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Regulation of Body Weight in Humans

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Jéquier, Eric, and Luc Tappy. Regulation of Body Weight in Humans. *Physiol. Rev.* 79: 451–480, 1999.—The mechanisms involved in body weight regulation in humans include genetic, physiological, and behavioral factors. Stability of body weight and body composition requires that energy intake matches energy expenditure and that nutrient balance is achieved. Human obesity is usually associated with high rates of energy expenditure. In adult individuals, protein and carbohydrate stores vary relatively little, whereas adipose tissue mass may change markedly. A feedback regulatory loop with three distinct steps has been recently identified in rodents: *1*) a sensor that monitors the size of adipose tissue mass is represented by the amount of leptin synthesized by adipose cells (a protein encoded by the *ob* gene) which determines the plasma leptin levels; *2*) hypothalamic centers, with specific leptin receptors, which receive and integrate the intensity of the signal; and *3*) effector systems that influence the two determinants of energy balance, i.e., energy intake and energy expenditure. With the exception of a few very rare cases, the majority of obese human subjects have high plasma leptin levels that are related to the size of their adipose tissue mass. However, the expected regulatory responses (reduction in food intake and increase in energy expenditure) are not observed in obese individuals. Thus obese humans are resistant to the effect of endogenous leptin, despite unaltered hypothalamic leptin receptors. Whether defects in the leptin signaling cascade play a role in the development of human obesity is a field of great actual interest that needs further research. Present evidences suggest that genetic and environmental factors influence eating behavior of people prone to obesity and that diets that are high in fat or energy dense undermine body weight regulation by promoting an overconsumption of energy relative to need.

I. INTRODUCTION

A. Is There a Set Point for Body Weight?

The maintenance of an adequate body weight is a major determinant of the survival of higher organisms including mammals. Stability of body weight and body composition over long periods of time requires that energy intake matches energy expenditure. In human adults, there are mechanisms that tend to maintain energy intake and energy expenditure in balance. It is important to emphasize that body weight regulation not only requires the maintenance of energy balance, but also nutrient balance must be achieved, i.e., the mixture of fuel oxidized must be adjusted to match the composition of energy ingested (83). The concept of a regulated set point has been extensively used in studies of body temperature regulation (107). Deviations of internal temperature below or above a set-point temperature elicit appropriate changes in heat production and in heat losses to correct the temperature changes and to defend the internal set-point temperature. Changes in environmental conditions or in the work load during exercise can induce acute alterations in body temperature that trigger thermoregulatory responses within minutes or hours. The goal is clearly to maintain internal temperature within a physiological range and to avoid detrimental variations of internal temperature.

The study of body weight regulation differs in many aspects from that of thermoregulation. First, the regulated variable, body weight, is obviously not homogeneous, since it includes various tissues that are composed of proteins, carbohydrates, fats, water, and minerals. Acute changes in body weight can result from alterations in fluid balance, such as dehydration during prolonged exercise without adequate water intake; the mechanisms of water balance are well known and allow adjustment of body fluids within a few hours. Body weight regulation, as described in this review, concerns maintenance of body energy. Because protein and carbohydrate stores in adults vary relatively little, body weight regulation mainly concerns adipose tissue mass. Chronic imbalance between energy intake and energy expenditure results in changes in adipose tissue mass. Therefore, body weight regulation implies that the adipose tissue mass is "sensed," leading to appropriate responses in individuals who maintain body weight and body composition constant during prolonged periods of time.

Maintenance of energy homeostasis implies a long-term regulation of energy balance. There is preponderant evidence for the existence of an adipose tissue mass control with signals that come in part from adipose tissue and that act on hypothalamic receptors with effectors in the autonomic nervous system. The control of adipose tissue mass requires a highly integrated and redundant neurohumoral system that minimizes the effects of short-term fluctuations in energy balance. A major breakthrough in obesity research has been the identification of genetic loci at which specific mutations cause obesity in mice and rats. The cloning of the *ob* gene and identification of its encoded protein leptin (283) have provided a feedback signaling system reflecting the amount of adipose energy stores (228). A second important discovery is the finding that the *ob* gene product leptin acts via hypothalamic receptors to inhibit feeding, increase thermogenesis, and decrease body weight in rodents. Thus, for the first time in body weight regulation research, a feedback regulatory loop with three distinct steps has been identified: *1*) a sensor that monitors the level of energy, *2*) hypothalamic centers that receive and integrate through leptin receptors the intensity of the signal, and *3*) effector systems that influence the two determinants of energy balance, i.e., energy intake and energy expenditure.

The afferent limb of the regulatory loop of body weight regulation consists of hormones that are secreted in proportion to body fat mass. Leptin, the *ob* gene protein produced by adipose cells, fulfills this criteria, since its plasma concentration in humans is proportional to body adiposity (228). The hypothalamic targets are leptin-responsive neurons. The binding of leptin to its receptor alters the expression of several genes producing specific neuropeptides [neuropeptide Y (NPY), agouti-related peptides, proopiomelanocortin (POMC) products including α-melanocyte-stimulating hormone (MSH) and other melanocortin-4 receptor ligands, corticotropin-releasing hormone (CRH), melanocyte concentrating hormone, orexin, and tubby (TUB) (42)] that modulate food intake and energy expenditure (276). The efferent limb of the regulatory loop is represented by neuronal network containing neurons with specific receptors for the hypothalamic neuropeptides mentioned above. The autonomic nervous system is also implicated in this efferent limb; leptin increases sympathetic nervous system (SNS) activity (109), which mediates its action on energy expenditure, whereas NPY, acting on the paraventricular nucleus (PVN) NPY receptors, reduces SNS outflow to brown adipose tissue (13).

When energy stores decrease, due to prolonged nutritional deprivation, one expects a stimulated food-seeking behavior and a decreased resting energy expenditure. In contrast, with nutritional abundance, a feature of most developed countries, one observes a high prevalence of obesity; furthermore, the recent increase in the incidence of obesity in many developing countries suggests that the mechanisms of body weight regulation are easily altered when food availability increases. There is evidence that a high-fat diet overrides satiety mechanisms; however, the concomitant decline in physical activity and the modern inactive life-styles are also important factors that parallel

the secular trends in obesity (196). A variety of genetic, dietary, and life-style factors contribute to determine the steady state of weight maintenance at which the daily oxidation of a fuel mix matches the amount and the composition of the nutrients of the diet (83, 84). Thus it can be concluded that the size of the adipose tissue mass is not under a strict set-point control.

Despite these recent advances in the understanding of the physiology of body weight regulation, obesity prevalence is increasing in many countries, which indicates that the prevention of excessive body weight gain and the treatment of obesity have not improved over the last decades. This area of great public health relevance has not yet benefited from the remarkable advances in the understanding of the physiopathology of obesity. It is hoped that recent developments in molecular and cellular biology will result in new therapeutic approaches that not only improve the efficacy of weight loss strategies but that may also reset body weight regulation to a new lower set point.

B. Does Metabolic Efficiency Vary Between Individuals?

The first law of thermodynamics applies to animals. In simple terms, it describes the conservation of energy. It can be stated as follows: changes in energy store equal energy intake minus energy expenditure.

In this simplified equation, "energy intake" means "metabolizable energy," i.e., energy intake minus fecal energy minus urinary energy. Metabolizable energy represents 90–95% of energy intake, depending on the composition of the diet, the amount of nondigestible fibers, and the degree of nutrients cooking. In individuals without disease of the gastrointestinal tract, the efficiency of macronutrients intestinal absorption varies little, and therefore, obesity is not due to a particular high level of nutrient absorption. A positive change in energy store results either from an excessive energy intake and/or a reduced energy expenditure. Whether obesity results from a chronic excess of energy intake or from reduced energy needs has been much discussed over the last decade (195). Many investigators assumed that the demonstration of a reduced energy expenditure in genetically obese rodents (46, 253) is a phenomenon also applicable to humans. However, the regulation of energy expenditure in young rodents and in adult humans is carried out by different mechanisms. Although in young rats dietary-induced thermogenesis (the increase in energy expenditure after feeding) is dependent on the activation of brown adipose tissue through stimulation of the SNS (213), there is no convincing evidence that this tissue is functional in adult humans. The recent discovery in adult man of uncoupling proteins (UCP-2 and UCP-3) (27, 28, 87, 169, 258, 263, 284) that are present in various tissues may open new developments in the field of the control of energy expenditure, but their potential role in body weight regulation is still uncertain.

The concept of reduced energy needs in obese individuals was supported by studies showing low levels of self-recorded food intake in weight-stable obese individuals (149, 195). It was, however, established that obese subjects underreport their true food intake (149, 231), and therefore, reliable assessment of caloric intake of obese individuals in everyday life is practically impossible to obtain.

In weight-stable obese individuals, energy needs have been indirectly calculated from measurements of energy expenditure. According to the above-mentioned energy balance equation, energy intake equals energy expenditure when body energy stores are constant. With the use of a respiration chamber (121), a method based on indirect calorimetry which allows continuous measurement of gas exchanges, it was clearly demonstrated that obese individuals expend more energy over 24 h than lean persons (122, 123, 195, 200). This indicates that energy needs of obese individuals are higher than those of lean persons, mainly because the former have a higher basal metabolic rate than the latter due to an enlarged fat-free mass (200, 201). When adjusted for fat-free mass, lean and obese individuals have similar basal metabolic rate (195, 200). During a period of weight gain, total energy expenditure (TEE) of the subjects increases until it reaches the level of energy intake (Fig. 1). The rise of energy expenditure in individuals who gain weight is a homeostatic mechanism that contributes to limit the increase in body weight.

Total energy expenditure includes three components: basal metabolic rate, the energy used for physical activity, and dietary-induced thermogenesis. During exercise, the efficiency with which skeletal muscle converts chemical energy (i.e., ATP) into mechanical work is relatively low, amounting to ~25%. The energetic efficiency with which obese individuals perform physical exercise is similar to that observed in healthy lean individuals and is not altered by weight loss (96).

Within the restrained space of a respiration chamber, spontaneous physical activity is limited. Therefore, it is important to measure TEE in free-living people. The doubly labeled water technique allows one to reach this goal (195). Several studies using this technique have confirmed that TEE is elevated in obese compared with sedentary lean people (10, 11, 149). Nevertheless, suprabasal energy expenditure, which mainly corresponds to energy expended in physical activity, was shown to be low in obese individuals, suggesting that they perform less exercise than lean individuals (220–222). The results of these studies show that obesity is not primarily due to energy saving mechanisms; it can be inferred from these data that the positive energy balance that leads to obesity is mainly due

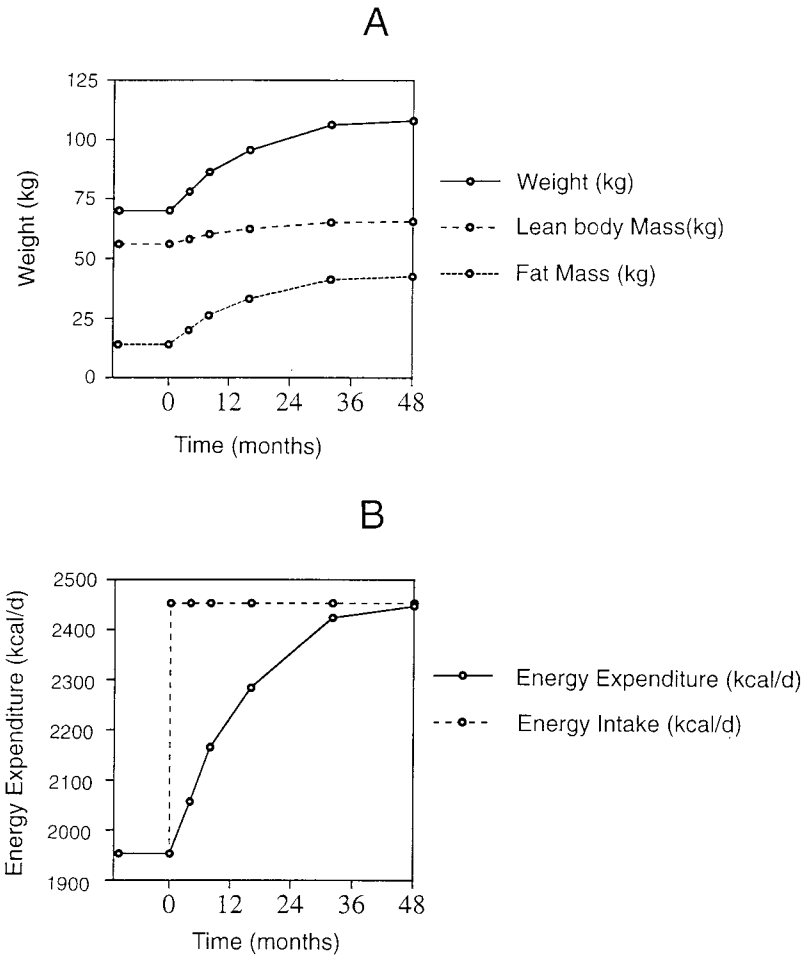

FIG. 1. *A*: schematic model of composition of weight gain over a 48-mo period illustrating dynamic phase of weight gain in an obese individual. Relative increase in lean body mass and fat mass was calculated from mean composition of weight gain, i.e., 75% fat mass, 25% lean body mass. *B*: schematic model of increase in total energy expenditure that accompanies weight gain when energy intake is chronically elevated. In this model, body weight reaches a new steady state (*A*) after 48 mo of excessive energy intake when energy expenditure reaches level of energy intake.

to an excessive energy intake. Individuals with a "low basal metabolic rate" for their body size (202) or people with a low spontaneous physical activity (285) have been found subsequently to gain more weight than those with high TEE. This illustrates that people with a "low resting energy expenditure" and the least active individuals have a greater risk to develop obesity than more active people. It does not mean, however, that an increased metabolic efficiency is the primary cause of obesity. In summary, present evidence shows that both excessive energy intake and low energy expenditure can contribute to the positive energy balance that leads to excessive body weight gain; it is, however, likely that the main mechanism is an excessive energy intake.

Dietary-induced thermogenesis can be markedly stimulated in young rodents when they have access to a highly palatable high-fat diet (213). In humans, in contrast, when food is ingested in excess of energy needs, the thermogenic response is limited to a maximal value of 25% of the excess energy intake (204). This means that at least 75% of the excess energy intake is stored. Carbohydrate overfeeding slightly stimulates the SNS activity (223) and the production of 3,3′,5-triiodothyronine (67). The contribution of these physiological responses to the rise in energy expenditure is low in adult humans, probably because brown adipose tissue is not functional.

The long-term effects of experimental perturbation of body weight show some degree of adaptation of energy expenditure and are in a direction tending to return the subjects to their initial weight (144). After a 10% gain in weight, nonresting energy expenditure of nonobese and obese subjects increased markedly, whereas the resting energy expenditure was less augmented. After a 10 or 20% loss in weight, both nonresting and resting expenditure decreased (144). Formerly, obese persons may require 10–15% fewer calories to maintain the newly reached "normal" body weight than a person who has never been obese (143, 144, 271). The frequently observed recidivism of obesity in obese subjects who lost weight is explained in part by this reduction of energy expenditure (266). Overall, these studies show that modulation of energy expenditure contributes to minimize energy deposition during overfeeding or energy mobilization from body stores during underfeeding. These changes in energy expenditure serve as homeostatic mechanisms that limit weight gain or weight loss. The above-mentioned studies

do not rule out the existence of subtle differences in metabolic efficiency between individuals. The available evidence, however, indicates that metabolic efficiency is not a major determinant of body weight in humans and that alterations in the control of food intake play a major role in the development of human obesity.

II. NUTRIENT BALANCE

A. Why Macronutrients Can Be Considered Separately

Maintenance of a constant body weight and body composition requires that energy and nutrient balances are achieved. The concept of nutrient balance stems from the fact that each of the three macronutrients (carbohydrate, fat, and protein) is either oxidized or stored in its own compartment. The conversion of a nutrient into another for storage does not represent important metabolic pathways (85). Although it is commonly believed that hepatic de novo lipogenesis is a mechanism by which fat accumulation occurs in humans, recent evidence indicates that only a few percent of glucose carbon atoms are converted into fatty acids and leave the liver as very-low-density lipoprotein (VLDL) triglycerides (111, 112). The de novo lipogenic response to a high-carbohydrate, low-fat diet is stimulated as compared with a high-fat diet (117), but the total amount of de novo fatty acids synthesized remains low and does not exceed 12 g/day. Furthermore, during carbohydrate overfeeding, the hepatic de novo lipogenesis was found not to exceed 5–10 g fatty acids synthesized per day (1, 230). De novo lipogenesis may occur during simultaneous lipid oxidation and will not result in net lipid deposition unless the amount of fat synthesized exceeds that of fat oxidized. Net lipogenesis, corresponding to accretion of lipid stores from carbohydrate, can be documented by the presence of respiratory quotients higher than 1.0. Such a net lipogenesis has been observed in humans only during periods of forced massive overfeeding, a condition which does not occur in everyday life (2). Recent observations indicate that hepatic lipogenesis accounts for only a minor portion of total fat synthesis in these conditions, suggesting that adipose tissue lipogenesis may play an important role (1).

The conversion of carbohydrate into fat is an energy-requiring process, in which 25% of the energy content of carbohydrates is converted into heat (82). In contrast, the deposition of dietary triglycerides into adipose tissue requires very little energy (0–2%). As a consequence, de novo lipogenesis from carbohydrate would be very unfavorable to increase body fat stores.

The metabolic responses to dietary carbohydrate and fat differ markedly. Dietary carbohydrate stimulates insulin release, a response which serves to limit the rise in glycemia. The increase in plasma insulin concentration promotes glucose uptake in insulin-sensitive tissues (40) and inhibits hepatic glucose production (50). Insulin stimulates glucose transport in muscle and glycogen synthesis in both muscle and liver. In addition, insulin decreases the release of free fatty acids from adipose tissue by inhibiting hormone-sensitive lipase and stimulates triacylglycerol uptake in adipose tissue by activating lipoprotein lipase. The postprandial rise in glycemia and in insulinemia, in combination with a reduced plasma free fatty acids concentration, results in an increase in the proportion of energy derived from carbohydrate oxidation and in a decrease in that derived from fat oxidation in the whole body.

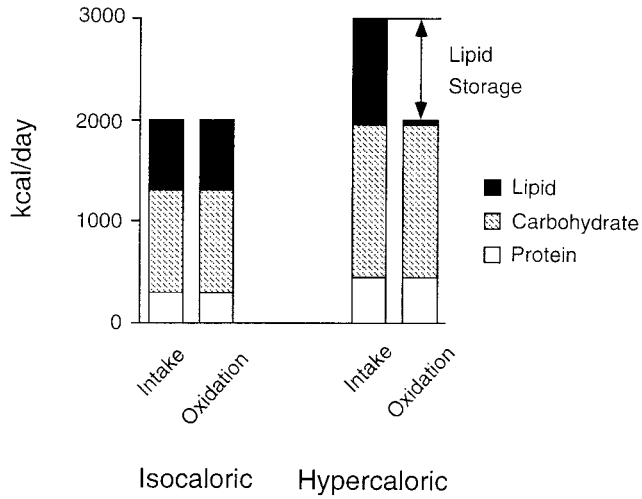

FIG. 2. Protein, carbohydrate, and lipid intake and oxidation in healthy young men on a control day with isocaloric energy intake and on 9th day of a 1,000 kcal/day excess energy intake (hypercaloric condition). Data show that protein and carbohydrate balances are achieved in both conditions, whereas fat balance is only present in isocaloric condition. In hypercaloric condition, fat oxidation is inhibited and a large lipid storage is demonstrated.

Although a high-carbohydrate meal promotes carbohydrate oxidation, by contrast the metabolic responses after a high-fat meal mainly consist in a stimulation of fat storage without stimulation of fat oxidation (81, 224) (Fig. 2). Only very high-fat meals induce a weak increase in fat oxidation (103), the majority of fat intake being stored in adipose tissue. It can be concluded that carbohydrate balance has a priority over fat balance. Furthermore, nitrogen balance tends to be maintained within a few days, even in the presence of changes in the amount of daily protein intake (83); when the minimum dietary protein requirement is met, the body's protein mass remains stable. There is ample evidence showing that in adult humans, variations in energy balance are reflected by changes in fat balance, the protein and carbohydrate balances being achieved within a few days (83). The weight gain, which characterizes the development of obesity, mainly results from the accumulation of dietary fat in

adipose tissue; the latter is due to the inability to oxidize the total amount of the daily fat intake.

B. Fat Balance: A Key Process in Body Weight Regulation

The importance of dietary fat in the development of obesity is further emphasized by most epidemiological studies which show a positive association between fat intake and body weight (152). The fuel mix oxidized may also influence body weight regulation. In certain groups of sedentary individuals, a high insulin sensitivity was associated with subsequent weight gain (205, 232, 286). These subjects have a high mean respiratory quotient measured over 24 h in a respiratory chamber (232, 286), indicating an increased carbohydrate oxidation and a reduced lipid oxidation. Thus both excess of fat intake and low fat oxidation are two factors that favor weight gain and therefore the development of obesity. The mechanisms controlling fat oxidation are therefore of great importance in the context of body weight regulation.

The rate of glucose and of fatty acids oxidation is dependent on their respective availability (120). More than 30 years ago, Randle et al. (199) proposed the concept of the "glucose-fatty acid cycle." According to this concept, the release of fatty acids from adipose tissue triacylglycerol (or from muscle triacylglycerol) imposes a limitation on glucose metabolism, by decreasing muscle glucose uptake and oxidation, while lipid oxidation is stimulated. When plasma free fatty acids (FFA) levels increase, this mechanism favors muscle FFA uptake, and FFA compete with glucose for oxidation. The enhanced FFA oxidation produces an increased acetyl CoA-to-CoA-SH ratio and an augmentation of cytoplasmic citrate concentration. The elevated concentration of acetyl CoA activates pyruvate dehydrogenase kinase, which phosphorylates and thus inhibits pyruvate dehydrogenase (PDH). Glucose metabolism is inhibited at two important steps. *1*) The increase in cytoplasmic citrate concentration inhibits phosphofructokinase, which results in an increased glucose-6-phosphate concentration; as a consequence, hexokinase is inhibited and finally glucose uptake is impaired. *2*) Inhibition of PDH impairs the entry of pyruvate into oxidative metabolism and thus contributes to inhibit glucose oxidation.

In the whole body, the total rate of fat oxidation is dependent on the concentration of plasma free fatty acids (101, 199). However, the utilization of triacylglycerol deposits in various tissues, such as skeletal muscle, also influences total body fat oxidation. A mechanism that tends to increase total body fat oxidation is the enlargement of the adipose tissue mass (225). The increased free fatty acids release into the circulation in obese subjects is not a straightforward matter of quantity of adipose tissue. The elevated plasma free fatty acids concentration is most pronounced in abdominal obesity (14), a condition which is often associated with insulin resistance and hyperinsulinemia. The paradoxical issue is the presence of a systemic elevation of plasma free fatty acids levels associated with hyperinsulinemia, since insulin is a very efficient inhibitor of free fatty acids mobilization. The lipolytic driving forces in patients with abdominal obesity dominate the inhibitory action of insulin. The visceral adipose tissue was shown to be more sensitive to lipolytic stimuli than subcutaneous depot fat (185, 206, 207). In addition, cells from visceral adipose tissues are less sensitive to the inhibitory action of insulin on lipolysis than adipose cells from subcutaneous adipose tissue. This seems to be associated with a low density of insulin receptors (24, 25). As a result of the elevated lipolysis in visceral adipose tissue of abdominally obese patients, the liver is exposed through the portal circulation to excess FFA concentrations. This is known to stimulate gluconeogenesis, which depends on the oxidation of fatty acids in the liver as an energy source; the resulting increased hepatic glucose output reflects insulin resistance in the liver (79). It is interesting that the high insulin secretion and insulin resistance in various tissues are probably secondary to the obese state because most data on individuals who have lost weight show a complete reversal of these phenomena.

Body weight eventually reaches a near-constant level in obese individuals in spite of an excess of energy and fat intake (83). Two homeostatic mechanisms have been described that are related to the composition of the body weight gain (~75% fat and 25% fat free mass). *1*) The enlargement of the fat free mass is accompanied by an increase in basal metabolic rate and, therefore, an enhanced total energy expenditure (122, 195, 200). *2*) The increase in the fat mass is accompanied by an enhanced rate of FFA release into the circulation, which contributes to stimulate fat oxidation (Fig. 3). Thus the enhanced fat oxidation observed in obese individuals in the resting state might serve as a lipostatic mechanism in individuals who are gaining weight. This metabolic adaptation eventually allows fat oxidation to rise to a level matching fat intake, thus limiting further weight gain. Studies on the relationship between fat mass and fat oxidation showed that a 10-kg increase in fat mass corresponds to a stimulation of fat oxidation of ~20 g/day (225). Thus enlargement of body fat serves as a mechanism that contributes to equilibrate fat balance in individuals with a chronic excess of fat intake. Whether the signal for the increased fat oxidation is the rise in plasma FFA levels or an hormonal message related to adipocyte hypertrophy and hyperplasia is not yet established (88). A positive energy balance, particularly due to carbohydrate overfeeding, also stimulates sympathetic activity (204), a mechanism

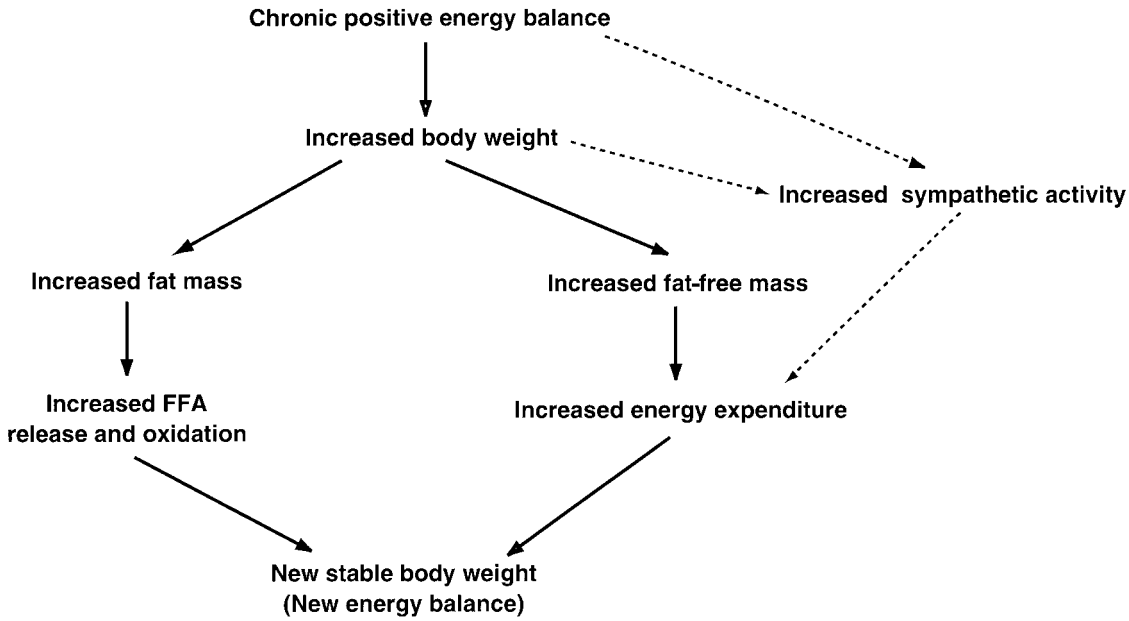

FIG. 3. Metabolic consequences of a chronic positive energy balance on fat mass and fat-free mass. Induced increases in free fatty acid (FFA) release, FFA oxidation, and energy expenditure eventually leads to a new stable body weight, resulting from a new energy balance. Solid arrows, main mechanisms; dashed arrows, mechanisms mainly operating in rodents.

which may contribute to increase energy expenditure (Fig. 3).

Carbohydrates, fats, and proteins are not the only macronutrients that provide energy. Ethanol is a fourth macronutrient that accounts for up to 10% of total energy intake among consumers of ethanol. When ethanol intake is light to moderate, this substrate is metabolized primarily by the alcohol dehydrogenase system. In a study carried out in a respiration chamber (249), we showed that both the addition of ethanol to the diet and the substitution of ethanol for 25% of energy needs led to a decrease in lipid oxidation. Ethanol ingestion in excess of energy needs therefore favors fat storage and weight gain and can be considered as a risk factor for the development of obesity (175).

III. ROLES OF WHITE AND BROWN ADIPOSE TISSUES

A. White Adipose Tissue

In adult individuals, variations in body weight mainly result from changes in the adipose tissue mass. The development of obesity corresponds to an increase in body weight, with ~75% of the weight gained corresponding to fat deposition as subcutaneous or intra-abdominal (visceral) adipose tissue and 25% as lean tissues (122). During the phase of adipose tissue deposition, fat balance must be positive, i.e., fat intake exceeds fat oxidation. An increase in adipose tissue mass results either from an enhanced deposition of triglycerides (TG) into adipocytes or from a rate of lipolysis in adipocytes lower than the rate of FFA esterification. This may occur because of excessive calorie and fat intake or decreased calorie and fat oxidation (secondary to a sedentary life-style for instance). The resulting positive fat balance leads to expansion of adipose tissue mass and volume.

It was initially suggested that adipocytes could multiply and proliferate only during infancy and childhood and that obesity developing during this period led to a so-called hyperplastic obesity characterized by increased adipocytes number with minor increases in adipocyte size. In contrast, obesity developing in adult was thought to increase adipocyte size exclusively, resulting in hypertrophic obesity (115). It has however been unequivocally demonstrated that new adipocytes can differentiate from fibroblast-like preadipocytes at any period of life and that the development of obesity in adults is also accompanied by substantial differentiation of preadipocytes into adipocytes. Several growth factors have been identified that regulate preadipocyte differentiation. They include, among others, epidermal growth factor, vitamin D_3, vitamin A, fibroblast growth factors, and peroxisome proliferation activator receptor. The role of these growth factors in the development of obesity remains unknown presently (124).

Although fat mass represents 8–20 kg in lean individuals, it may be considerably higher in obese subjects in whom >100 kg fat may be stored. Fat storage is located

mainly in adipocytes of the subcutaneous adipose tissue and the intraperitoneal cavity. Although adipose tissue mass in obese individuals can be considerable, only a minor fraction (<10%) corresponds to active protoplasmic tissue, the largest proportion corresponding to intracellular inert TG depots. As a consequence, adipose tissue metabolism, whether in terms of total energy consumed, or of substrate oxidation, represents only a minor fraction of the metabolism of the whole organism. Adipose tissue, however, occupies a central place in metabolic regulation by its role in the storage and mobilization of fatty acids and glycerol. Fatty acids released from adipose tissue may in turn modulate glucose metabolism at distinct sites. A brief description of adipose tissue metabolism illustrates how the main energy depot of the body is controlled.

The study of adipose tissue metabolism in vivo has proven to be difficult because this tissue is diffusely present in the organism. Moreover, considerable metabolic variability may exist according to the localization of adipocytes. Thus abdominal subcutaneous and intra-abdominal adipocytes are more actively lipolytic than subcutaneous femoral or gluteal adipocytes (8, 206–208, 239, 267). Studies of the metabolism of adipocytes have been performed in vitro on isolated adipocytes that yielded results that are not always consistent with investigations in vivo. Two major techniques have been recently developed to assess adipose tissue metabolism in vivo. One of these techniques, adipose tissue catheterization, rests on the identification of a subcutaneous major vein, the superficial epigastric vein, which drains essentially subcutaneous adipose tissue and can be catheterized with relative ease (89, 91). Simultaneous catheterization of an artery allows one to calculate arteriovenous differences across the adipose tissue capillary bed for various substrates. This measurement, coupled to a determination of adipose tissue blood flow with the radioactive xenon clearance (139), allows one to assess substrate exchanges in adipose tissue under various conditions. Adipose tissue metabolism can also be assessed in vivo using the microdialysis technique (7).

With these techniques, some basic aspects of subcutaneous adipose tissue metabolism have been elucidated in human beings. It has been shown from catheterization studies that adipose tissue extracts both glucose and oxygen from the interstitial fluid (62, 63, 89, 91). This suggests that glucose oxidation provides a large portion of adipocyte energy expenditure. Acetate and ketone bodies are also extracted from the blood and, if oxidized, may contribute significantly to adipocyte energy expenditure (62, 63). Several observations also indicate that adipocytes produce substantial amounts of lactate (113, 156). A major role of adipose tissue is to release stored TG as FFA as energy substrates for the tissues and organs of the body in the postabsorptive state. Control of the hydrolysis of TG stored within the adipocyte is carried out by the enzyme hormone-sensitive lipase (HSL). This enzyme is activated by phosphorylation, and the protein kinase responsible for this activation is activated by increases in cAMP elicited by the interactions of catecholamine on the adipocytes membrane receptors (90, 92). In contrast, adenosine and PGE_2 interact with membrane receptors coupled to inhibitory G proteins and impair cAMP generation, and hence lipolysis (155). Insulin inactivates HSL, whereas growth hormone and cortisol activate it. Activation of HSL stimulates the hydrolysis of TG, with subsequent release of FFA and glycerol into the interstitial fluid. Catecholamines (norepinephrine and epinephrine) appear to be the most potent activator of lipolysis in adipose tissues. In vitro studies of isolated adipocyte indicate that adipocytes bear both α_2- and β-receptors in their plasma membrane. Activation of β-receptors activates lipolysis (6, 7), whereas α_2-receptor activation appears to exert a tonic inhibition of lipolysis (136).

After ingestion of a meal, lipolysis is strongly suppressed as indicated by a decreased net glycerol output from adipose tissue (89, 91). This inhibition of lipolysis is mainly insulin mediated and is accompanied by a shift in adipose tissue metabolism from a tissue releasing carbon atoms to a tissue with a net uptake of carbon atoms. This is explained by activation of the enzyme lipoprotein lipase (LPL) at the surface of endothelial cells, an activation which is secondary to insulin action. As a consequence of LPL activation, TG circulating as chylomicrons or VLDL are hydrolyzed to fatty acids and glycerol. Fatty acids are subsequently transported into fat cells, where they are reesterified to TG. The glycerol molecules used for esterification are issued from adipose tissue glycolysis because the lack of glycerol kinase in adipocytes prevents the phosphorylation of exogenous glycerol into glycerophosphate. The efficiency with which circulating TG is deposited in adipose tissue might influence body weight gain.

Because hydrolysis of TG circulating as chylomicron and VLDL is the first step of TG deposition in adipose tissue, this has led investigators to consider the possibility that alterations of LPL might be at the origin of excess fat deposition in obesity. It has been reported that LPL activity is increased in fat biopsies obtained from obese patients (72). However, to contribute to the pathogenesis of obesity, LPL activity should be increased before the development of obesity, a condition which of course cannot be studied conveniently. As a substitute, several studies have assessed LPL activity in obese patients after weight reduction. These studies have led to contradictory results but have altogether not substantiated the hypothesis of an increased basal LPL activity in adipose tissue as a mechanism responsible for the development of obesity (72). It has even been reported that adipose tissue of obese patients presents a blunted activation of LPL in response to insulin (61, 72, 194). This tissue is resistant to insulin's

action, since both the insulin inhibition of lipolysis and the insulin activation of LPL are blunted. In obese patients, insulin resistance of adipose tissue may limit further weight gain by impeding net fat storage in adipose tissue. As a result of insulin resistance, clearance of TG circulating as lipoprotein is impaired. Furthermore, impaired suppression of lipolysis leads to increased turnover of FFA, which exceeds lipid oxidation. Increased reesterification of FFA ensues at the level of liver cells, and TG are secreted into the circulation as VLDL. These alterations of adipose tissue metabolism probably contribute significantly to the hypertriglyceridemia that is frequently encountered in obese patients.

B. Brown Adipose Tissue

There are two major phenotypes of adipose tissue: white and brown adipocytes. Whereas the former is primarily an energy depot, the latter is characterized morphologically by a more abundant cytoplasm containing multiple small lipid droplets. These cells have a unique mitochondrial machinery that allows them to uncouple oxidative phosphorylation from ATP synthesis. This uncoupling of oxidative phosphorylation is due to the presence in the mitochondria of brown fat cells of an uncoupling protein denominated uncoupling protein-1 (UCP-1) that can be activated to dissipate the proton gradient across the mitochondrial membrane to generate heat. Uncoupling of oxidative phosphorylation in brown adipose tissue can be switched on by sympathetic stimulation, itself elicited by cold exposure, dietary factors (overfeeding), and stress (such as endotoxin administration). The SNS acts on the brown adipocytes through a distinct subtype of β-adrenergic receptors (β_3-receptors). As a result of β_3-receptor activation, UCP-1 is activated, and its synthesis is stimulated at the level of gene transcription (209).

Brown adipose tissue is present in significant amounts in several animal species and in newborn humans, in whom it plays a role in thermoregulatory process during cold exposure. Its existence in adult humans remains however controversial, although it has been observed in patients with pheochromocytoma. Recently, it was demonstrated that adipocytes expressing the UCP-1, and hence bearing the brown adipocyte phenotype, were diffusely present in adipose tissue of nonhuman primates (264). Surprisingly, these brown adipocytes lacked β_3-receptor, with the consequence that their function remains mysterious.

The hypothesis that a defective stimulation of brown adipose tissue may play a role in the pathogenesis of obesity and its metabolic complications has regained new interest recently, when it was reported that genetic anomalies of the uncoupling protein (UCP-1) or of the β_3-receptor may possibly be associated with human obesity. Several reports have indicated that a mutation of the β_3-receptor (of unknown functional consequence) is present in ~10% of the general population (54, 268, 275). Although the prevalence of the mutation was not found to be increased in the obese population, it was associated in these early reports with a more rapid weight gain or with the development of non-insulin-dependent diabetes mellitus at a younger age. Several subsequent studies, however, failed to detect an association between obesity or resting metabolic rate and this mutation (99, 114, 147, 176, 210, 280). A polymorphism of the uncoupling protein gene has also been identified. Coexistence of a mutation of β_3-receptor and of a variant of the uncoupling protein gene was shown to be associated with a high weight gain during adult life (52). Although intriguing, these observations however do not suggest that these mutations play a major role in the pathogenesis of obesity.

Recently, the genes of two additional uncoupling proteins have been identified (27, 28, 87). These proteins have been denominated UCP-2 and UCP-3. In contrast to UCP-1, which is expressed exclusively in brown adipose tissue, UCP-2 is present in several tissues, including the liver, skeletal muscle, and white adipose tissue, whereas UCP-3 is expressed predominantly in skeletal muscle (263). Surprisingly, UCP-2 and UCP-3 gene expression have been shown to be induced by fasting and suppressed by feeding (27, 28) and are therefore unlikely to be involved in energy-dissipating mechanisms in response to alterations of energy balance. Furthermore, UCP-2 mRNA was also observed to be increased in adipose tissue of obese patients. No correlation was observed between UCP-2 or UCP-3 mRNA levels and resting metabolic rate (169). The search for associations between polymorphisms of these UCP and obesity has to date been negative (258). Further studies of the physiology and biochemistry of these proteins will be required to evaluate their potential role in obesity.

IV. CONTROL OF FOOD INTAKE

A. Satiation and Satiety

Most people who maintain a stable body weight spontaneously adapt their energy intake to a large range of energy expenditure through accurate mechanisms of control of food intake. A detailed presentation of the physiology of appetite lies outside the scope of this review (18, 21). Appetite is a complex phenomenon arising from a sequence of interactions among peripheral and central mechanisms. The gastrointestinal tract contains chemo- and mechanoreceptors that relay the information about its nutrient content to the brain mainly via the vagus nerve (167). Impairment of appetite or satiety may arise from peripheral or central mechanisms.

The amount of energy ingested over 24 h depends on two major variables: the size of individual meals and the frequency with which meals are ingested. These two variables are regulated by distinct mechanisms. Hunger can be defined as the sensation felt by an individual that drives him to search for and ingest food. This sensation is elicited after a variable period following the absorption of the nutrients ingested with the previous meal. Although its mechanisms remain poorly understood, it has been repeatedly observed that a slight fall in plasma glucose concentration precedes the initiation of food intake in both rats and humans (41). After the ingestion of a certain amount of food, a suppression of hunger occurs that will lead to the termination of food intake. This process is referred to as satiation, and the mechanisms that underlie it are the major determinants of meal size. The time of satiation is followed by a period of variable duration that is characterized by the absence of hunger; this is referred to as satiety. Termination of the period of satiety coincides with the resurgence of the feeling of hunger, leading to consumption of the next meal, thus resuming the cycle of food intake (18, 21). The mechanisms that promote satiation are different from those that determine the duration of satiety; thus meals size and meals frequency are controlled by different factors.

The overall process of food intake control is governed by complex and intricate mechanisms. Not only the macronutrients composition, size, and caloric density of the meals but also their organoleptic properties (sight, smell, taste, and texture) play an important role in the determination of satiation. In addition, it has been demonstrated that individuals who were voluntarily overfed or underfed over extended periods of time to achieve significant changes in body weight tend to restore their usual body weight within a period of several weeks or months; such individuals spontaneously reduced or increased their food intake when placed again on an ad libitum diet until their body weight came back to initial values (33). Thus, in addition to the effects of nutrients ingested with the previous meals, body size and body composition obviously play a more chronic role in the control of food intake. Furthermore, it is evident that, in our modern civilizations, food intake is not invariably the result of hunger. Numerous situations may lead to food or drink consumption as a result of social activities (68). Alterations of food intake may also result from complex psychodynamic stimuli, as is probably the case in anorexia nervosa and bulimia nervosa.

B. Hypothalamic and Brain Stem Centers

It has been recognized for several decades that hypothalamic and brain stem centers control food intake and energy expenditure in animals and in humans. In the rat, it was observed that electrical stimulation of the lateral hypothalamic area triggered food and drink intake while electrical stimulation of the ventromedial area induced termination of food intake (34, 36, 38). It was further observed that physical or chemical lesions of the ventromedial area of the hypothalamus in rats led to the development of obesity and insulin resistance (9, 98). Such lesions induced marked alterations of the feeding behavior, in such a way that affected animals consumed a large excess of calories when given palatable food in sufficient amounts, but failed to actively search for food during food deprivation. They also displayed neuroendocrine abnormalities; lesions of the ventromedial hypothalamic (VMH) area were characterized by early hyperinsulinemia, which was mediated by an increased vagal activity and could be prevented by vagotomy, and a decreased overall sympathetic activity and brown adipose tissue thermogenesis (9).

Since these early experiments, the concept of the existence of a satiety center and a feeding center has considerably evolved, and it is now recognized that food intake control is regulated by complex interactions (for review, see Ref. 146). Nuclei within the lower brain stem integrate and relay information between peripheral autonomic/endocrine organs and other forebrain structures. Nuclei in the pars-midbrain and the thalamus interpret this information in relation to the sensory properties of food. Hypothalamic nuclei respond to neural inputs as well as to circulating hormones and substrates. Finally, forebrain nuclei such as the amygdala and the frontal cortex are involved in the aversive or positive aspects of food intake (146). Various inputs, including neural inputs from the vagal nerve, hormones, and possibly substrate concentration changes inform these regulatory centers on the metabolic status of the body (146).

Recently, several neuropeptides involved in the regulation of food intake have been identified, and several others probably remain to be discovered. Of these neuropeptides, NPY is likely to play an important role (146, 215, 252, 261, 270). It is so far the most potent stimulus for food intake identified within the central nervous system (CNS). Neuropeptide Y release from the arcuate nucleus is increased in virtually all situations associated with a drive for feeding, such as fasting or hypoglycemia. In contrast, nutrient absorption induces a feedback inhibition of NPY secretion, which coincides with termination of food intake (71, 215). Insulin appears to be tightly associated with these changes in NPY secretion. Central insulin administration invariably decreases NPY mRNA levels in the arcuate nucleus, while insulinopenia increases it (226, 229). Neuropeptide Y in turn is able to alter energy metabolism through effects exerted at the level of the CNS (13, 282). Neuropeptide Y, however, does not appear to be the only factor responsible for the control of energy intake, as indicated by the recent observa-

tion that transgenic mice deficient in NPY did not display marked alterations in feeding behavior (74). It is likely that NPY interacts with other regulatory peptides and with normal inputs in a yet unexplained fashion. Control of food intake must rely on several feedback loops, because it is an essential process that is needed for the survival of individuals.

Recently, genetic models of animal obesity have led to the identification of two other peptides tightly involved in the regulation of food intake. Obesity in the yellow (A y/a) mouse is caused by a promoter rearrangement of the agouti locus, resulting in constitutive, ectopic expression of the agouti peptide. This peptide acts as an antagonist of the melanocortin-4 receptor in hypothalamic cells and increases feeding behavior. This led to the recognition that desacetyl-α-MSH, produced by POMC neurons in the arcuate nucleus, exerts a tonic inhibition of food intake (39, 157, 181). More recently, obesity in the *ob/ob* mouse led to the discovery of the OB gene product, which has been renamed since "leptin." This peptide, synthesized in adipose cells, exerts several actions on energy homeostasy and on the neuroendocrine system, which is discussed in more detail in section v (43, 105, 190, 283).

Other central effectors, galanin (135), catecholamines (145), and opioid peptides, have been shown to exert potent antagonist actions on food intake and more particularly on fat intake. More recently, glucagon-like peptide-1 (GLP-1) has also been shown to be a potent inhibitor of food intake (257). Other factors, among which glucocorticoids (by acting on the mineralocorticoid type receptor within the CNS) and growth hormone releasing hormone (GHRH) potentiate food intake (66, 138), but their role in body weight regulation remains uncertain.

Signals from metabolic origin may also contribute to the sensation of satiety in mammals such as the degree of oxidative metabolism of glucose and FFA in the liver (94). It has been shown that inhibition of fat oxidation by methyl palmoxirate (95) or 2-mercaptoacetate (137) causes an increase in feeding. Suppression of appetite resulting from this mechanism is, however, not necessarily due to the ingestion of fat, since fuels derived from internal adipose stores, as it occurs during fasting, may also provide FFA for oxidative liver metabolism. There has been a large interest in the search of peripheral satiety signals arising from adipose tissue that could reflect the degree of repletion of fat stores, and therefore be candidates for feedback signals in a regulatory bop. Substances such as satietin (129), adipsin (60), and oleoyl-estrone (219) are produced by adipose cells, but their role in appetite control is uncertain.

C. Effects of Nutrients on Food Intake

The influence of nutrients on subsequent food intake has been extensively studied by covertly altering the food composition or energy content of a meal and observing the changes in the subsequent food composition of the next meal. It has generally been observed that an acute deficit in energy intake is rapidly compensated in the subsequent meals. Although the results of the published studies are somewhat disparate, it has been generally observed that a selective deficit in one of the major macronutrients did not trigger a specific increase of the intake of this specific macronutrient, but rather a compensatory ingestion of an equivalent number of calories from a mixed diet (16–18, 20, 21). In contrast, excessive intake of nutrients generally decreases subsequent food intake.

There is a hierarchy regarding the ability of the various macronutrients to suppress subsequent food intake. Proteins display the most potent effect to delay subsequent nutrient ingestion. Carbohydrates, whether administered orally or parenterally, are also able to significantly increase the early satiety period and to decrease the amount of food ingested at the next meal (19). Lipids appear to have less potent satiating effects. Of interest, intravenous infusion of lipid emulsion failed to alter voluntary food intake, while intraduodenal administration of lipid was effective (274). This indicates that gut factors may be responsible for the satiating effects of lipids. Stimulation of cholecystokinin (CCK) secretion by enteral lipid is likely to play a significant role in this regard. The role of CCK as an hormone that mediates satiation and early-phase satiety has been recently emphasized. The intake of protein and fat stimulates the release of CCK from cells in the mucosa of the upper small intestine. This hormone activates CCK-A receptors in the pyloric region of the stomach; the signal is then transmitted via vagal afferents to the nucleus of the tractus solitarius, where it is relayed to the PVN and to the VMH (238). Another peptide, enterostatin, appears to selectively reduce intake of a high-fat diet (75). Enterostatin is produced from pancreatic procolipase, a cofactor for lipase that is necessary for optimal fat digestion; procolipase is cleaved by trypsin to colipase and the pentapeptide enterostatin. Thus a peripheral satiety signal can be generated during fat digestion and may delay the subsequent feeling of hunger.

Nutrients exert both immediate and delayed effects on subsequent food intake. Acute changes in body weight induced by forced overfeeding as part of an experimental setting (32) or of a ritual procedure (189) are subsequently corrected by a diminution of spontaneous food intake. This indicates that there are signals that inform the nervous centers controlling food intake on body weight or body composition. These mechanisms remain to date largely not elucidated. The recently identified adipose tissue peptide leptin (283) was shown in animals to act as a signal related to the size of the adipose tissue mass that is sensed by hypothalamic centers. Whether

leptin plays a role in the long-term regulation of body weight in humans is uncertain (108), except in those rare humans who have a deficiency of leptin production (171, 241) or a truncated leptin receptor (53).

D. Influence of the Increasing Proportion of Dietary Fat on Energy Intake

Diet composition differs markedly among countries and cultures. Traditional African diets for instance are characterized by a high content in carbohydrate and fibers, and it is interesting to note that obesity is virtually absent in societies eating this type of food. In contrast, in industrialized countries, fat may represent 40% or more of total calories ingested, and obesity is highly prevalent. Such a trend toward increasing both dietary fat and the prevalence of obesity has also been reported among people of the high socioeconomic classes in many developing countries over the past decade (172, 193). Although such dietary changes are usually paralleled by significant reduction in physical activity, it raises the suspicion that dietary composition, and in particular the increasing proportion of fat, may be a major determinant of the energy content of the daily food intake. This hypothesis is indeed supported by several observations. First, the effect of altering the fat content of the meals was monitored in healthy lean human subjects. It was observed that when food items with a high fat content were presented, subjects ate 30% more calories per day compared with what they ate when presented with food items with a higher carbohydrate content (70, 211, 256). Interestingly, this excessive amount of calories ingested on a high-fat diet were consumed as a smaller volume, as well as a smaller weight of food due to the higher energy density of high-fat foods (20). Second, it has been reported in several surveys that the diet composition of obese subjects contain a higher proportion of fat than that of lean individuals (152). This observation strongly suggests that a habitual high dietary fat content may lead individuals to obesity due to the lower satiating effect of fat compared with carbohydrates (100, 152, 178, 179). Third, the observation that obese subjects lose weight when placed on an ad libitum high-carbohydrate diet further supports the hypothesis that high-carbohydrate, low-fat diets are more satiating than high-fat diets (159, 197).

Several recent studies have investigated why individuals fed a high-fat diet ingest an excessive amount of calories (18–21). A higher energy density of the diet (i.e., more calories consumed for a given volume or weight of food ingested) may be the simplest explanation (194, 243, 244). An alteration in energy density of the meals results in a parallel change in energy intake. Isoenergetically dense diets with varying fat content induced similar mean daily energy intakes in healthy male volunteers, which indicates that it is not the high proportion of fat per se that leads to overfeeding (243). However, as mentioned earlier, fat feeding also may induce satiety, so the reason why the satiety mechanisms become ineffective remains presently unclear. It has been proposed that, as fat exerts its satiating effect through mechanisms elicited in the gut, the delayed gastric emptying due to a high-fat meal results in satiation signals that intervene too late during the course of the meal; as a result, a large amount of calories has already been ingested before satiation is elicited (20). Another hypothesis was proposed by Flatt (86). This author proposed that food intake is mainly regulated to maintain a constant glycogen content in the body. From a teleological point of view, this hypothesis appears reasonable with regard to the key role played by glucose as the main brain nutrient and the small capacity to store glucose as glycogen in the body. The amount of carbohydrate ingested every day is known to be the major determinant of body glycogen stores in both humans and animals. As a consequence of obligatory glucose oxidation, eating a diet containing a high percent of calories as fat and a low percent of calories as carbohydrate would lead to a larger amount of food energy to get sufficient carbohydrate intake to maintain glycogen stores. This may explain a chronic excess of caloric intake and the development of obesity when food with a high fat content is available. This theory has received experimental support from animal studies, but human studies have remained controversial, mainly due to the fact that it is difficult to assess spontaneous food intake under the laboratory conditions that are needed to measure substrate oxidation rates simultaneously (242, 244, 245, 256).

Although it appears that obese subjects generally consume a diet with a higher fat content than lean subjects, such a high-fat diet per se does not appear sufficient to explain the development of obesity. In a recent dietary survey, adult males from the United Kingdom were partitioned into groups consuming either a high-fat diet or a low-fat diet on a spontaneous basis. It was observed that obesity was almost absent in individuals eating a low-fat diet. However, among those consuming a high-fat diet, only a minor portion was obese and a large portion of these people had a normal weight (21). This may indicate that a defect in the mechanisms responsible for the satiety to fat is not a major cause for the development of obesity. Another possibility to explain the absence of obesity in individuals consuming a high-fat diet is a high capacity to stimulate fat oxidation (285). Individuals with a high capacity to oxidize fat appear to have a low risk of weight gain when exposed to a high-fat diet (205). Obesity results from alterations of the mechanisms that normally allow one to adapt spontaneous food intake to energy needs. However, because of the complexity of the various factors that are involved in this regulation, it appears unlikely that a single defect can be responsible for the

development of obesity. This concept is important to consider when one studies the genetics of obesity. Most studies suggest that obesity is a polygenic disorder (29); only in very rare situations, a single gene defect is responsible for the development of human obesity (53, 171).

V. ROLE OF LEPTIN IN BODY WEIGHT REGULATION

A. Genes and Environment

The role of genetics in human body weight regulation has been much studied over the last decade. The research interest has been focused on the genetics of obesity because of the high prevalence of this disease. It is likely that the genes involved in weight gain increase the susceptibility of an individual to the development of obesity when exposed to environmental conditions that favor a positive energy balance. Adoption (31, 247) and twin studies (32, 246) have shown that human obesity has a genetic component. The level of heritability, which describes the fraction of the population variation in a trait that can be explained by genetic transmission, varies for body mass index, between 25 and 40% (30). Age-related changes in body fatness and total body fat during young adult life are heritable (76, 77), which supports the concept of a genetic basis for obesity. Recent studies have shown that both the body fat mass and the partitioning between central and peripheral fat depots are influenced by genetics (191). The ability to store energy as fat in adipose tissue has been an important mechanism to individual survival and reproductive capacity. It is therefore likely that mutations of genes that favor energy storage and metabolic efficiency have conferred a survival advantage to individuals when food supply was scarce and during periods of famine. The combined influence of an easy access to energy-dense foods and of a decrease in physical activity has made these genes maladaptive (212). Thus obesity is most likely a polygenic disease characterized by interactions between genetic and environmental factors. The list of candidate genes that are associated with obesity is increasing (30), but more years of research are needed to identify the important genes and the mutations of genes that favor excess body fat content and the distribution of abdominal versus gluteal fat.

B. *Ob* Gene and Ob Protein: Studies in Animals

The discovery and the cloning of specific genes responsible for excessive fatness in animal models of obesity have greatly led to a renewed interest in genetic factors involved in the development of human obesity. The recent isolation and the cloning of the obese (*ob*) gene (283) that induces obesity and diabetes in mice when mutated has attracted particular attention. The *ob* gene is mostly expressed in white adipose tissue, and it might function as part of a signaling pathway from adipose tissue that acts to regulate body weight. The *ob* gene is also moderately expressed in brown adipose tissue (170). The gene product, the Ob protein leptin, is a circulating factor that may control food intake and energy expenditure. In the *ob/ob* mouse, two separate mutations in the *ob* gene result in either a premature stop codon or the total absence of *ob* mRNA. Without leptin, the mouse overeats, resulting in the obese phenotype. There is extensive homology of the *ob* gene among vertebrates that suggests that its function is highly conserved. When the human genome was screened, an *ob* homolog, 83% identical to the mouse *ob* gene, was found, confirming *ob* as a highly preserved gene (166, 283). The *ob* gene product leptin is a 16-kDa protein that is present in mouse and human plasma; it is however undetectable in plasma from *ob/ob* mice (105). In contrast, the development of obesity in another line of mice, the *db/db* mouse, is secondary to a mutation of the leptin receptor. In these animals, leptin plasma levels are markedly increased secondary to a resistance to the effects of leptin (105).

The demonstration that leptin plays a role in mouse body weight regulation stems from the observation that its chronic injection into *ob/ob* mice causes the animals to lose weight and maintain their weight loss (43, 105, 190). Leptin appears to have a dual action; it decreases the animal food intake and increases its energy expenditure, causing the animal to oxidize more fat (Fig. 4). It has been reported that the metabolic effects of leptin (stimulation of metabolic rate with normalization of body temperature) in treated *ob/ob* mice precede its effects on appetite and body weight (190). When leptin was injected at the doses of 5 $\mu g \cdot g^{-1} \cdot day^{-1}$ intraperitoneally for 33 days, *ob/ob* mice exhibited a decrease in body weight within 4 days, and lost 40% of their body weight after 33 days (105). Food intake of treated *ob/ob* mice was less than that of control mice after 2 days and stabilized at 40% the intake of control mice at all points after 4 days. In another experiment, untreated *ob/ob* mice were pair-fed with *ob/ob* mice receiving leptin injections. The latter lost more weight than the former, indicating that leptin not only decreases food intake but also increases energy expenditure (105).

In contrast to the *ob/ob* mice in whom leptin injections resulted in a significant weight loss, there was no effect of leptin injections on body weight or food intake in *db/db* mice. These results were expected in view of the high plasma levels of leptin in *db/db* mice, indicating a state of resistance to leptin action. These studies illustrate the fact that there is a range of sensitivities to the effects of injected leptin on body weight. The *ob/ob* mice that are leptin deficient are very sensitive to leptin injections.

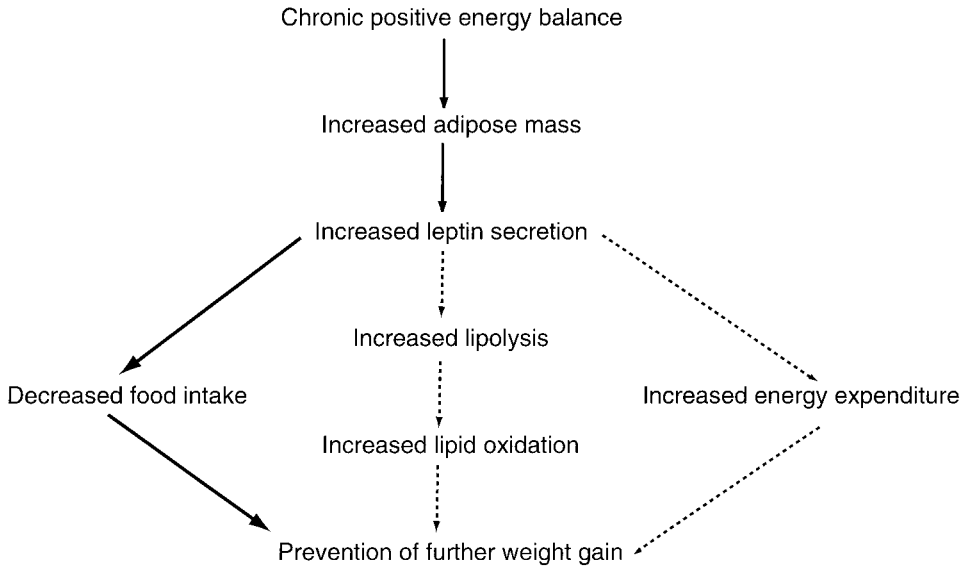

FIG. 4. Schematic representation of effects of leptin limiting further weight gain in response to a condition of chronic positive energy balance. This concept is mainly supported by data obtained in rodents. Solid arrows, main mechanisms; dashed arrows, less important mechanisms.

Control mice, with a physiological production of leptin, are less sensitive than *ob/ob* mice to the weight-lowering effect of leptin injections. Finally, *db/db* mice that have high plasma leptin levels and alterations in hypothalamic leptin receptor are resistant to leptin injections.

The *ob/ob* mice are characterized by a low resting metabolic rate, hypothermia, and hypoactivity. All these metabolic defects are rapidly normalized by leptin treatment (190). With the highest dose of leptin (10 μg/g), hyperglycemia and hyperinsulinemia were also brought back to normal levels in *ob/ob* mice; in addition, the metabolic effects of leptin preceded the effect on appetite control (190). Leptin administration also reduced food intake and body weight in diet-induced obese (DIO) mice (43). These results show that this circulating protein may play a role in the regulation of feeding behavior not only in animals with an altered leptin synthesis but also in animals with dietary obesity of nongenetic origin.

C. Central Effects of Leptin

Leptin acts on targets in the CNS, as shown by injections of leptin into the lateral ventricle of *ob/ob* mice which induce a suppression of eating within 30 min after injection and which lasts more than 6 h (43). Tartaglia et al. (251) have identified and cloned a leptin-binding receptor (OB-R) that is expressed in the mouse choroid plexus and in hypothalamus. These authors have genetically mapped the gene encoding OB-R to the 5-cm interval that contains the *db* locus. The *db/db* mice have high plasma levels of leptin and have been shown to be resistant to recombinant leptin (43, 105, 190, 240). It was shown that the *db* gene encodes the receptor for leptin and that a mutation of this gene could be responsible for leptin resistance. The OB-R protein is a large single membrane-spanning receptor (110) of the class I cytokine receptor family. The OB-R protein has a short intracellular domain; in some tissues, alternatively spliced forms of mouse OB-R exist with longer intracellular domains, as was found for a human OB-Rb homolog that is characterized by a long intracellular domain (251). The long leptin receptor isoform (OB-Rb) is most abundantly expressed in the hypothalamus and is the receptor that signals and mediates the central effects of leptin. The OB-Rb was identified in the arcuate, lateral, ventromedial, and dorsomedial nuclei of the hypothalamus (168, 227). The long receptor form present in humans can have sequence polymorphisms, which are of unclear significance (56). The other major spliced isoforms of OB-R are Ob-Ra and Ob-Re. Ob-Ra has the shorter intracellular domain and Ob-Re has no transmembrane domain and is a soluble form of receptor (250).

It was shown that Ob-R mRNA are expressed in a variety of peripheral tissues, which suggests that leptin may also act outside the brain (250). Peripheral effects of leptin have indeed been demonstrated on adrenocortical cells (26) and on pancreatic β-cells (134). The localization of the OB-R within the hypothalamus is of particular interest, since the hypothalamic nuclei are major sites of control of both food intake and energy expenditure (37). One mechanism by which leptin may regulate food intake and energy expenditure is inhibition of hypothalamic NPY synthesis and release (240). Hypothalamic NPY stimulates food intake and decreases thermogenesis (282). Stephens et al. (240) showed that the level of pre-pro-NPY mRNA in cells from the arcuate nucleus of the hypothalamus was increased in the *ob/ob* mouse and decreased significantly during leptin administration for 30 days, suggesting that

leptin inhibits NPY synthesis and release. Therefore, the hormone leptin might act by inhibiting the synthesis and release of NPY in the arcuate nucleus of the hypothalamus (270). This mechanism could explain the reduction in appetite and the increase in energy expenditure induced by leptin administration in *ob/ob* and DIO mice. Furthermore, Cusin et al. (64) reported that intracerebroventricular bolus injection of leptin in lean rats resulted in a decrease in NPY levels in its sites of synthesis (arcuate nucleus) of the hypothalamus; this effect was associated with a marked weight loss.

Although a role for NPY in mediating the central effects of leptin is well established in rodents (216, 270), recent investigations on mutant mice deficient for NPY indicate that this neuropeptide may not be essential for leptin actions on feeding behavior (74). These NPY-deficient mice showed normal food intake, body weight, and adiposity. In addition, treatment of these mutant mice with leptin for 5 days significantly reduced their food intake, body weight, and adipose tissue mass. Thus leptin can suppress feeding and promote weight loss via signaling pathways that are independent of those using NPY.

Recently, a mutation was identified in the hypothalamic leptin receptor gene of *db/db* mice: the insertion of an additional 106-bp nucleotide sequence in the PCR product (49, 140). In the *fa/fa* rat, a single-base substitution that results in an amino acid change of the leptin receptor was described (192). This amino acid substitution could affect the dimerization of the receptor (which is involved in signal transduction of this class of receptors) and may be the cause of obesity in *fa/fa* rats. The *fa*-type receptor exhibits a reduced leptin-binding affinity and a reduced signal transduction (277). The extent to which the reduced affinity of the Ob-Rb-*fa* for leptin contributes to the metabolic disorders of *fa/fa* rats needs to be studied further.

D. Modulation of the Central Effects of Leptin

Evidence has accumulated in animal studies showing that leptin acts centrally to decrease feeding and stimulate brown adipose tissue activity, and these effects may be mediated, at least in part, by inhibition of NPY production by neurons in the arcuate nucleus (270). In contrast, intracerebroventricular administration of NPY induces sustained hyperphagia and excessive weight gain in rats (216, 270). In addition, leptin production by white adipose tissue is increased following a 6-day intracerebroventricular NPY infusion. Thus leptin acts centrally to decrease NPY synthesis and NPY concentrations in the arcuate nucleus and PVN; it is likely that reduced NPY release in the PVN mediates leptin's hypophagic and thermogenic effects. Conversely, NPY-induced obesity results in raised plasma leptin concentrations. Leptin, produced by white adipose tissue, and the NPYergic arcuate nucleus-PVN neurons may interact in a homeostatic loop to regulate body fat mass.

Recently, glucocorticoids were shown to act as counterregulatory hormones of the central effects of leptin. Leptin injected intracerebroventricularly in normal rats induced modest reductions in body weight and food intake. In contrast, the same dose of leptin (3 μg as a bolus given intracerebroventricularly) had very potent and long-lasting effects in decreasing body weight and food intake when administered to adrenalectomized animals (281). Furthermore, glucocorticoid administration to adrenalectomized rats inhibited these potent effects of leptin. These data show that glucocorticoids have an inhibitory role on the central actions of leptin.

The latter results support the concept that glucocorticoids modulate the central effects of leptin. Under normal conditions, glucocorticoids may prevent the hypophagic action of leptin. This may explain why patients with a lack of glucocorticoids production (Addison disease) are often hypophagic. In contrast, obesity is often accompanied by various degrees of hypercorticism, which may contribute to decrease the central responsiveness to leptin. Thus leptin resistance that is observed in obese patients may be in part due to a modulatory role of glucocorticoids (15, 47, 188).

E. Peripheral Effects of Leptin

The leptin receptor isoforms are widely expressed in a variety of organs and tissues (125, 251, 269), which suggests that leptin may have actions on extraneuronal tissues (233). In normal Wistar rats, sustained hyperleptinemia at 8 ng/mg induced for 28 days by infusing a recombinant adenovirus containing the rat leptin cDNA induced disappearance of body fat. In contrast, control rats pair-fed to the hyperleptinemic rats retained ~50% body fat (48). This effect may be due to the thermogenic effect of hyperleptinemia (105), but it raises the possibility of a specific effect of hyperleptinemia on fat storage in adipocytes. Leptin strongly stimulates lipolysis in white adipose tissue fat pads from lean Zucker *Fa/fa* rats, whereas no increase in lipolysis was observed in the fat pads from obese *fa/fa* rats, which harbor an inactivating mutation of the OB-Rb (234). Recent data suggest that the weight-reducing action of leptin results not only from an endocrine hypothalamic mode of action, but also through an auto- or paracrine pathway by stimulating lipolysis in white adipose tissue (97, 173). Furthermore, by regulating the expression of enzymes of FFA oxidation, leptin may control intracellular TG content of adipocytes (284).

The various effects of leptin on the hypothalamic-pituitary axis concern many functions unrelated to body

weight control, such as the induction of the onset of puberty (3, 164). The activity of the hypothalamic-pituitary-adrenal axis in humans varies inversely to the serum leptin levels (150). It is interesting to mention that glucocorticoids in high doses increase leptin expression in vitro (237, 265) and in vivo (187), whereas leptin inhibits glucocorticoid production, suggesting the existence of a negative-feedback loop between leptin and glucocorticoids.

F. Regulation of Leptin Production in Rodents

In *ob/ob* mice, an overproduction of mutated mRNA was observed, suggesting that the absence of functional leptin activates the mutated *ob* gene expression (283) or that the increased adipose tissue mass explains this overproduction of mutated mRNA. Similarly, in *db/db* mice and *fa/fa* rats, which are characterized by altered hypothalamic leptin receptors, there is an upregulation of *ob* mRNA in adipose tissue. Thus, in the presence of either a nonfunctional leptin, or of altered leptin receptors, *ob* mRNA is upregulated. This upregulation of *ob* mRNA may be linked to the increased food intake of these mutants and possibly to the resulting increase in insulin secretion. The influence of food intake on *ob* gene expression is further supported by experiences showing that fed normal rats had twice as much *ob* mRNA in adipose tissue as did fasted fats (217). In addition, *ob* gene expression exhibited diurnal variation, increasing during the night, after rats started eating, and decreasing during the light period, when rats did not feed. Fasting decreased *ob* mRNA level, and refeeding fasted rats restored *ob* mRNA within 4 h to levels of fed animals.

In rats, insulin is an important stimulus for *ob* gene expression: injection of insulin into fasted rats doubled leptin mRNA in adipose tissue cells within 4 h (217). In hyperphagic, hyperinsulinemic rats such as Zucker (*fa/fa*) rats (174) or in VMH-lesioned rats (98), the high plasma insulin levels may explain the increased *ob* gene expression measured in adipose tissue of these animal models of obesity. The upregulation of *ob* gene in VMH-lesioned rats shows that fat accumulation in nongenetically obese animals can be associated with increased leptin production (98). In rats, insulin stimulates leptin secretion (162, 217, 265), whereas leptin suppresses the secretion of insulin from pancreatic islet cells (73, 126). There is an adipoinsular axis by which the adipose tissue mass induces the β-cells to secrete less insulin. This does not occur, however, in human obesity, since high leptin levels are observed in the presence of hyperinsulinemia.

The lack of leptin in *ob/ob* mice and the mutated Ob-Rb in *db/db* mice might explain the early development of hyperinsulinemia in these animals due to the absence of a leptin-suppressive effect on insulin secretion. These results also show that a functional leptin receptor (Ob-Rb) is present in pancreatic islets and suggest that leptin overproduction, particularly from adipose tissue, may inhibit both basal and glucose-stimulated insulin secretion. Hyperleptinemia might be a link between obesity and diabetes (73, 186).

Stimulation of *ob* mRNA expression is an early signal in rats, since it occurs after a single meal (217). This early response, however, cannot explain long-term body weight regulation. A signal proportional to adipose tissue size is needed to act in a long-term homeostatic mechanism of body weight regulation. To get more insight into the relationship between the size of the adipose tissue mass and the expression of *ob* mRNA, Frederich et al. (93) assessed plasma levels of leptin and expression of *ob* mRNA in adipose cells of rodents in response to a variety of perturbations that affect body mass. Fasting of mice for 24 h induced a marked fall in plasma levels of leptin. This regulation is at least in part at the transcription level, since fasting of mice and rats was accompanied by a reduction of *ob* mRNA expression in white adipose cells. Refeeding restored the expression of *ob* mRNA to control levels.

Another model to study the influence of adipose tissue mass on *ob* mRNA expression is the investigation of obese mice due to neonatal treatment with monosodium glutamate (MSG). These mice do not have hyperphagia but become obese due to a defect in hypothalamic control of energy expenditure (254). These MSG-induced obesity mice have increased expression of *ob* mRNA in adipose tissue and increased circulating leptin concentrations. When MSG-obese mice are treated by caloric restriction or by thermogenic drugs, the loss of excess body weight is accompanied by a return of *ob* mRNA expression toward normal levels. These studies confirm the relationship between adipose tissue mass and the regulation of leptin secretion. Leptin could be an adipostat signal that decreases with starvation and rises with obesity; leptin plasma levels reflect, therefore, the size of energy stores in adipose tissue.

The *ob* mRNA expression in vitro is also upregulated by glucocorticoids such as dexamethasone and cortisol (237). Increased glucocorticoids directly stimulate leptin secretion from adipose tissue, which then inhibits NPY release in the hypothalamus (240). This could explain the increased leptin expression in genetic models of rodent obesity, such as the Zucker *fa/fa* rat (174), since these animals are characterized by hyperglucocorticoidism. In contrast, increases in intracellular cAMP result in inhibition of *ob* mRNA expression (237). Thus, when lipolysis is stimulated in white adipose tissue by the SNS or by circulating epinephrine, the stimulation of adrenergic β_3-receptors leads to a reduction of *ob* mRNA expression and of leptin production (165). Norepinephrine and isoproterenol, two catecholamines with a strong lipolytic action,

inhibit leptin gene expression by the activation of a guanine nucleotide-binding regulatory protein G_s-coupled pathway in 3T3-L1 adipocytes (133). These results suggest that a signaling pathway that results in activation of protein kinase A regulates leptin gene expression in 3T3-L1 adipocytes.

G. Regulation of Leptin Production in Humans

In humans, serum leptin concentration is related to the size of adipose tissue mass in the body (57, 59, 106, 127, 153, 154, 160). The mechanisms by which the increase in adipose tissue is translated into an increase in serum leptin concentration involve both the number of adipose cells and the induction of *ob* mRNA per cell. Obese individuals have an increase in adipose cells number; in addition, a significantly greater amount of *ob* mRNA was found in adipocytes from obese subjects than in those from normal-weight subjects (59). There is evidence that the small fat cells express less *ob* mRNA than large fat cells (106). Excess fat mass in the massively obese subjects results from adipocyte hyperplasia and hypertrophy. It is likely that when small fat cells fill with lipid, there is a threshold size that causes the stimulation of *ob* gene expression. The mechanisms that produce appropriate transcription factors when fat cell size increases are not yet identified; they may involve metabolites of triacylglycerol, such as diacylglycerol and FFA (151). Cell stretching may also be a signal (5), because an increased tension exogenously applied on cells can induce signaling (148). *Ob* mRNA levels were found in mature adipocytes but not in stroma-vascular cells (166). No significant amount of *ob* mRNA was detected in the brain, heart, lung, liver, stomach, pancreas, spleen, small intestine, kidney, prostate, testis, colon, or skeletal muscle (166).

Thus the expression of the *ob* gene appears to be limited to adipose cells in humans. In obese subjects, *ob* gene expression is increased, and the rate of leptin production is directly related to adiposity (127). However, a large portion of the interindividual variability in plasma leptin concentration is not accounted for by differences in body fatness. Gender is an important factor determining plasma leptin, with women having markedly higher leptin concentrations than men for any given degree of fat mass (214). Furthermore, plasma leptin in women increases during the luteal phase of the menstrual cycle. Thus sex hormones play a role in the regulation of leptin secretion by adipose cells (104). Other yet unrecognized factors are likely to be involved. The rate of leptin clearance from plasma is independent of body mass and adiposity. Thus the elevated plasma leptin concentration associated with obesity (59, 160) is due to an upregulation of leptin production both by increased total body fat mass and by overexpression of the obese gene per unit of fat mass (57, 59, 106, 108, 153, 154). Regional differences in leptin production rates may exist. Masuzaki et al. (166) reported that the *ob* mRNA level in the subcutaneous adipose tissue was higher than in the omental, retroperitoneal, and mesenteric adipose tissues, but Lönnqvist et al. (153) found no statistically significant differences in *ob* expression between subcutaneous and omental adipose tissue in obese subjects.

An important question is to know whether an abnormal leptin synthesis due to a mutation of the *ob* gene exists in humans. No defect in the adipose tissue mRNA for leptin was found in more than 100 subjects (58). Thus, in most cases of obesity, this metabolic disorder does not result from a defect in the function of the *ob* gene in the adipose tissue. Recently, in two severely obese children, members of the same highly consanguineous pedigree, a homozygous frame-shift mutation involving the deletion of a single guanine nucleotide in codon 133 of the gene for leptin was found (171). The serum leptin levels of these two children were very low despite their elevated fat mass. Both children had a normal body weight at birth, but their weight deviated from predicted centiles by 3 or 4 mo of age. One of these children, at 2 yr of age, weighed 29 kg (>99.6th centile). Both children had a clear history of marked hyperphagia. Assessment of their energy expenditure has not been possible, but they were not hypothermic (mean body temperatures were within the normal range, 36–37°C). Fasting plasma glucose was normal in both children, but the 8-yr-old girl had elevated plasma insulin levels, indicating the presence of insulin resistance. Recently, another missense mutation in the leptin gene was described that induced low plasma leptin and morbid obesity in three affected members of a Turkish family (241). Congenital deficiency of leptin in humans results in a phenotype very similar to that of *ob/ob* mice (marked obesity, hyperinsulinemia, insulin resistance, and hyperphagia). This study shows that leptin must critically influence energy balance and body weight regulation in prepubertal humans. However, this genetic alteration is a very rare mutation, since it has not been observed in a large number of obese subjects (56, 106, 153, 166) before the publication by Montague et al. (171).

In other disorders of body weight regulation, such as anorexia nervosa, both serum and cerebrospinal fluid (CSF) leptin levels correlate with the body mass index of the patients (80, 163). Interestingly, CSF-to-serum leptin ratio was highest before weight gain in anorexia nervosa patients and decreased as the patients gained weight, suggesting that CSF leptin may contribute to reduce food intake in patients with anorexia nervosa (163).

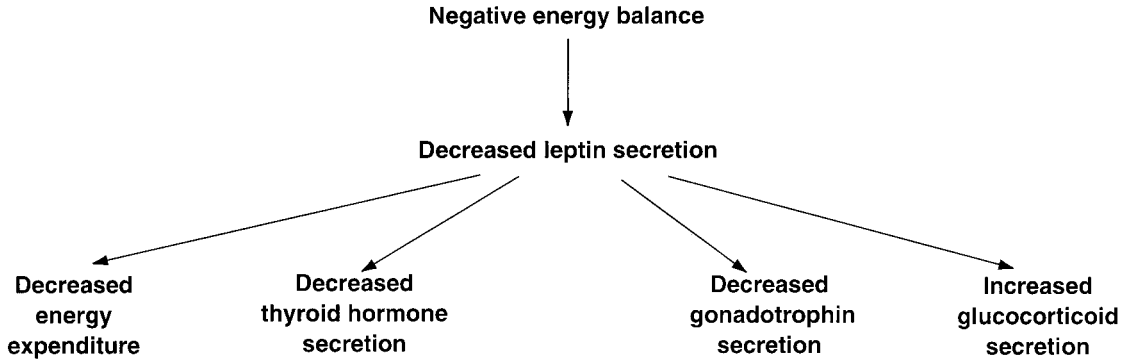

FIG. 5. Schematic representation of effects of leptin in response to a condition of negative energy balance.

H. Short-Term Changes in Leptin Production in Humans

Serum leptin concentration is not only dependent on the size of adipose tissue mass, since fasting decreases leptin concentration without marked changes in the body lipid content. A decrease of 10% in body weight was associated with 53% reduction in serum leptin (59). This large change in serum leptin concentration in the presence of a small reduction in adipose tissue mass suggests that leptin secretion is regulated by factors unrelated to adipose tissue mass. One important factor is caloric intake; a reduced energy intake is accompanied by a lower fasting serum insulin concentration, which may alter serum leptin secretion both in experimental animals and in humans (23, 131, 255). The decline in leptin levels could be responsible for the decrease in energy expenditure that is induced by weight loss (144). The decrease of leptin expression and levels in starvation leads to energy conservation by decreasing thyroid hormone-induced thermogenesis and gonadotrophin secretion while at the same time increasing secretion of glucocorticoids that mobilize energy stores (4, 88, 141, 279). Thus adaptation to fasting seems to require a sharp decline in leptin levels (Fig. 5).

In contrast to experiences in rats, the postprandial rise in serum insulin concentration is not associated in humans with changes in serum leptin levels (59). It was reported (235) that leptin in humans is secreted in circadian rhythms with a nocturnal rise over daytime secretion. These results confirm that the changes in leptin plasma concentrations during a 24-h period are not influenced by meal ingestion and meal-related increases in circulating insulin concentration. Leptin plasma concentrations are low around noon and begin to rise toward 3 P.M.; they reach maximal values during the night. The nocturnal increase in plasma leptin concentration precedes the early morning rise of ACTH and cortisol (273). Thus the rise in plasma leptin does not result from induction of *ob* gene expression by cortisol. It is of interest, however, to mention that in vitro, experiments in human adipose cells show that cortisol is a stimulator of leptin gene expression and may potentiate the effect of insulin on leptin production (265).

While in rats the short-term rhythmicity of adipose tissue *ob* mRNA expression is related to increases in insulin plasma levels due to food intake (217), this does not occur in humans. A 3-h euglycemic hyperinsulinemic clamp did not increase leptin mRNA levels in human adipose tissue (262), showing that *ob* gene expression is not acutely regulated by insulin in human subjects (65). To test whether insulin may exert a long-term effect on *ob* gene expression, Kolaczynski et al. (132) confirmed that a 5-h euglycemic hyperinsulinemic clamp with insulin infusion rates up to 1,200 $mU \cdot m^{-1} \cdot min^{-1}$ had no effect on circulating levels of leptin, but during prolonged hyperinsulinemic clamps, a rise in leptin concentration was observed after 48 h of hyperinsulinemia. In addition, biopsies of subcutaneous abdominal adipose tissue were incubated in the presence of hyperinsulinemia (100 nM). There was no effect on leptin release into the medium up to 72 h; however, a twofold increase in leptin release to the medium was observed by 96th hour of culture. This increase in leptin release was preceded by a 200% insulin-induced increase in *ob* gene expression. Therefore, in humans, insulin does not seem to act directly on *ob* gene expression; insulin appears to act more through a trophic effect on adipocytes (162).

The control of human leptin expression is more related to adipocyte hypertrophy and hyperplasia (154) than to acute changes in plasma insulinemia (106). It is therefore unlikely that leptin plays a role as an acute satiety factor, since its release into the circulation is not affected by meal sizes. Another condition that results in a change in adipose tissue *ob* gene expression is short-term (36-h) fast, which was associated with a decrease in plasma leptin concentration (131). Refeeding the subjects restored plasma leptin to baseline values. The reduction of *ob* gene expression during fasting was not correlated with the decrease in plasma insulin concentration or with the rise of β-hydroxybutyrate concentration. The mecha-

nisms of the inhibition of *ob* gene expression during fasting are not yet known. It was, however, reported that low-rate infusions of glucose during fasting, which prevented the decrease in plasma glucose and insulin concentrations, also prevented the drop in plasma leptin concentrations (23). After a sustained weight loss due to an ad libitum low-fat diet, plasma leptin concentration decreased in parallel with plasma insulin (108).

I. Resistance to Leptin Action in Humans

In obese individuals, the high plasma leptin levels do not induce the appropriate expected responses, i.e., a reduction in food intake and an increase in energy expenditure. If these responses were present, we would expect a weight loss and a correction of the obese state. It appears, therefore, that obese humans are resistant to the effects of endogenous leptin (59). Among the possible mechanisms that can result in leptin resistance, one may consider a defect in the transporter system that facilitates the transport of leptin, a 146-amino acid protein, through the blood-brain barrier (BBB) (12). The receptor Ob-Ra, expressed in choroid plexus, that acts to transport leptin to the CSF (140), could be altered. Caro et al. (44) showed that leptin enters the brain by a saturable transport system. Schwartz et al. (227) demonstrated that the efficiency of leptin uptake, measured as CSF-to-plasma ratio, was lower in obese than in lean individuals. If the leptin concentration in the CSF is similar to the hypothalamic interstitial leptin concentration, this transport defect may explain why obese individuals do not have the expected responses (such as a reduction in food intake and an increase in resting energy expenditure) to their hyperleptinemia. There is a threshold plasma leptin concentration (~25 ng/ml) above which the uptake of leptin into the CSF does not increase anymore in spite of high values of leptinemia. Thus, in patients with morbid obesity, an increase in leptin production by the enlarged fat mass would be futile (59).

It has been suggested that hyperleptinemia might downregulate the leptin transporters in *db/db* mice (158, 161). Another implication is that the use of leptin to treat obesity might be ineffective, if endogenous leptin has already saturated its transporters. The transport system that mediates delivery of circulating leptin to brain cells may involve leptin binding sites in the choroid plexus and leptomeninges (69, 158, 161, 251). These leptin receptors allow the distribution of leptin into the CSF, but they may not be involved in the delivery of circulating leptin into brain interstitial fluid that bathes hypothalamic receptors. Golden et al. (102) demonstrated that a leptin receptor functions at the brain capillary endothelium that comprises the BBB. Therefore, the BBB leptin receptor might function in parallel with the leptin receptor at the choroid plexus epithelium, which comprises the blood-CSF barrier. The transport system of leptin through the BBB is also saturable, as shown by studies of ^{125}I-leptin transport into brain in vivo in the mouse (12). Further studies are needed to determine the activity of the BBB leptin receptor in obesity models and in human obesity.

Resistance to leptin is also observed in a diet-induced model of obesity (93). Obesity was induced in two strains of mice by exposure to a 45% fat diet up to 56 days (260). Peripherally administered leptin inhibited food intake in both strains after 4 days of exposure to a high-fat diet, but the mice became resistant to peripheral leptin administration after 16 days of a high-fat diet. In contrast, a leptin dose 4,000 times smaller (i.e., 0.1 μg) given directly into the CNS through intracerebroventricular cannula was very active in inhibiting food intake and decreasing body weight in these diet-induced obese mice. These results support the hypothesis that the transport system that allows leptin to enter the brain is saturable (12). Thus diet-induced obese mice show a time-dependent development of resistance to peripherally administered leptin, whereas these animals are responsive to centrally administered leptin. These results suggest, if applicable to humans, that obese individuals who exhibit resistance to the effects of their elevated endogenous leptin may respond to a leptin analog that can penetrate into the CNS (260).

Leptin circulates both in bound and free forms. Curiously, a significantly higher proportion of leptin circulates in the bound form in lean compared with obese subjects (236). In obese individuals, the majority of leptin circulates in the free form, the bioactive protein, but obese subjects are nevertheless resistant to leptin effects. In obesity, it is possible that the serum leptin-binding sites are saturated. With increasing circulating leptin levels, leptin may "spill over" into the free pool (116). A possible role for binding proteins could be to facilitate the transport of leptin across the BBB to its hypothalamic sites of action. Further studies are needed to isolate the binding leptin proteins and to assess their possible role in modulating leptin actions.

Another possible site of leptin resistance in humans could be a defect located in the hypothalamic leptin receptor. The hypothalamic leptin receptor has several alternatively spliced forms (140), as illustrated by the abnormally spliced Ob-Rb receptor, expressed in the hypothalamus of *db/db* mice. The mutation of the *db* gene generates a truncated version of the Ob-Rb receptor, lacking most of the intracellular domain (49). Thus an abnormal splicing of the Ob-Rb transcript in the obese *db/db* mouse is associated with obesity. In addition, the *fa/fa* Zucker rat presents a missense mutation that lies within the rat Ob-Rb receptor (192). This mutation causes obesity in the Zucker (*fa/fa*) rat (51), and, as a consequence of the mutated receptor, the expression of the *ob* gene is

markedly augmented in adipose tissue of this animal (180).

These examples show that a variety of leptin receptor defects may result in obesity in genetic models of obesity. The search for possible variations in the human hypothalamic Ob receptor should indicate whether such mutations may explain the development of obese phenotypes in humans. Considine et al. (56) recently studied the expression of hypothalamic Ob receptor in lean and obese individuals. The full-length leptin receptor, as identified by Tartaglia et al. (251), was expressed in human hypothalamus. There was no difference in the amount of leptin-receptor mRNA between lean and obese individuals, and there was no correlation between leptin-receptor gene expression and body mass index. In addition, no abnormal splicing of the human Ob hypothalamic receptor was observed. More specifically, the authors were unable to detect the insertion of an additional 106-bp nucleotide sequence in the PCR product of the Ob receptor gene derived from the obese subjects, which rules out the possibility that the *db/db* mouse leptin-receptor defect is commonly present in human obesity. The *fa/fa* rat mutation, a single-base substitution that results in an amino acid change (192), was not detected in any of the obese subjects studied (56). Although sequence variations were detected in several regions of the human leptin receptor, most variations were single-base substitutions that did not result in a change of amino acid. There was a single base substitution that was detected in most obese and lean individuals, a change of a glutamine for an arginine in the leptin-receptor protein. It is not likely that this polymorphism results in leptin resistance, because this change was common in lean and obese subjects, and most subjects were heterozygous for the base change. Recently, a homozygous mutation in the human leptin receptor gene that results in a truncated leptin receptor lacking both the transmembrane and the intracellular domains was reported in three girls (53). This mutation resulted in an early-onset morbid obesity and a lack of pubertal development. These rare cases of morbid obesity show that leptin plays a role in the regulation of body weight in humans (183).

Because most cases of human obesity are not associated with an impaired leptin production or altered Ob receptors, a defect could lie in the leptin signaling cascade. It was mentioned above that a role of leptin is to decrease NPY in normal animals (227, 240). A defect in leptin signaling due to leptin resistance induces overexpression of hypothalamic NPY in *db/db* mice, a mechanism that is implicated in the pathogenesis of the obesity syndrome (227). Whether a similar mechanism may exist in humans is not known. It is, however, not certain whether NPY is the leptin transducer system because mice deficient for NPY have normal food intake and body weight (74). Maintenance of energy balance is so fundamentally important for the survival of animals and humans that such an important regulation is dependent on a multidimensional system with overlapping control pathways (45). The fact that many peptides influence food intake supports this concept (35, 37); therefore, a pharmacological approach for decreasing food intake that consists in inhibiting a single pathway is bound to have a limited effect on body weight regulation.

J. Does Leptin Play a Role in Human Obesity?

The importance of leptin in the regulation of body weight in humans is still far from being understood. Leptin is not an acute satiety factor, since its plasma concentration does not change after eating. Leptin plasma levels appear to represent a long-term integrative signal of the size of the adipose tissue mass; this signal can be sensed by hypothalamic leptin receptors and thus serves as a message proportional to energy stores that can be received and integrated at regulatory sites in the CNS.

If the leptin signal is "too small" for the regulatory sites, one might expect that body weight may rise until the leptin signal corresponds to a "set point" value. This hypothesis was tested by Ravussin et al. (203), who showed that relatively low plasma leptin concentrations precede weight gain in Pima Indians. Individuals with relatively low plasma leptin concentrations may have less inhibitory effects on food intake. They tend to overeat and thus increase their body fat mass until the resulting increase in plasma leptin concentration reaches a level that suppresses further overeating by acting on hypothalamic regulatory centers. Recently, Surwit et al. (248) showed that in A/J and B/6 mice, there was a direct relationship between the ability to increase plasma leptin levels in response to a high-fat diet and the resistance to the development of obesity.

Another mechanism by which increased plasma concentrations of leptin may contribute to energy balance in individuals who overeat is through an increase in energy expenditure. There is strong evidence in rodents that leptin stimulates energy expenditure in brown adipose tissue. Leptin administration to obese mice made deficient in brown adipose tissue was ineffective in reducing weight, suggesting that activation of brown adipose tissue thermogenesis is central in leptin actions in rodents. The existence and functional significance of brown adipose tissue in humans are, however, controversial. If this tissue is really virtually absent in humans, several of the actions of leptin reported in mice and rats may indeed not apply to humans. No relationship between plasma leptin concentration and resting energy expenditure (normalized for body composition) has been reported in humans, suggesting that leptin does not affect basal energy-consuming processes.

Salbe et al. (218), however, found in 5-yr-old children that plasma leptin concentrations correlated with total energy expenditure, independently of the percent of body fat. Yet, leptin was not correlated with resting energy expenditure in these children. This led to the conclusion that children who were more physically active had higher plasma leptin concentrations. These findings support the concept that leptin may play a role in the control of energy expenditure in humans by a central stimulation of physical activity.

Many investigators reported that leptin is secreted by adipocytes in proportion to their TG stores, which constitutes a long-term stable signal for leptin brain receptors. In addition, there are also short-term changes in plasma leptin levels that occur with restriction of energy intake over a few days; the changes in leptin expression are out of proportion to changing fat stores (108, 130–132, 198). Therefore, factors that are extrinsic to the adipocyte can regulate leptin gene expression both in vitro and in vivo; these include insulin (22, 108, 132, 162) and glucocorticoids (237) that stimulate leptin mRNA synthesis as well as adrenergic receptors agonists that inhibit leptin gene expression (165). In humans, the decrease in leptin and insulin concentrations with sustained weight loss correlated significantly, independent of changes in body fat (108). Thus reduced insulin concentration and improved insulin sensitivity may be responsible for the reduction in plasma leptin concentration that accompanies weight loss. This mechanism of control of leptin production is supported by the observations that prolonged changes in plasma insulin concentration are necessary to elicit changes in plasma leptin concentration (132, 259).

The present evidence shows that leptin is neither an acute satiety factor, since its production does not increase after meal ingestion, nor a precise indicator of the adipose tissue mass, since plasma leptin concentration decreases relatively more after energy restriction than the reduction in the fat mass. Therefore, the simple view that leptin is part of a closed loop that informs the brain how much fat the body has (45) does not take into account short-term changes in plasma leptin concentrations due to either energy restriction (108) or to energy overfeeding (131).

It is likely that the responsiveness to leptin may vary according to metabolic conditions or to genetic background. In animal models of obesity, obese *ob/ob* mice with a lack of leptin production are very sensitive to leptin administration, whereas obese *db/db* mice with elevated plasma leptin concentration are unresponsive to exogenous leptin because they lack functioning leptin receptors (49, 51, 140). The DIO mice and the normal mice are moderately responsive to exogenous leptin administration. Caro et al. (45) suggested that the majority of obese humans should respond to leptin administration in a similar manner as DIO mice. A very small group of very severe obese individuals with a lack of leptin production (171) should be very sensitive to exogenous leptin administration. Whether metabolic or hormonal conditions may modify leptin responsiveness in animals and in humans is of particular interest and needs to be further studied.

VI. OTHER GENES IMPLICATED IN THE PATHOGENESIS OF ANIMAL OR HUMAN OBESITY

Other genetic alterations than those of the leptin signaling pathway have been identified, which led to the development of excess body weight in rodents or in humans. The yellow (A y/a) mouse is characterized by alterations of hair pigmentation as well as by the development of obesity and impaired glucose metabolism. The mutated gene responsible for this phenotype is the agouti locus, which encodes for a 131-amino acid peptide called agouti signaling protein (ASP). The mutation affects the gene promoter and leads to ectopic overexpression of functionally and structurally unaltered ASP (278). At the level of the hair follicle, ASP increases the synthesis of pheomelanin, which is responsible for the yellow color of these animals. This action is secondary to the blockade of the action of MSH at the level of its receptor MC1R. The development of obesity can be attributed to overexpression of ASP in the brain, which interferes with the action of α-MSH on a distinct melanocortin receptor MC4R (78, 118). It appears, therefore, that excess ASP in the brain of A y/a mice interferes with signal generation by MSH at MC4R, a signal which normally inhibits food intake.

In another form of genetic obesity in the rat, the Fat mutation of the gene coding for carboxypeptidase E (CPE) has been identified (177). Carboxypeptidase E is involved in the cleavage of prohormones such as proinsulin or POMC. It is thought that mutation of CPE leading to loss of prohormone cleavage activity causes body weight gain by decreasing the synthesis of brain peptides acting as suppressors of food intake. Decreases in brain levels of α-MSH, GLP-1, CRH, or melanin concentrating hormone may possibly be involved (142). In humans, a case of genetically determined obesity secondary to a mutation of an enzyme implicated in prohormone cleavage has been recently described. Homozygous mutation of the prohormone convertase 1 gene leads to early massive obesity, hypoglycemia, as well as to hypogonadism and hypocortisolism. Increased proinsulinemia secondary to impaired cleavage of proinsulin is thought to lead to hypoglycemia due to insulin-like activity. The development of obesity may be secondary to impaired production of α-MSH and/or GLP-1 from POMC and proglucagon, respectively (119, 184).

Such single-gene mutations appear to be exceptionally at the origin of human obesity. Their identification in

rodents and humans, however, allows us to gain invaluable insights into the mechanisms responsible for the control of body weight, and it can be foreseen that the study of other genetic mutations will further extend the number of brain peptides involved in the control of food intake and/or energy expenditure. The tubby mutation, which leads to the relatively late development of obesity in affected mice (55), may be such an opportunity. The gene mutated in tubby mice codes for a protein of a novel class, the function of which remains unknown presently, and which is expressed at high levels in the hypothalamus (128). It can therefore be expected that elucidation of the role of this peptide, or of peptides coded by other genes yet to be discovered at the origin of obesity, will greatly enhance our understanding of body weight homeostasy in the future.

VII. CONCLUSIONS

The regulation of body weight requires long-term regulation of energy balance. It is important to emphasize that many individuals, whether lean or obese, maintain their body weight within small limits during long periods of time. If energy intake exceeded expenditure by 1% daily for 1 yr, then the result would be a storage of ~9,000 kcal or ~1.15 kg of adipose tissue (212). The mean weight gain by the average American man or woman between the ages of 25 and 55 years is ~9 kg, which represents a mean excess of ~0.3% of ingested calories over energy expenditure (212).

This high precision of the control of energy balance is achieved by several regulatory loops. Weight regulation is characterized by its integrated and redundant nature. Many pathways participate in homeostatic responses that tend to maintain adequate fuel stores. The combined responses that control energy intake and energy expenditure to maintain energy homeostasis have conferred a survival advantage during human evolution. Now, food availability has increased in many countries and advances in technology and transportation have reduced the need for physical activity in daily life. These two factors pose a great challenge for body weight regulation and are probably the main reasons that account for the increasing prevalence of obesity worldwide. The problem is that the impact of these factors on obesity prevalence cannot be proven by adequate data, because both dietary intake and physical activity are difficult to measure on a population-wide scale. In addition, the mean daily imbalance that can lead to obesity over a period of several years is very small and beyond the range of measurement precision of available methodology.

The question of great practical interest is to know whether the recent developments following the characterization of obesity-associated gene products that has revealed new biochemical pathways and molecular targets for pharmacological interventions will lead to new successful treatments of human obesity. Until now, the pharmacological approach to treat obesity has been a failure. New antiobesity drugs are being developed based on the recent advances in molecular biology. One new target is to act on leptin receptors or on the leptin signaling pathway. A drug that stimulates the leptin pathway may contribute to suppress appetite, increase metabolic rate, and reduce the amount of body fat (42). Because obesity may result from reduced hypothalamic responsiveness to leptin, a low-molecular-weight drug that goes through the BBB and acts on leptin receptors or on the leptin signaling pathway might be an interesting therapeutic approach. Another potential target is the development of antagonists of NPY receptor subtypes that mediate the effects of NPY on food intake. The increase of energy expenditure by stimulating the synthesis of UCP-2 and UCP-3 is another potential target to treat obesity (28, 87, 263).

Although new antiobesity drugs may contribute to improve the treatment of obesity, it is important to emphasize the main physiological characteristics of body weight regulation; variations in energy balance are mainly reflected by the difference between fat intake and fat oxidation. With de novo lipogenesis not being an important pathway in humans under conditions of ad libitum food intake, body weight gain almost entirely results from the deposition of dietary fat in adipose tissue. It is, therefore, a research priority to identify factors that determine fat intake and those that control fat oxidation.

Further research is needed in the field of the control of food intake. The satiating capacity of protein and carbohydrate appears to be greater than that of fat. Energy intake is related to the meal energy density, and obviously the fat content of a meal is a major determinant of its energy density. Thus energy-dense diets favor overconsumption of energy, and a high-fat diet does not promote fat oxidation. The logical conclusions for the prevention and the treatment of obesity are *1*) to reduce the energy density of the everyday diet and *2*) to stimulate fat oxidation by promoting a sufficient level of physical activity.

Future perspectives in the field of body weight regulation research should aim to link the genetic approach with metabolic studies on fat balance. A major challenge is to elucidate whether individuals who maintain a normal body weight and body composition in spite of eating an energy-dense diet have specific genetic markers. The control of lipid mobilization and the fuel partitioning between fat and carbohydrate oxidation at the cellular levels need to be studied further. A new approach in the pharmacological treatment of obesity may consist of stimulating fat oxidation while sparing carbohydrate stores. This may delay the feeling of hunger and contribute to reduce energy intake.

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