energy intake and the possible effect of dietary fat on weight *because its effect on* total energy intake are often mixed up.

In this respect, most studies found that macronutrient composition does not influence weight loss when the energy content of an energy-deficit diet is stable (75;76). However, it was also shown that men who were overfed with fat gained more weight than men who were overfed

with carbohydrates to the same energy level. Furthermore, obese individuals were found to be more prone to the effect of fat overfeeding than non-obese individuals (61).

In conclusion, it remains unclear whether macronutrient composition of the diet influences body weight independently of total energy intake. Long-term studies are necessary to elucidate this issue.

## **5.2 The effect of dietary fibre on satiety**

Dietary fibre can be defined as dietary components that are not enzymatically broken down to absorbable units in the stomach and small intestine. Most dietary fibres are fermentable. This means that the undigested dietary fibres are fermented in the large intestine by anaerobic bacteria. Subsequently short-chain fatty acids and gasses are formed. These fatty acids are absorbed and used as energy source. A part of dietary fibres are soluble and dissolve to some extent in water or forms a gel (77).

Several potential physiological effects of dietary fibre on energy balance are proposed.

Dietary fibres are able to reduce the energy density of foods, which may increase satiety because of their high volume (see chapter 5.1) (62;77). The capacity to bind water leads to an additional lowering of energy density and also promotes gastric distension (77). Furthermore, dietary fibres may trigger maximal sensory stimulation in the mouth due to the increased need for chewing and so promote satiety (62;77). Increased chewing may also promote gastric distension through the subsequent production of saliva and gastric acid (77).

Furthermore, soluble dietary fibres lead to slower gastric emptying by forming a viscous gel matrix. The resulting extended period of nutrient absorption may increase satiety (62;77).

Additionally, some dietary fibres reduce the absorption of fat and protein by decreasing the physical contact between these nutrients and the bowel absorptive surface (77).

Gut hormones may mediate the delayed gastric emptying and slow bowel transit time caused by dietary fibre. One candidate is the satiety stimulating hormone glucagon-like peptide 1 which is secreted in the gastrointestinal tract in response to several stimuli including fermentable fibres (77) (see chapter 3.1.1). Therefore, dietary fibres may affect energy balance through stimulation of hormone secretion. Indeed, most human studies that investigated the effect of dietary fibre on hunger and satiety found an increase in satiety between meals and/or a decrease in hunger after consumption of a high-fibre diet compared to a low-fibre diet (77).

## **5.3 The effect of food texture (liquid vs. solid) on satiety**

The rise in obesity rates has also been attributed to the growing consumption of sweetened beverages (66;78). Therefore, it has been suggested that the provision of energy in liquid form plays a role in the obesity epidemic (66). Calories in liquid form may affect satiety less strongly than solid foods with the same caloric content (62).

However, the physiological responses that mediate satiety after consumption a liquid are conflicting. The absence of chewing and the higher rate of gastric emptying may induce weaker responses in the gastrointestinal tract that would otherwise lead to inhibition of further energy intake (66;67). However, simultaneous ingestion of solid and liquid foods may delay the rate of gastric emptying and increase satiety. Furthermore, liquids often have a low-energy density. This aspect also suggests that liquids compared with solid foods may have a greater ability to induce satiety (see 5.4.).

This uncertainty about the satiating ability of liquids is also reflected by discrepancies in observational studies. While some studies showed that solids are more satiating than liquids, other studies showed that liquids are more satiating than solid foods. Some of these

discrepancies may result from the timing of consumption. It seems that high-volume liquids consumed with or close before a meal may promote satiety, whereas liquids consumed between meals (as occurring often for soft-drinks) may be less satiating and may lead to over-consumption of calories.

## **5.4 The effect of the energy density of foods on satiety**

The energy density of food may influence energy balance by affecting gastric emptying in humans. High-energy dense foods reduce the gastric emptying rate in ml/minute, but the rate at which energy leaves the stomach (kcal/minute) is increased. The exact consequences of an increased rate of caloric emptying are unclear. It has been speculated that it causes an increase in the rate of nutrient digestion and absorption and that it accelerates the return of hunger after consumption of high-energy dense foods (59).

Energy density is predominantly influenced by fat and water content. Furthermore, the energy density of a food can be influenced by dietary fibre and sucrose content. Studies that investigated the effect of energy density by altering fat content found a lower total energy intake after ad libitum consumption of low energy dense diets (79). One study found the decrease in intake to be independent of macronutrient composition and that a similar volume of food daily was consumed across the conditions (80). Other studies that provided meals with fixed energy content but changing volumes also showed a reduced energy intake after consumption of higher volumes (i.e. a low-energy density meal) (81-84). A nice example is a 2-day study among women who had free access to a main entree varying in energy density (low, medium, high) due to alterations in the proportion of low-fibre vegetables to pasta (85). They consumed a similar weight of food across the three conditions. In contrast, studies that provided meals with fixed volumes but different energy contents found no consistent difference in subsequent satiety or hunger between test meals (59). Based on these results it seems that the greater volume of low-energy dense foods is mainly responsible for the effects of these foods on hunger and satiety. This hypothesis is supported by the finding that stomach distension causes neural signals inducing satiety (60-62).

Additionally, energy density may influence total energy intake through less physiological mechanisms. Firstly, high-energy dense foods tend to be more palatable than low-density foods. Secondly, high-energy dense foods have a small volume in relation to their energy content and therefore may easily be over-consumed(59;61). In this respect one needs to keep in mind that low-fat counterparts of high-fat foods often have comparable energy densities, since they are based on sugars or high-refined carbohydrates. Therefore they may also easily be over-consumed (73;78).

In conclusion, the energy density of the diet seems to be a very important factor for mediating satiety and energy intake.

## **5.5 The Atkins diet**

Despite the scientific debate about the effect of macronutrient composition of the diet on body weight (see 5.2), high-protein, high-fat, low-carbohydrate diets have become increasingly popular. At the moment, the Atkins diet is the most famous and a very popular low-carbohydrate diet. Therefore, the Atkins diet which will be discussed in more detail.

### 5.5.1 The Atkins philosophy

Atkins claims that most overweight individuals do not overeat, but that the intake of large amounts of carbohydrates are the cause of overweight. He was convinced that carbohydrates are addictive and result in increased hunger and food intake. Additionally, carbohydrate overeating causes elevated blood glucose levels, increased insulin production (hyperinsulinemia) and insulin resistance. Hyperinsulinemia is by Atkins held responsible for the resistance to loose weight. Reducing the intake of carbohydrates prevents overproduction of insulin and there are other metabolic advantages on a low-carbohydrate diet according to Atkins. The intake of carbohydrates should be restricted severely enough to produce ketosis. This is the formation of ketones as a result of rapid fat breakdown into glycerol and fatty acids, and is a physiological response to the lack of glucose as energy source (as in starvation). In such a situation the body adapts to the use of ketones as alternative energy source. As the use of body fat stores as a fuel in stead of dietary carbohydrates costs more energy, this allows, according to Atkins, overweight individuals to eat as many or more calories as before starting the diet, yet still lose weight. It would furthermore result in preservation of lean body mass and decreased appetite, blood glucose and insulin levels (67). In practice, the Atkins philosophy is based on four principles, i.e. weight loss, weight maintenance, good health and disease prevention. The cornerstone of this nutritional approach is a four-phase eating plan together with vitamin and mineral supplementation and regular exercise. The four phases are induction, ongoing weight loss, pre-maintenance and lifetime maintenance. In the induction phase, carbohydrate consumption is restricted to 20 grams per day, whereas the daily requirement of carbohydrates is 180 gram. Daily carbohydrate intake, in the form of nutrient-dense (amount of nutrients per calorie) and fibre-rich foods, is gradually increased in phase 2 to the extent that moderate weight loss is sustained. In phase 3 further increased until weight loss stops and a lower stable weight is maintained. The final phase, lifetime maintenance, consist of controlling carbohydrate intake, while selecting from a wide variety of foods.

### 5.5.2 Scientific evidence for the Atkins Philosophy

Of interest is to what extent the statements made by Atkins are supported by scientific evidence. Atkins puts the blame on carbohydrate overeating as the cause of hyperinsulinemia and finally obesity. Insulin is secreted after consumption of carbohydrates and proteins. However, the relationship between hyperinsulinemia and weight gain is not clear yet. Whereas some studies found a positive association between increase in insulin and increase in body weight (86;87), another study found a negative relationship (88). Even when hyperinsulinemia is the cause of the metabolic imbalance causing overweight, the composition of the weight loss diet (high or low carbohydrate) may play a role in the absolute reduction of insulin but not in the amount of weight loss (67). A recent systematic review found no favourable effects of low-carbohydrate diets on fasting serum glucose or insulin levels (89).

In May 2003, two randomised trials evaluating the effect of low-carbohydrate diets on weight loss were published in The New England Journal of Medicine by Samaha et al. (90) and Foster et al. (91). The press presented these results as scientific evidence for Atkins diet effectiveness. However, this conclusion is rather premature. Both studies found that after six months of follow-up obese individuals on a low-carbohydrate diet showed greater weight loss than obese individuals on a low-fat diet (respectively 7.0 vs. 3.2 kg and 5.8 Vs 1.9) (90;91). However, on the long-term (after one-year follow-up) these differences seem to be diminished and were no longer statistically significant (4.4 Vs 2.5 kg) (91).

Atkins claims that weight loss can be reached with equal or higher energy intake as compared to pre-weight loss intake. However, in the study of Samaha et al. the increased weight loss after six months in the low-carbohydrate group could be explained by the greater reduction in energy intake in this group compared with the low-fat group (90). Mechanisms responsible for the greater decrease in energy intake among the low-carbohydrate group may be alterations in satiety signals, but even so the monotony of this diet. Unfortunately, in the other study by Foster et al. food intake was not measured (91).

In addition to the lack of convincing results, both studies had shortcomings. They had a high dropout rate of about 40% percent on both diets, which underlines the difficulty of long-term compliance to both low-carbohydrate and low-fat diets. Furthermore, the low-carbohydrate diet in the study of Samaha et al. varied only slightly from the baseline diet in macronutrient distribution. It was not representative of the usual Atkins diet, whereas the low-fat diet used by the control group was not representative for a low-fat diet (91). Furthermore, the allegation that carbohydrate intake but not caloric intake is important with regard to weight control is in contradiction with results from a recent systematic review on the effect of low-carbohydrate diets for the treatment of obesity (89). This review demonstrated that weight loss was not predicted by carbohydrate content, but by caloric intake, diet duration and baseline body weight.

In conclusion, to this day there is no scientific evidence for a metabolic advantage on a low-carbohydrate diet with regard to weight loss.

### **5.5.3 Potential risks of the Atkins diet**

Little is known about the adverse effects of long-term use of low-carbohydrate diets. Several adverse effects have been proposed on lipids, glucose, insulin, liver and kidneys and it is important to investigate those potential risks.

Low-carbohydrate diets stimulate the formation of ketones. Excretion of ketones requires minerals, such as calcium, that must come from either food or body stores, which are predominantly found in the bones. Consequently, individuals with prolonged formation of ketones may risk calcium loss from bone (92). Furthermore, accumulation of ketones may result in abnormal insulin and glucose metabolism and impaired liver and kidney function (89).

Additionally, diets low in carbohydrates and high in fat (often high in saturated fat) may adversely affect serum lipid profiles. Increased intake of saturated fat is known to cause an increase in LDL-cholesterol. However, both randomised trials described above, found no effect of low-carbohydrate diets on total or LDL-cholesterol. In fact, lipid profile improved more in the Atkins group as compared to the low-fat group, characterised by a larger increase in HDL-cholesterol and/or a greater decrease in triglyceride concentrations (90;91). The larger improvements in these lipid fractions in the Atkins group may be the result of lowering carbohydrate intake, which was already associated with short-term decrease in HDL-cholesterol and triglyceride in earlier studies. It could also be the result of the larger (but temporary) weight loss after 6 months in the Atkins group (93). Furthermore, the effects of the Atkins diet on plasma lipids when body weight remains stable are unknown. Therefore, long term adverse effects on plasma lipid levels cannot yet be excluded.

Finally, diets low in carbohydrate contain fewer vitamins, minerals and dietary fibres than recommended (67).

In conclusion, long-term follow-up studies are needed to get a better understanding of the efficacy and safety of low-carbohydrate diets for the loss and/or control of body weight.

## 5.6 Infant-feeding and obesity later in life

It has been suggested that breastfeeding (versus formula feeding) may affect energy balance, for several reasons. Firstly it has been assumed that breastfed children are better able to self-regulate their energy intake. Breastfeeding allows the infant to control the amount of milk consumed based on internal satiety signals. Conversely, bottle-fed infants are encouraged to finish their bottle, while their satiety signals indicate that they have eaten enough. However, this assumption is hard to study and direct evidence for or against it is lacking.

Secondly, the way of infant-feeding may influence hormone levels or other endogenous factors. It has been reported that formula-fed infants have higher plasma insulin concentrations than breastfed infants, although it is not known how long this difference persists. These higher concentrations would be expected to stimulate fat deposition and the early development of adipocytes (94;95). Furthermore, breast milk contains bioactive factors, which may modulate factors that inhibit adipocyte differentiation in vitro (e.g. epidermal growth factor and tumour necrosis factor  $\alpha$ ) (95). Breastfeeding may also affect leptin metabolism in early and later life. High leptin levels reduce energy intake (see chapter 3.1.2). Among breastfed infants, leptin levels were significantly higher than in bottle-fed infants (96). Furthermore, plasma leptin concentrations at 13 to 16 years of age were associated with early diet of preterm infants. Children who consumed more breast milk had a lower ratio of leptin concentration to fat mass than children who consumed less breast milk in early life (97). These findings may indicate that leptin concentrations are programmable by breastfeeding. However, not all studies reported differences in leptin levels according to the way of infant feeding (98;99).

Finally, at 3 to 6 months of age the intake of proteins of breastfed infants was about 66% to 70% lower than the intake of bottle-fed infants. A high intake of protein tends to be to stimulate higher insulin secretion and has been associated with higher risk of childhood obesity (95;100;101).

These results suggest that breastfeeding would reduce the risk of childhood obesity compared with formula-feeding. This hypothesis has been widely studied, but results remain inconclusive. Recently, a review on this topic was published which included only large-scale ( $\geq 100$  infants per feeding group) studies that evaluated relatively long-term effects ( $> 3$  years of age) (94). Of the eleven selected studies, eight showed that breastfed children have a lower risk of childhood obesity than formula-fed children. However, a shortcoming of these studies is their observational character and therefore the susceptibility to confounding (102). An important potential confounding variable is maternal obesity. Among overweight mothers breastfeeding is less common due to partly socio-economic factors and failure to initiate lactation. Therefore, the observed association between formula-feeding and overweight may in fact be explained by the fact that formula-fed infants more often have overweight mothers. Children from overweight mothers might be genetically susceptible to the development of overweight, have other diets, or are less active in early life.

In conclusion, conclusive evidence for an association between breastfeeding and overweight is lacking to date.





## **6. Influences of physical activity on energy balance**

This chapter describes potential effects of physical activity on several aspects of energy balance, such as appetite, food preferences and energy intake. Furthermore, the possible role of nonexercise activity thermogenesis in explaining differences in weight gain is discussed.

### **6.1 Physical activity, appetite and energy intake**

There is ample evidence that food restriction gives rise to hunger and increased food intake (103;104). Similarly, there is a common belief that an increase in energy expenditure through physical activity may be followed by an increase in energy intake to compensate for the energy used. However, most of the studies that investigated changes in energy intake after physical activity found no increase in hunger or energy intake as a result of the energy deficit induced by exercise (105). One of the criticisms on especially the short-term studies is that energy intake was not monitored long enough after the intervention. It has been suggested that it takes a longer time to compensate for the energy loss by exercise (106). However, also most long-term intervention studies did not find an increase in energy intake after physical activity.

In contrast, during and shortly after a period of vigorous or intense exercise hunger seems to be suppressed instead of increased. The suppression of hunger is temporary and there appears to be no effect on subsequent food intake. This phenomenon has not been observed after moderate exercise. Only under extreme circumstances, such as expeditions or long distance-swimming, appetite is suppressed so that participants are not able to take sufficient amounts of energy to counterbalance energy expenditure (105). However, these extreme conditions are clearly not typical of day-to-day living.

Overall, the body of evidence points to a weak coupling between energy intake and energy expenditure induced by physical activity. These results suggest that the body does not possess a rapidly acting physiological mechanism that automatically matches energy intake to energy deficit caused by exercise (105;107;108). This also indicates that food intake is not downregulated when physical activity decreases. There is indeed some evidence that energy intake remains stable when energy expenditure is manipulated (105).

Exercise has, on the other hand, been associated with alterations in food selection and food preferences. Exercise may induce an increase in the selection of carbohydrates. The underlying hypothesis is that the energy source that is used mostly during exercise stimulates the selection for a particular nutrient. Thus utilisation of glycogen stimulates the consumption of carbohydrates. There is indeed some evidence that carbohydrate intake is increased with prolonged exercise. Whether this is the result of physiological an/or cognitive factors remains, however, unclear (109).

### **6.2 Physical activity and changes in steady state energy balance**

Although the body does not seem to possess a rapidly acting physiological mechanism that automatically matches energy intake to energy deficit caused by exercise, observational studies clearly show that 'the higher the level of physical activity the higher the energy intake'. Therefore, it seems that in the long run, energy intake must compensate for the accumulating energy expense through physical activity. This is necessary, since the body cannot tolerate a permanent loss in body weight. Figure 5 describes the theoretical impact of

exercise on body weight as proposed by Blundell and colleagues (105). Initially, a steady state exists in which energy intake matches energy expenditure (A-B). After the level of exercise has increased, body weight decreases as the higher energy expenditure by physical activity is not compensated by an increase in energy intake (adjustment period B-C). A combination of behavioural and physiologic alterations brings about a new equilibrium (C-D). Several physiological effects contribute to the establishment of this new steady state. Firstly, resting metabolic rate decreases when total body mass decreases. Furthermore, the energy cost of physical activity itself decreases because of an increase in maximal oxygen consumption ( $VO_{2max}$ ). As a result the net exercise-induced energy-deficit of the same amount of exercise (measured by time and intensity) gets smaller, and weight reduction levels off (105;107).

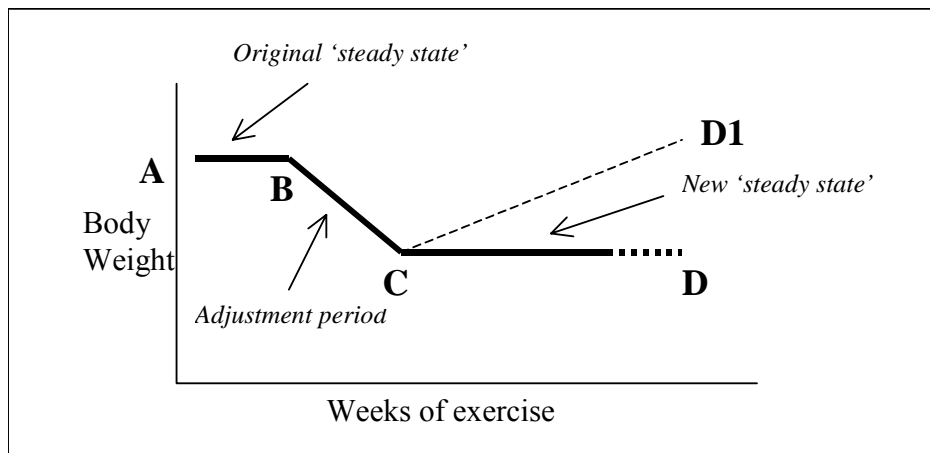


Figure 5. Schematic diagram to illustrate the theoretical impact of exercise on body weight. Adapted from (105).

The line A-B indicates the initial steady state. Point B represents the start of the period with increased exercise. Line B-C indicates the adjustment period when the higher energy expenditure is not compensated by increased energy intake. Point C represents the time at which through behavioural and metabolic alterations a new steady state (C-D) is reached. If exercise is abandoned at point C the weight would be restored to point D1 (or possible above it).

It is unlikely that the above mentioned physiological processes that reduce the exercise-induced energy deficit are solely responsible for reaching the new level of energy balance, i.e. the new steady state. On the long term, small changes in food intake may occur, that are difficult to observe by current methods.

### 6.3 Effect of physical activity on leptin levels

Indications for an effect of exercise on the long-term regulation of energy intake comes from the observation that energy expenditure was inversely associated with plasma leptin levels (110). Additionally, a decrease in leptin levels was observed in postmenopausal women who entered a three-day exercise program (111). In contrast, in obese youth no change in leptin was found after 8-month physical training (112). Also in a small study among obese women no acute effect of exercise on leptin levels was observed (113). However, during the exercise trial, and not during the control trial, high leptin levels were associated with significant lower subsequent food intake, perceptions of hunger and desire to eat as well as with higher perceptions of satiety and fullness.

In conclusion, although these findings support the involvement of leptin in the regulation of appetite and food intake in response to physical activity, definite conclusions cannot be drawn yet.

## 6.4 Nonexercise activity thermogenesis (NEAT)

In general, intervention studies on physical activity increase energy expenditure by increasing conscious activities. However, total physical activity also includes energy loss through heat production (thermogenesis) that accompanies nonvolitional exercise, such as the activities of daily living, fidgeting, spontaneous muscle contraction, and maintaining posture when not recumbent. This type of thermogenesis has been defined as Non-Exercise Activity Thermogenesis (NEAT). It has been hypothesised that differences in weight gain due to overfeeding between individuals can be explained by individual differences in the potential to increase NEAT (114;115).

Ravussin already mentioned nonvolitional physical activity as a determinant of vulnerability to obesity in 1986 (115). Levine and co-workers further investigated this issue in a study among 16 nonobese volunteers. They observed a large interindividual difference (range 1.4 - 7.2 kg) in weight gain to a 1000 kcal per day in excess of weight-maintenance requirements for 8 weeks (114). Assuming that conscious physical activity remained constant, he concluded that changes in NEAT accounted for a large part of these interindividual differences in weight gain. However, Levine's study had several shortcomings and needs to be replicated (116;117). Furthermore, the mechanisms responsible for the activation of NEAT are largely unknown to date. Involvement of leptin has been proposed (76), but this may be unlikely because in the experiment of Levine et al. changes in NEAT with overfeeding were not related to changes in leptin levels (118). A recent discovery was that in rats orexin A can act in the paraventricular nucleus of the brain to increase NEAT (119). Clearly, more research is needed to elucidated the suggested role of NEAT in weight regulation.



## **7. Influences of smoking (cessation) on energy balance**

In 2002, 34% of Dutch men and 28% of Dutch women smoked (120). Quitting rates are often lower for female than for male smokers, and women are more likely to relapse (121). There is evidence that poorer cessation rates of women are partly due to their greater concern regarding weight gain after cessation (121). General weight concerns predict smoking initiation in adolescent girls, and nearly 40% of young female smokers specifically report that they smoke to control body weight. More knowledge about the mechanisms by which smoking exerts its effect on energy balance may lead to better prediction and control of weight gain after smoking cessation, thus increasing the likelihood of maintaining smoking abstinence (122).

### **7.1 Relation between smoking (cessation) and body weight.**

Subjects who smoke have a lower body weight than subjects who do not smoke or who quit smoking (123). The mean difference in weight between smokers and non-smokers is about 3 to 4 kg and becomes more pronounced with advancing age (124;125). Women seem to be more susceptible to the weight reducing effects of smoking than men (125). Conversely, after smoking cessation, weight gain is observed in 70-80% of the people who quit and predominantly among women (123;124). The mean weight gain corresponds with the weight loss that is associated with smoking (122;124). The rate of weight gain appears to be rapid in the first weeks or months after smoking cessation and plateaus after 6 months (124). Nicotine is one of more than 3800 compounds in tobacco smoke and is held responsible for the weight reducing effect of smoking (124). Additionally, weight gain after smoking cessation seems to result from the withdrawal of nicotine. This effect of nicotine has been shown in animals since a long time (126). In humans this effect was confirmed after the introduction of nicotine replacement therapy. Nicotine replacement therapy is associated with attenuated weight gain following smoking cessation (127), but not in all studies (128). The effect of smoking (cessation) may be explained by changes in food intake, metabolic rate and/or the consumption of food as a substitute for the psychological effects of tobacco (129). These possibilities will be further described below.

### **7.2 Smoking cessation and energy intake**

There is no evidence that caloric intake is lower in smokers compared with non-smokers. In fact, a meta-analysis showed that smokers reported a 4.9% higher energy intake than non-smokers (130). Additionally, food intake seems not to be reduced after acute nicotine intake among smokers (124). However, in non-smokers nicotine may acutely reduce food intake. These findings suggest that among smokers long-term continuous exposure to nicotine may lead to the development of tolerance to an anorectic effect of nicotine (124). Smoking cessation has consistently been associated with a significant increase in energy intake - in a stable range of 200-400 kcal/day - less than one month after quitting (131). The magnitude of the increase in energy intake after smoking cessation accounts for most of the weight gain typically observed soon after cessation (about 0.5 kg per week, total weight gain 3.6 - 4.5 kg) (121). However, this consistent increase in caloric intake is not as apparent in long-term studies. Self-reported increases in eating or hunger feelings show a similar pattern, i.e. a sharp rise during the first few weeks after cessation followed by a gradual decline

towards pre-cessation levels (124). Important predictors of the increase in caloric intake after smoking cessation are female gender, restrained eating behaviour (the tendency to keep caloric intake low to maintain an unrealistically low body weight), and unemployment status (131).

Smoking relapse appears to be accompanied by a short-term decrease in food intake (124). However, this finding should be interpreted with caution, because those who are able to resist smoking may differ from those who are not able to resist smoking with regard to the ability to control eating behaviour.

Several studies investigated the effects of smoking cessation on the composition of the diet, but the results remain inconsistent (125). Some studies have found an increase in carbohydrate or fat intake among smokers, whereas others have not (125). An increase in consumption of sweet-tasting high-fat foods has also been observed (131). There is some evidence that the observed increase in caloric intake after smoking cessation appears specifically to be due to an increase in between-meal snacking. It has been shown that during smoking abstinence between-meal snack intake increased 94% among women and 50% among men (121).

In summary, changes in smoking status temporarily increase (cessation) or decrease (relapse or possibly smoking initiation) food intake. Nevertheless, smokers report a higher intake of energy than non-smokers, despite their lower body weight. Therefore these short-term changes may not pertain for a long time.

### **7.3 Smoking cessation and hunger feelings**

As an explanation for the weight reducing effect of smoking, it has been proposed that smoking reduces feelings of hunger. In the first weeks of smoking cessation self-reported hunger feelings may be increased (131). However, data from a more recent study suggest that smoking has no (acute) effect on hunger (132). Hence, smoking may prolong satiety after a meal and therefore inhibit subsequent caloric intake (125). It has been hypothesised that the reducing effect of nicotine on hunger feelings occurs after a meal - thus in the fed state -, but not during fasting. This suggests that smokers might reduce snacking, but not their consumption during temporally spaced meals. This hypothesis is supported by a study in which smokers ate less as between meal snacks, but as much or more during meals when they were allowed to smoke compared to when they were deprived of smoking (131).

It has also been suggested that the desire for smoking may be misattributed as the desire for food (125). The easy availability of food and feelings of craving for smoking (and thus for food) may play a more prominent role in increased food intake after smoking cessation than any pharmacological/physiological effect of nicotine.

### **7.4 Smoking cessation and energy expenditure**

Effects of smoking on energy expenditure can be distinguished in acute and chronic effects. Studies examining the acute metabolic effect of nicotine intake show that nicotine causes a small but significant and transient ( $\leq 30$  min) increase in energy expenditure at rest ( $< 10\%$  resting metabolic rate). Estimates of the magnitude of this effect are different across studies (122). This is possibly due to variable intake of nicotine, which is determined by depth of smoke inhalation (122). However, given the typical interval of  $\sim 30$  min between cigarettes for many heavy smokers, resting metabolic rate may remain elevated throughout the day because of these acute effects. The acute effects of smoking at rest, averaging about 6% or

70 kcal/day, explain 0.3 kg weight gain per month after quitting (124). However, these studies have been carried out under fasting and quiet rest. Normally, smokers are more likely to smoke in situations that influence energy balance, such as during or after casual physical activity or after a meal. Studies suggest that the acute effects of smoking on energy expenditure are enhanced during casual physical activity, while after food intake these effects may be reduced (122;133). The metabolic effects of smoking during waking hours (including effects during casual physical activity) have been estimated to account for ~140-200 kcal/day in excess for the average smoker. This could produce a weight gain of ~1.5 kg after 2 months of cessation (122).

Despite the *acute* effects on energy expenditure as described above, there is no clear evidence for a *chronic* metabolic effect of smoking as well as its constituent nicotine. Among women smoking cessation of 1 month was associated with a significant decrease in resting metabolic rate (134). Relapse caused return of metabolic rate to precessation levels, but maintaining abstinence was associated with remaining reduced metabolic rate. In contrast, most studies found no significant differences in resting metabolic rate between smokers and non-smokers and this rate declines very little after smoking cessation (122;133;135). However, the abstinence of smoking is not verified in most studies. Furthermore, there is some evidence that the relation between smoking and resting metabolic rate depends on the number of cigarettes smoked (136). All in all, current data do not indicate a chronic metabolic effect caused by smoking.

In summary, smoking causes an acute increase in energy expenditure, but this increase seems temporary and may be insufficient to explain the observed weight gain after smoking cessation.

## **7.5 Effects of nicotine on factors involved in the regulation of energy balance**

The previous paragraphs described that smoking causes an acute increase in energy expenditure and that smoking cessation or relapse causes alterations in food intake. Of interest are the potential physiological mechanisms behind these nicotine-induced alterations in energy balance.

Nicotine exerts its physiological effect through action on nicotinic acetylcholine receptors (nAChRs) within the central nervous system. As a consequence the release of neurotransmitters is modulated. Different subtypes of nAChRs are expressed at several neurones that play an important role in regulation of energy balance. They may exert different effects on energy balance after stimulation by nicotine (123). In this paragraph the potential effect of nicotine on the short- and long-term system that controls food intake, energy expenditure and fat oxidation will be discussed.

### **7.5.1 Effect of nicotine on mechanisms that regulate energy intake**

Already in early studies, chronic nicotine administration in rats caused an increase in levels of plasma CCK, a gut peptide that is released to mediate satiety (see chapter 3.1.1) (137). However, little is further known about the possible effect of nicotine on the short-term regulation system of food intake. Nicotine has also been connected with the system that regulates long-term energy intake, through the finding that nicotine administration caused an increase in  $\alpha$ -MSH secretion in mice brain (137).  $\alpha$ -MSH is involved in the melanocortin pathway that plays an important role in regulation of food intake (see chapter 3.1.3). Since the discovery of leptin and its role in regulation of energy balance it has been proposed that the weight reducing effect of nicotine may be mediated by an increase in leptin levels.

However, despite the fact that a few studies indeed reported higher leptin levels among smokers (138;139), most epidemiological studies have found that smokers have lower leptin levels compared with non-smokers (140-145). Furthermore, smoking cessation was not associated with decreased, but increased leptin levels in two prospective studies (139;146). Differences in body weight between smokers and non-smokers seem not to be responsible for this apparent inconsistency because most studies adjusted for body mass index. A possible explanation may be found in the hypothesis that nicotine increases the sensitivity of leptin receptors in the hypothalamus (123). Furthermore, catecholamines are induced by smoking, and have been found to decrease leptin levels in humans (137). Nevertheless, it remains uncertain whether alterations in leptin levels due to smoking contribute to the lower weight of smokers and post-cessation weight gain.

Further evidence for the involvement of the melanocortin pathway in the effect of nicotine on energy balance comes from the observation that the appetite reducing effect of nicotine seems to occur in the lateral hypothalamus (147). Food intake is significantly decreased after administration of nicotine into this brain area. Furthermore, within the lateral hypothalamus the expression of neuropeptides and their receptors involved in regulation of energy balance are affected by nicotine. The effect of nicotine on melanin-concentrating hormone expression is still unclear. Chronic treatment of rats with nicotine appears to result in an increase in the expression of NPY and orexins, but a simultaneous decrease in the number of NPY-receptors and orexin-A receptors (123;137). This might explain their reduced appetite and energy intake. Nicotine also seems to increase the release of serotonin in the lateral hypothalamus and therefore may cause early meal termination through acting as a false indicator of satiety (123).

In conclusion, the effect of nicotine on food intake seems to be mediated by neuronal pathways of which is known that they are involved in regulation of energy balance.

### **7.5.2 Effects of nicotine on the regulation of energy expenditure**

Nicotine increases circulating catecholamines as noradrenaline or adrenaline. The elevation in catecholamines was found to last for >20 min which may explain the time course of metabolic increase after smoking. However, in heavy smokers catecholamines may remain elevated throughout the day. The increase in catecholamines may affect substrate oxidation and the sympathetic nervous system, and therefore energy expenditure.

Fat oxidation measured by indirect calorimetry seems to be higher in smokers than in non-smokers and increases with increasing nicotine uptake (133). Furthermore, the increased noradrenaline levels by nicotine cause an increase in lipolysis (122). This increased lipolysis by nicotine seems partly to be due to stimulation of  $\beta$ -adrenergic receptors (ADRBs, see 3.2.2) (148).

The stimulation of ADRBs, which belong to sympathetic nervous system, may also occur in tissues involved in thermogenesis (129). In rats it was found that nicotine increases thermogenesis. Furthermore, nicotine seems to increase UCP1 protein in mice. However, to what extent this finding contributes to the regulation of thermogenesis in humans is yet unknown. Whether UCP2 and UCP3 expression levels are affected by nicotine remains to be determined (123;137).

In paragraph 7.4 it was mentioned that the acute effect of smoking on energy expenditure is enhanced during light physical activity. This was partly due to an increase in circulating plasma catecholamines induced by smoking (149). Physical activity causes a change in blood flow away from liver and kidneys (organs involved in nicotine metabolism), and towards



muscle. This results in an enhanced presence of nicotine in the circulation and may be responsible for the extended effects observed during activity (122).

In conclusion, smoking causes release of catecholamines that are predominantly responsible for its positive effect on energy expenditure.



## 8. Conclusions

*The aim of this report is to describe the physiological mechanisms involved in the regulation of maintaining energy balance. Knowledge about these mechanisms may well offer clues to the prevention of obesity.*

- ▶ Key components of the energy balance system are energy intake, energy expenditure and energy storage. The central nervous system plays an important role in the regulation of energy balance through effects on feeding behaviour and energy expenditure.
- ▶ Energy intake is predominantly regulated in the hypothalamus.
  - Short-term control of food intake depends on neural and endocrine signals that are released from the gastrointestinal tract, such as CCK, GLP1, ghrelin and PYY. They are involved in meal initiation, meal termination and the determination of between-meal intervals.
  - The appetite suppressant PYY and the appetite stimulator ghrelin may both be reduced in obese individuals. Long term studies are needed to investigate the potentials to use ghrelin antagonists and PYY in the treatment of obesity.
  - Long-term regulation of food intake balances energy intake and energy expenditure and is mainly regulated by the melanocortin pathway. Leptin and insulin activate this pathway and act via two distinct populations of neurones (AgRP/NPY and  $\alpha$ -MSH(POMC)/CART) on the melanocortin 4 receptor within the arcuate nucleus of the hypothalamus. This receptor may alter the expression of neuropeptides in other parts of the hypothalamus (e.g. MCH, orexins, CRH and TRH) and thereby affect energy intake.
  - Obese subjects mostly have relative high leptin levels and are thought to be insensitive to leptin.
  - The short- and long-term system of food intake seems to be interrelated, but the crosstalk between those systems is not well studied.
- ▶ Daily energy expenditure consists of three components, of which at least one (adaptive thermogenesis) is under physiological control.
  - The sympathetic nervous system may, via  $\beta$ -adrenergic receptors, regulate adaptive thermogenesis, that is the energy expended to digest, metabolise and store food, in human skeletal muscle.
  - Lower sympathetic nervous system activity and/or lower sensitivity to a certain level of sympathetic activity may be involved in the aetiology of obesity.
  - Uncoupling proteins may be able to influence the efficiency of energy expenditure and so influence energy balance. However, the potential role of uncoupling proteins in human obesity needs to be further elucidated before any conclusions can be drawn.
  - Physical activity can create an energy deficit. On the short-term, the body does not possess a rapidly acting physiological mechanism that matches energy intake to the energy deficit. In the long-term, leptin may be involved in the establishment of a new steady state in which energy intake matches energy expenditure.
  - The hypothesis that nonvolitional (unconscious) activity such as fidgeting and spontaneous muscle contraction is a determinant of vulnerability to obesity is interesting, but needs to be further studied before any conclusions can be drawn.

- ▶ The neural pathway that controls food intake also affects energy expenditure.
- Leptin affects energy expenditure through altering the activity of peptides that are involved in pathways through which fat is metabolised e.g. AMPK and SCD-1
- ▶ At low levels of energy expenditure it is more difficult to maintain body weight than at high levels of energy expenditure.
- Based on the literature the physiological mechanisms involved in the regulation of energy intake and/or expenditure at low levels of physical activity do not differ from those at high levels.
- However, at low levels of energy expenditure it is easier to overeat.
- Furthermore, evidence suggests that the physiological response to weight loss (even when already overweight) is more vigorous than the response to weight gain.
- Therefore, it is hard to counteract the effects of even a small excess in energy intake relative to energy expenditure.

*Another aim of this literature study is to identify factors that influence energy balance, due to their effect on the physiological regulation of food intake and/or energy expenditure, such as dietary components and smoking.*

- ▶ Based on results of scientific research the following seem to be important dietary factors that may influence energy balance
- *Dietary fibres* increase the need for chewing which may result in maximal sensory stimulation in the mouth and increased gastric distension. Furthermore, dietary fibres slow down the rate of gastric emptying and thereby decrease the rate of nutrient absorption, which may increase satiety.
- Of all macronutrients, *dietary fat* most easily leads to excess energy intake, because fat seems to be less satiating on the short-term, has a lower priority of oxidation and the energy costs for absorbing, processing and storing fat are lower than for carbohydrates and proteins.
- *Liquids* may be less satiating when not consumed with or close to a meal than solids due to the absence of chewing and an increased rate of gastric emptying. Therefore, consumption of beverages containing a lot of energy between meals may unconsciously lead to caloric over-consumption.
- Finally, low *energy dense* foods possibly decrease the rate of gastric (caloric) emptying and slow the return of hunger, mostly because of their higher volume. The energy density of foods is mostly determined by their dietary fibre, fat and water content. Dietary fibres are able to lower energy density of foods through their capacity to bind water and so increase volume, while fat is more than twice as energy dense as protein and carbohydrate.
- ▶ Convincing evidence for some other dietary factors that theoretically can affect physiological mechanism that regulate energy balance is lacking.
- *Breastfeeding* may have a protective effect on overweight in later life, but more research is needed to elucidate this issue.
- *The Atkins diet* (low-carbohydrate) is a very popular diet for weight loss today. However, there is no scientific evidence for Atkins theory and long-term information about the efficacy and safety of this diet is lacking and therefore those diets should not be recommended.

- Besides dietary factors, smoking may affect energy balance.
- Smoking causes a decrease in body weight of about 3 to 4 kg, which is regained after smoking cessation.
- Nicotine reduces appetite possibly by increasing the sensitivity to leptin and/or by enhancing the release of serotonin during feeding. Furthermore, in the lateral hypothalamus nicotine may attenuate NPY and orexin signalling due to down-regulation of their receptors.
- It is not clear whether these physiological changes are sufficient to explain the observed changes in *energy intake* and body weight due to altering of smoking status. Possibly, psychological factors play a role.
- *Energy expenditure* is acutely increased through nicotine, but no chronic effect on basal metabolic rate has been observed. Nicotine may alter energy expenditure via changes in sympathetic nervous system activity and fat oxidation. However, the magnitude of this effect and the significance for body weight is uncertain.



## 9. Recommendations

- ▶ Due to a more vigorous physiological response to weight loss than to weight gain, it is very difficult to loose weight. Therefore prevention of overweight and obesity should be emphasised.
- ▶ Based on physiological mechanisms, effective strategies to prevent a positive energy balance or maintain an obtained weight after weight loss are:
  - Diets that are low in energy density, low in fat, high in fibre. Additionally, avoid large amounts of energy-containing liquids between meals. This is in accordance with the recommendations of the Netherlands Nutrition Centre.
  - Increase physical activity or maintain it at high levels. There is no evidence that the physiological mechanisms regulating energy balance differ at low levels of energy expenditure from those at high levels, but it is easier to overeat at low levels of energy expenditure.
- ▶ It may be necessary to update this review in several years' time because:
  - The research on the regulation of energy balance is a very dynamic area. New information is becoming available daily.
  - In the near future, it is expected that our knowledge about the physiological mechanisms that regulate energy balance in humans will expand. Until now, most knowledge on this issue is derived from animal studies. Although animal studies helps us further to clarify specific pathways because of a large overlap in these pathways between rodents and mammals, the findings can not always be translated to the human situation.
  - The effect of different dietary factors (e.g. macronutrients, dietary fibre) on hormones (e.g. PYY, ghrelin) and neuropeptides (e.g. NPY) that are involved in the short- and long-term regulation of food intake will become more clear in the future. For example, the influence of different types of carbohydrates (e.g. fructose, sucrose, glycemic index) and fat (saturated, mono- and polyunsaturated) on short and long-term satiety are topics that are now studied widely.
  - The crosstalk between the short-and the long-term system that regulates energy balance may become more clear and probably more external factors and their influences on the regulatory mechanism of energy balance will be identified.





## References

1. Blokstra A, Schuit AJ. Factsheet Overgewicht; Prevalentie en trend. 2003; RIVM report 260301/f1/2003. [http://www.rivm.nl/vtv/data/kompas/determinanten/endogeen/lichaamsgewicht/factsheet\\_overgewicht.pdf](http://www.rivm.nl/vtv/data/kompas/determinanten/endogeen/lichaamsgewicht/factsheet_overgewicht.pdf)
2. Seidell JC, Visscher TL. Nutrition and health--obesity. *Ned Tijdschr Geneeskd* 2003;147:281-6.
3. Health Council of the Netherlands. Overweight and obesity. The Hague, Health Council of the Netherlands, 2003: publication no. 2003/07.
4. Peters JC, Wyatt HR, Donahoo WT, Hill JO. From instinct to intellect: the challenge of maintaining healthy weight in the modern world. *Obes Rev* 2002;3:69-74.
5. Garrow JS, James WPT. Human Nutrition and Dietetics. ninth ed. Churchill Livingstone; 1993.
6. McArdle WD, Katch FI, Katch VL. Exercise physiology: energy, nutrition, and human performance. third ed. Lea & Febiger; 1991.
7. Flatt JP. Use and storage of carbohydrate and fat. *Am J Clin Nutr* 1995;61(Suppl):952S-9S.
8. Flatt JP. McCollum Award Lecture, 1995: diet, lifestyle, and weight maintenance. *Am J Clin Nutr* 1995;62:820-36.
9. Goran MI. Genetic influences on human energy expenditure and substrate utilization. *Behav Genet* 1997;27:389-99.
10. Schrauwen P, Walder K, Ravussin E. Human uncoupling proteins and obesity. *Obes Res* 1999;7:97-105.
11. Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 1989;49(Suppl):968-75.
12. Prins JB, O'Rahilly S. Regulation of adipose cell number in man. *Clin Sci* 1997;92:3-11.
13. Naslund E, Hellstrom PM, Kral JG. The gut and food intake: an update for surgeons. *J Gastrointest Surg* 2001;5:556-67.
14. Kamphuis, M. The sense of dietary fat; food intake and body weight regulation. Thesis, Maastricht University; 2003.
15. Marx J. Cellular warriors at the battle of the bulge. *Science* 2003;299:846-9.
16. Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 2001;104:531-43.

17. Verdicch C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J Clin Endocrinol Metab* 2001;86:4382-9.
18. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002;418:650-4.
19. Porte D Jr, Baskin DG, Schwartz MW. Leptin and insulin action in the central nervous system. *Nutr Rev* 2002;60(Pt 2):S20-9; discussion S68-84, 85-7.
20. Flier JS, Maratos-Flier E. The stomach speaks--ghrelin and weight regulation. *N Engl J Med* 2002;346:1662-3.
21. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661-71.
22. Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science* 1998;280:1378-83.
23. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;402:656-60.
24. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000;407:908-13.
25. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002;346:1623-30.
26. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763-70.
27. Korner J, Leibel RL. To eat or not to eat - how the gut talks to the brain. *N Engl J Med* 2003;349:926-8.
28. Niswender KD, Schwartz MW. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol* 2003;24:1-10.
29. Friedman JM. The function of leptin in nutrition, weight, and physiology. *Nutr Rev* 2002;60(Pt 2):S1-14; discussion S68-84, 85-7.
30. Emond M, Schwartz GJ, Ladenheim EE, Moran TH. Central leptin modulates behavioral and neural responsivity to CCK. *Am J Physiol* 1999;276 (Pt 2):R1545-9.
31. Fruhbeck G, Gomez-Ambrosi J. Control of body weight: a physiologic and transgenic perspective. *Diabetologia* 2003;46:143-72.

32. Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, et al. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 2003;144:1506-12.
33. Cummings DE, Schwartz MW. Melanocortins and body weight: a tale of two receptors. *Nat Genet* 2000;26:8-9.
34. Bray GA. Obesity is a chronic, relapsing neurochemical disease. *Int J Obes Relat Metab Disord* 2004;28:34-8.
35. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* 2003;349:941-8.
36. Hofbauer KG. Molecular pathways to obesity. *Int J Obes Relat Metab Disord* 2002;26 (Suppl 2):S18-27.
37. Lowell BB, Bachman ES. Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* 2003;278:29385-8.
38. Schrauwen P, Hesselink M. UCP2 and UCP3 in muscle controlling body metabolism. *J Exp Biol* 2002;205(Pt 15):2275-85.
39. Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M, Langin D. Increased uncoupling protein-2 and -3 mRNA expression during fasting in obese and lean humans. *J Clin Invest* 1997;100:2665-70.
40. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB. betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 2002;297:843-5.
41. van Baak MA. The peripheral sympathetic nervous system in human obesity. *Obes Rev* 2001;2:3-14.
42. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature* 2000;404:652-60.
43. Kurokawa N, Nakai K, Kameo S, Liu ZM, Satoh H. Association of BMI with the beta3-adrenergic receptor gene polymorphism in Japanese: meta-analysis. *Obes Res* 2001;9:741-5.
44. Schiffelers SL, Saris WH, Boomsma F, van Baak MA. beta(1)- and beta(2)-Adrenoceptor-mediated thermogenesis and lipid utilization in obese and lean men. *J Clin Endocrinol Metab* 2001;86:2191-9.
45. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL. Is the energy homeostasis system inherently biased toward weight gain? *Diabetes* 2003;52:232-8.
46. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002;415:339-43.

47. Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foulfelle F, Ferre P, Birnbaum MJ, et al. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 2004;428:569-74.
48. Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 2002;297:240-3.
49. Ntambi JM, Miyazaki M, Stoeckl JP, Lan H, Kendziora CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci USA* 2002;99:11482-6.
50. Levine AS, Kotz CM, Gosnell BA. Sugars: hedonic aspects, neuroregulation, and energy balance. *Am J Clin Nutr* 2003;78:834S-42S.
51. Hill JO, Peters JC. Environmental contributions to the obesity epidemic. *Science* 1998;280:1371-4.
52. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, Dussault J, Moorjani S, Pinault S, Fournier G. The response to long-term overfeeding in identical twins. *N Engl J Med* 1990;322:1477-82.
53. Snyder EE, Walts B, Perusse L, Chagnon YC, Weisnagel SJ, Rankinen T, Bouchard C. The human obesity gene map: the 2003 update. *Obes Res* 2004;12:369-439.
54. O'Rahilly S, Farooqi IS, Yeo GS, Challis BG. Minireview: human obesity-lessons from monogenic disorders. *Endocrinology* 2003;144:3757-64.
55. Froguel P, Boutin P. Genetics of pathways regulating body weight in the development of obesity in humans. *Exp Biol Med* 2001;226:991-6.
56. Fujisawa T, Ikegami H, Kawaguchi Y, Ogihara T. Meta-analysis of the association of Trp64Arg polymorphism of beta 3-adrenergic receptor gene with body mass index. *J Clin Endocrinol Metab* 1998;83(7):2441-4.
57. Allison DB, Heo M, Faith MS, Pietrobelli A. Meta-analysis of the association of the Trp64Arg polymorphism in the beta3 adrenergic receptor with body mass index. *Int J Obes Relat Metab Disord* 1998;22:559-66.
58. Chagnon YC, Rankinen T, Snyder EE, Weisnagel SJ, Perusse L, Bouchard C. The human obesity gene map: the 2002 update. *Obes Res* 2003;11:313-67.
59. Yao M, Roberts SB. Dietary energy density and weight regulation. *Nutr Rev* 2001;59 (Pt 1):247-58.
60. Jequier E. Pathways to obesity. *Int J Obes Relat Metab Disord* 2002;26 (Suppl 2): S12-7.
61. Golay A, Bobbioni E. The role of dietary fat in obesity. *Int J Obes Relat Metab Disord* 1997;21 (Suppl 3):S2-11.
62. Hill JO, Peters JC. Biomarkers and functional foods for obesity and diabetes.

Br J Nutr 2002;88 (Suppl 2):S213-8.

63. Melanson KJ, Westerterp-Plantenga MS, Saris WH, Smith FJ, Campfield LA. Blood glucose patterns and appetite in time-blinded humans: carbohydrate versus fat. *Am J Physiol* 1999;277(Pt 2):R337-45.
64. Lawton CL, Delargy HJ, Brockman J, Smith FC, Blundell JE. The degree of saturation of fatty acids influences post-ingestive satiety. *Br J Nutr* 2000;83:473-82.
65. Flint A, Helt B, Raben A, Toubro S, Astrup A. Effects of different dietary fat types on postprandial appetite and energy expenditure. *Obes Res* 2003;11:1449-55.
66. Almiron-Roig E, Chen Y, Drewnowski A. Liquid calories and the failure of satiety: how good is the evidence? *Obes Rev* 2003;4:201-12.
67. Freedman MR, King J, Kennedy E. Popular diets: a scientific review. *Obes Res* 2001; 9 (Suppl 1):1S-40S.
68. Monteleone P, Bencivenga R, Longobardi N, Serritella C, Maj M. Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. *J Clin Endocrinol Metab* 2003;88:5510-4.
69. Coleman RA, Herrmann TS. Nutritional regulation of leptin in humans. *Diabetologia* 1999;42:639-46.
70. Kratz M, von Eckardstein A, Fobker M, Buyken A, Posny N, Schulte H, Assmann G, Wahrburg U. The impact of dietary fat composition on serum leptin concentrations in healthy nonobese men and women. *J Clin Endocrinol Metab* 2002;87:5008-14.
71. Park EI, Paisley EA, Mangian HJ, Swartz DA, Wu MX, O'Morchoe PJ, Behr SR, Visek WJ, Kaput J. Lipid level and type alter stearoyl CoA desaturase mRNA abundance differently in mice with distinct susceptibilities to diet-influenced diseases. *J Nutr* 1997;127:566-73.
72. Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ Jr, Lane MD. Differentiation-induced gene expression in 3T3-L1 preadipocytes. Characterization of a differentially expressed gene encoding stearoyl-CoA desaturase. *J Biol Chem* 1988;263:17291-300.
73. Willett WC. Dietary fat and obesity: lack of an important role. *Scan J Nutr* 2003;47: 58-67.
74. Astrup A. Dietary fat and obesity: still an important issue. *Scan J Nutr* 2003;47:50-7.
75. Atkins RC, Ornish D, Wadden T. Low-carb, low-fat diet gurus face off. Interview by Joan Stephenson. *JAMA* 2003;289:1767-8, 1773.
76. VanItallie TB. Resistance to weight gain during overfeeding: a NEAT explanation. *Nutr Rev* 2001;59:48-51.
77. Howarth NC, Saltzman E, Roberts SB. Dietary fiber and weight regulation. *Nutr Rev*

2001;59:129-39.

78. Saris WH. Sugars, energy metabolism, and body weight control. *Am J Clin Nutr* 2003;78:850S-7S.
79. Stubbs RJ, Harbron CG, Murgatroyd PR, Prentice AM. Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. *Am J Clin Nutr* 1995;62:316-29.
80. Bell EA, Rolls BJ. Energy density of foods affects energy intake across multiple levels of fat content in lean and obese women. *Am J Clin Nutr* 2001;73:1010-8.
81. Rolls BJ, Bell EA, Thorwart ML. Water incorporated into a food but not served with a food decreases energy intake in lean women. *Am J Clin Nutr* 1999;70:448-55.
82. Rolls BJ, Bell EA, Castellanos VH, Chow M, Pelkman CL, Thorwart ML. Energy density but not fat content of foods affected energy intake in lean and obese women. *Am J Clin Nutr* 1999;69:863-71.
83. Stubbs RJ, Johnstone AM, O'Reilly LM, Barton K, Reid C. The effect of covertly manipulating the energy density of mixed diets on ad libitum food intake in 'pseudo free-living' humans. *Int J Obes Relat Metab Disord* 1998;22:980-7.
84. Stubbs RJ, Johnstone AM, Harbron CG, Reid C. Covert manipulation of energy density of high carbohydrate diets in 'pseudo free-living' humans. *Int J Obes Relat Metab Disord* 1998;22:885-92.
85. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. Energy density of foods affects energy intake in normal-weight women. *Am J Clin Nutr* 1998;67:412-20.
86. Schwartz MW, Boyko EJ, Kahn SE, Ravussin E, Bogardus C. Reduced insulin secretion: an independent predictor of body weight gain. *J Clin Endocrinol Metab* 1995;80:1571-6.
87. Sigal RJ, El-Hashimy M, Martin BC, Soeldner JS, Krolewski AS, Warram JH. Acute postchallenge hyperinsulinemia predicts weight gain: a prospective study. *Diabetes* 1997;46:1025-9.
88. Swinburn BA, Nyomba BL, Saad MF, Zurlo F, Raz I, Knowler WC, Lillioja S, Bogardus C, Ravussin E. Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest* 1991;88:168-73.
89. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, Bravata DM. Efficacy and safety of low-carbohydrate diets: a systematic review. *JAMA* 2003;289:1837-50.
90. Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, Williams M, Gracely EJ, Stern L. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003;348:2074-81.
91. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO,

- Rader DJ, Edman JS, Klein S. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003;348:2082-90.
92. Bray GA. Low-carbohydrate diets and realities of weight loss. *JAMA* 2003;289:1853-5.
  93. Katan MB, Zock PL, Mensink RP. Effects of fats and fatty acids on blood lipids in humans: an overview. *Am J Clin Nutr* 1994;60( Suppl):1017S-22S.
  94. Dewey KG. Is breastfeeding protective against child obesity? *J Hum Lact* 2003;19:9-18.
  95. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, von Voss H. Breast feeding and obesity: cross sectional study. *BMJ* 1999 17;319:147-50.
  96. Savino F, Costamagna M, Prino A, Oggero R, Silvestro L. Leptin levels in breast-fed and formula-fed infants. *Acta Paediatr* 2002;91:897-902.
  97. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations in later life. *Am J Clin Nutr* 2002;75:993-9.
  98. Lonnerdal B, Havel PJ. Serum leptin concentrations in infants: effects of diet, sex, and adiposity. *Am J Clin Nutr* 2000;72:484-9.
  99. Shimizu T, Satoh Y, Shoji H, Hisata K, Tadokoro R, Shinohara K, Shiga S, Yamashiro Y. Lack of plasma leptin response to feeding in newborn infants. *J Paediatr Child Health* 2004;40:42-3.
  100. Scaglioni S, Agostoni C, Notaris RD, Radaelli G, Radice N, Valenti M, Giovannini M, Riva E. Early macronutrient intake and overweight at five years of age. *Int J Obes Relat Metab Disord* 2000;24:777-81.
  101. Rolland-Cachera MF, Deheeger M, Akrouit M, Bellisle F. Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. *Int J Obes Relat Metab Disord* 1995;19:573-8.
  102. Clifford TJ. Breast feeding and obesity. *BMJ* 2003;327:879-80.
  103. Lawton CL, Burley VJ, Wales JK, Blundell JE. Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. *Int J Obes Relat Metab Disord* 1993;17:409-16.
  104. Green SM, Burley VJ, Blundell JE. Effect of fat- and sucrose-containing foods on the size of eating episodes and energy intake in lean males: potential for causing overconsumption. *Eur J Clin Nutr* 1994;48:547-55.
  105. Blundell JE, King NA. Physical activity and regulation of food intake: current evidence. *Med Sci Sports Exerc* 1999;31(Suppl):S573-83.
  106. Edholm OG. Energy balance in man studies carried out by the Division of Human Physiology, National Institute for Medical Research. *J Hum Nutr* 1977; 31:413-31.
  107. Blundell JE, King NA. Exercise, appetite control, and energy balance.

Nutrition 2000;16:519-22.

108. King NA, Tremblay A, Blundell JE. Effects of exercise on appetite control: implications for energy balance. *Med Sci Sports Exerc* 1997;29:1076-89.
109. Blundell JE, King NA. Effects of exercise on appetite control: loose coupling between energy expenditure and energy intake. *Int J Obes Relat Metab Disord* 1998; 22 (Suppl 2):S22-9.
110. Franks PW, Farooqi IS, Luan J, Wong MY, Halsall I, O'Rahilly S, Wareham NJ. Does physical activity energy expenditure explain the between-individual variation in plasma leptin concentrations after adjusting for differences in body composition? *J Clin Endocrinol Metab* 2003;88:3258-63.
111. Koutsari C, Karpe F, Humphreys SM, Frayn KN, Hardman AE. Plasma leptin is influenced by diet composition and exercise. *Int J Obes Relat Metab Disord* 2003;27:901-6.
112. Barbeau P, Gutin B, Litaker MS, Ramsey LT, Cannady WE, Allison J, Lemmon CR, Owens S. Influence of physical training on plasma leptin in obese youths. *Can J Appl Physiol* 2003;28:382-96.
113. Tsofliou F, Pitsiladis YP, Malkova D, Wallace AM, Lean ME. Moderate physical activity permits acute coupling between serum leptin and appetite-satiety measures in obese women. *Int J Obes Relat Metab Disord* 2003;27:1332-9.
114. Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 1999;283:212-4.
115. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest* 1986;78:1568-78.
116. Cooke R. A fidgeter's calculation. *Science* 1999;284:1125-6.
117. Ravussin E, Danforth E Jr. Beyond sloth--physical activity and weight gain. *Science* 1999;283:184-5.
118. Levine JA, Eberhardt NL, Jensen MD. Leptin responses to overfeeding: relationship with body fat and nonexercise activity thermogenesis. *J Clin Endocrinol Metab* 1999;84:2751-4.
119. Kiwaki K, Kotz CM, Wang C, Lanningham-Foster L, Levine JA. Orexin A (hypocretin 1) injected into hypothalamic paraventricular nucleus and spontaneous physical activity in rats. *Am J Physiol Endocrinol Metab* 2003 2.
120. Blokstra A, Schuit AJ. Factsheet roken; Prevalentie en trend. 2003;RIVM Report 260301/f2/2003.
121. Perkins KA, Levine MD, Marcus MD, Shiffman S. Addressing women's concerns about weight gain due to smoking cessation. *J Subst Abuse Treat* 1997;14:173-82.



122. Perkins KA. Metabolic effects of cigarette smoking. *J Appl Physiol* 1992;72:401-9.
123. Jo YH, Talmage DA, Role LW. Nicotinic receptor-mediated effects on appetite and food intake. *J Neurobiol* 2002;53:618-32.
124. Perkins KA. Weight gain following smoking cessation. *J Consult Clin Psychol* 1993;61:768-77.
125. Kos J, Hasenfratz M, Battig K. Effects of a 2-day abstinence from smoking on dietary, cognitive, subjective, and physiologic parameters among younger and older female smokers. *Physiol Behav* 1997;61:671-8.
126. Winders SE, Grunberg NE. Effects of nicotine on body weight, food consumption and body composition in male rats. *Life Sci* 1990;46:1523-30.
127. Doherty K, Militello FS, Kinnunen T, Garvey AJ. Nicotine gum dose and weight gain after smoking cessation. *J Consult Clin Psychol* 1996;64:799-807.
128. Assali AR, Beigel Y, Schreiber R, Shafer Z, Fainaru M. Weight gain and insulin resistance during nicotine replacement therapy. *Clin Cardiol* 1999;22:357-60.
129. Jessen AB, Toubro S, Astrup A. Effect of chewing gum containing nicotine and caffeine on energy expenditure and substrate utilization in men. *Am J Clin Nutr* 2003;77:1442-7.
130. Dallongeville J, Marecaux N, Fruchart JC, Amouyel P. Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. *J Nutr* 1998;128:1450-7.
131. Perkins KA. Effects of tobacco smoking on caloric intake. *Br J Addict* 1992;87:193-205.
132. Perkins KA, Sexton JE, DiMarco A, Fonte C. Acute effects of tobacco smoking on hunger and eating in male and female smokers. *Appetite* 1994;22:149-58.
133. Jensen EX, Fusch C, Jaeger P, Peheim E, Horber FF. Impact of chronic cigarette smoking on body composition and fuel metabolism. *J Clin Endocrinol Metab* 1995;80:2181-5.
134. Moffatt RJ, Owens SG. Cessation from cigarette smoking: changes in body weight, body composition, resting metabolism, and energy consumption. *Metabolism* 1991;40:465-70.
135. Warwick PM, Edmundson HM, Thomson ES. No evidence for a chronic effect of smoking on energy expenditure. *Int J Obes Relat Metab Disord* 1995;19:198-201.
136. Blackburn H, Brozek J, Taylor HL. Common circulatory measurements in smokers and nonsmokers. *Circulation* 1960;23:1112-24.
137. Li MD, Kane JK, Konu O. Nicotine, body weight and potential implications in the treatment of obesity. *Curr Top Med Chem* 2003;3:899-919.

138. Nicklas BJ, Tomoyasu N, Muir J, Goldberg AP. Effects of cigarette smoking and its cessation on body weight and plasma leptin levels. *Metabolism* 1999;48:804-8.
139. Eliasson B, Smith U. Leptin levels in smokers and long-term users of nicotine gum. *Eur J Clin Invest* 1999;29:145-52.
140. Hodge AM, Westerman RA, de Courten MP, Collier GR, Zimmet PZ, Alberti KG. Is leptin sensitivity the link between smoking cessation and weight gain? *Int J Obes Relat Metab Disord* 1997;21:50-3.
141. Wei M, Stern MP, Haffner SM. Serum leptin levels in Mexican Americans and non-Hispanic whites: association with body mass index and cigarette smoking. *Ann Epidemiol* 1997;7:81-6.
142. Ruige JB, Dekker JM, Blum WF, Stehouwer CD, Nijpels G, Mooy J, Kostense PJ, Bouter LM, Heine RJ. Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study. The Hoorn Study. *Diabetes Care* 1999;22:1097-104.
143. Mantzoros CS, Liolios AD, Tritos NA, Kaklamani VG, Doulgerakis DE, Griveas I, Moses AC, Flier JS. Circulating insulin concentrations, smoking, and alcohol intake are important independent predictors of leptin in young healthy men. *Obes Res* 1998;6:179-86.
144. Chu NF, Stampfer MJ, Spiegelman D, Rifai N, Hotamisligil GS, Rimm EB. Dietary and lifestyle factors in relation to plasma leptin concentrations among normal weight and overweight men. *Int J Obes Relat Metab Disord* 2001;25:106-14.
145. Donahue RP, Zimmet P, Bean JA, Decourten M, DeCarlo Donahue RA, Collier G, Goldberg RB, Prineas RJ, Skyler J, Schneiderman N. Cigarette smoking, alcohol use, and physical activity in relation to serum leptin levels in a multiethnic population: The Miami Community Health Study. *Ann Epidemiol* 1999;9:108-13.
146. Perkins KA, Fonte C. Effects of smoking status and smoking cessation on leptin levels. *Nicotine Tob Res* 2002;4:459-66.
147. Miyata G, Meguid MM, Fetisov SO, Torelli GF, Kim HJ. Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. *Surgery* 1999;126:255-63.
148. Andersson K, Arner P. Systemic nicotine stimulates human adipose tissue lipolysis through local cholinergic and catecholaminergic receptors. *Int J Obes Relat Metab Disord* 2001;25:1225-32.
149. Walker JF, Collins LC, Rowell PP, Goldsmith LJ, Moffatt RJ, Stamford BA. The effect of smoking on energy expenditure and plasma catecholamine and nicotine levels during light physical activity. *Nicotine Tob Res* 1999;1:365-70.