Leptin

Leptin (Greek *leptos* meaning thin) is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin is one of the most important adipose derived hormones.

Leptin is expressed predominantly by adipocytes, which fits with the idea that body weight is sensed as the total mass of fat in the body. Smaller amounts of leptin are also secreted by cells in the epithelium of the stomach and in the placenta. Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as in T lymphocytes and vascular endothelial cells.

Physiologic Effects of Leptin

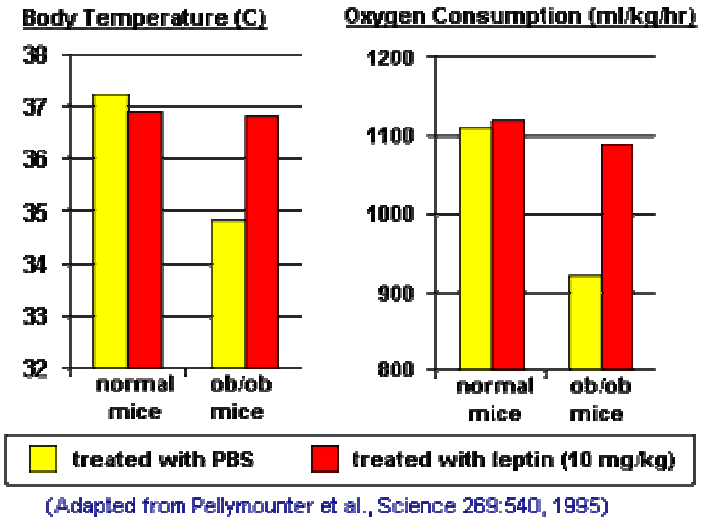
Regulation of Food Intake, Energy Expenditure and Body Weight

Leptin in an important component in the long term regulation of body weight. Genetically obese mice with inactivating mutations in the ob gene or the gene encoding the leptin receptor (db gene) have been known for many years and were instrumental in the initial cloning of the ob gene. Recent studies with obese and non-obese humans demonstrated a strong positive correlation of serum leptin concentrations with percentage of body fat, and also that there was a higher concentration of ob mRNA in fat from obese compared to thin subjects. It appears that as adipocytes increase in size due to accumulation of triglyceride, they synthesize more and more leptin. *In essence, leptin provides the body with an index of nutritional status.*

Leptin's effects on body weight are mediated through effects on hypothalamic centers that control feeding behavior and hunger, body temperature and energy expenditure. Soon after cloning the ob gene, its cDNA was expressed as protein in E coli and preliminary assessment of its effects undertaken. Daily injections of recombinant

mouse or human leptin into ob/ob mice (i.e. the obese mutants unable to synthesize leptin) led to a dramatic reduction in food intake within a few days, and to roughly a 50% reduction in body weight within a month. As depicted in the graph below, weight loss resulting from administration of leptin appears to result from a combination of at least two fundamental effects:

- Decreased hunger and food consumption, mediated at least in part by inhibition of neuropeptide Y synthesis. Neuropeptide Y is a very potent stimulator of feeding behavior.
- Increased energy expenditure, measured as increased oxygen consumption, higher body temperature and loss of adipose tissue mass.



(Adapted from Pellymounter et al., Science 269:540, 1995)

As expected, injections of leptin into db/db mice, which lack the leptin receptor, had no effect. When leptin was given to normal mice, they lost weight, showed profound depletion of adipose tissue and manifest increases in lean mass.

The mechanisms by which leptin exerts its effects on metabolism are largely unknown and are likely quite complex. In contrast to dieting, which results in loss of both fat and lean mass, treatment with leptin

promotes lipolysis in adipose tissue, but has no apparent effect on lean tissue.

The effects of leptin were observed by studying mutant obese mice that arose at random within a mouse colony at the Jackson Laboratory in 1950. These mice were massively obese and hyperphagic. Leptin itself was discovered in 1994 by Jeffrey M. Friedman and colleagues at the Rockefeller University through the study of those mutant mice. The *Ob(Lep)* gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans. Leptin is produced by adipose tissue and interacts with six types of receptor (LepRa–LepRf). LepRb is the only receptor isoform that contains active intracellular signaling domains. This receptor is present in a number of hypothalamic nuclei. Leptin binds to the ventromedial nucleus of the hypothalamus, known as the "**appetite center**." Leptin signals to the brain that the body has had enough to eat, or satiety. A very small group of humans possess homozygous mutations for the leptin gene which leads to a constant desire for food, resulting in severe obesity. This condition can be successfully treated by the administration of recombinant human leptin.

Thus, circulating leptin levels give the brain input regarding energy storage so it can regulate appetite and metabolism. Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and by increasing the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH). The NPY neurons are a key element in the regulation of appetite; small doses of NPY injected into the brains of experimental animals stimulates feeding, while selective destruction of the NPY neurons in mice causes them to become anorexic. Conversely, α-MSH is an important mediator of satiety, and differences in the gene for the receptor at which α-MSH acts in the brain are linked to obesity in humans.

Leptin is also regulated (downward) by melatonin during the night. Brazilian researchers found in 2004 that, in the presence of insulin,

"melatonin interacts with insulin and upregulates insulin-stimulated leptin expression."

Mechanism of action

It is unknown whether leptin can cross the blood-brain barrier to access receptor neurons, because the blood-brain barrier is somewhat absent in the area of the median eminence, close to where the NPY neurons of the arcuate nucleus are. It is generally thought that leptin might enter the brain at the choroid plexus, where there is intense expression of a form of leptin receptor molecule that could act as a transport mechanism.

Once leptin has bound to the Ob-Rb receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, presumably, effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible for increasing appetite. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them.

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin. These people are said to be resistant to the effects of leptin, in much the same way that people with type 2 diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some point so the body doesn't adequately receive the satiety feeling subsequently to eating.

In mice, leptin is also required for male and female fertility. In mammals, humans, puberty in females is linked to a critical level of body fat. When fat levels fall below this threshold (as in anorexia), the ovarian cycle stops and females stop menstruating.

Leptin is also strongly linked with angiogenesis, increasing VEGF levels.

Leptin and reproduction

Reproductive Function

It has long been known that starvation adversely affect reproductive function. For example, very low body fat in human females is often associated with cessation of menstrual cycles, and similar effects are seen in starving or nutritionally-deprived animals. Also, the onset of puberty is known to correlate with body condition as well as age.

Leptin concentrations are low in people and animals with low body fat, and leptin appears to be a significant regulator of reproductive function. These effects are probably due in part to the ability of leptin to enhance secretion of gonadotropin-releasing hormone, and thus luteinizing and follicle-stimulating hormones from the anterior pituitary.

One of the first demonstrations of leptin's effect on reproduction dealt with onset of puberty. Prepubertal mice treated with leptin became thin, as one would expect, but also reached reproductive maturity and began cycling significantly earlier than control mice. Additionally, some humans with inactivating mutations in the leptin receptor gene not only are obese, but fail to achieve puberty.

The body's fat cells, under normal conditions, are responsible for the constant production and release of leptin. This can also be produced by the placenta. Leptin levels rise during pregnancy and fall after parturition (childbirth). Leptin is also expressed in fetal membranes and the uterine tissue. Uterine contractions are inhibited by leptin.

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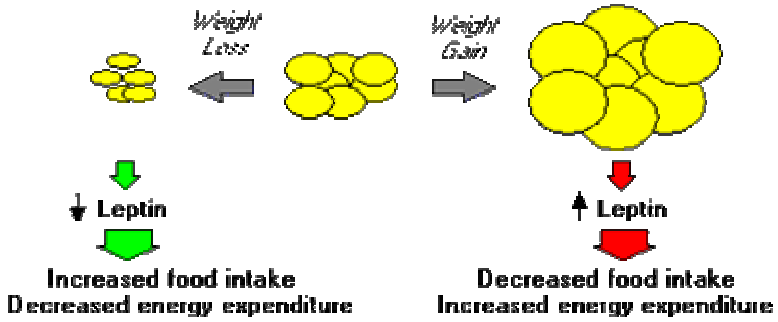
Control of Leptin Synthesis and Secretion

The amount of leptin expressed by adipocytes correlates well with the lipid content of the cells. Once synthesized, leptin is secreted through a constitutive pathway and not stored in the cell.



At this time, the mechanisms responsible for regulating leptin expression in adipocytes are unknown. It is likely that a number of hormones modulate ob gene expression, including glucocorticoids and insulin.

Recent discoveries

Professor Cappuccio of the University of Warwick has recently discovered that short sleep duration may lead to obesity through an increase of appetite via hormonal changes. Lack of sleep produces ghrelin which stimulates appetite and leads to less leptin to suppresses appetite.

Next to a biomarker for body fat, serum leptin levels also reflect individual energy balance. Several studies have shown that fasting or following a very low calorie diet (VLCD) lowers leptin levels. It might be that on short term leptin is an indicator of energy balance. This system is more sensitive to starvation than to overfeeding, i.e. leptin levels do not rise extensively after overfeeding. It might be that the dynamics of leptin due to an acute change in energy balance are related to appetite and eventually in food intake. Although this is a new hypothesis, there is already some data that supports it.

There is some recognition that leptin action is more decentralized than previously assumed. In addition to its endocrine action at a distance (from adipose tissue to brain), leptin also acts as a paracrine mediator. In fetal lung leptin is induced in the alveolar interstitial fibroblasts ("lipofibroblasts") by the action of PTHrP secreted by formative alveolar epithelium (endoderm) under moderate stretch. The leptin from the mesenchyme in turn acts back on the epithelium at the leptin receptor carried in the alveolar type II pneumocytes and induces surfactant expression which is one of the main functions of these type II pneumocytes. In addition to white adipose tissue -the major source of leptin, it can also be produced by brown adipose tissue, placenta (syncytiotrophoblasts), ovaries, skeletal muscle, stomach (lower part of fundic glands), mammary epithelial cells, bone marrow, pituitary and liver.

There is also evidence that leptin plays a role in hyperemesis gravidarum (severe morning sickness), in polycystic ovary syndrome

and a 2007 research suggest that hypothalamic leptin is implicated in bone growth.

The mechanisms by which leptin exerts its effects on metabolism are largely unknown and are likely quite complex. In contrast to dieting, which results in loss of both fat and lean mass, treatment with leptin promotes lipolysis in adipose tissue, but has no apparent effect on lean tissue.

Blood concentrations of leptin are usually increased in obese humans, suggesting that they are in some way insensitive to leptin, rather than suffering from leptin deficiency. Mutations in ob or db genes appear to be a very rare cause of morbid obesity in humans, but both have been described. The effect of such mutations on body weight is dramatic.

Modulation of T cells activity in immune system

The important role of Leptin/Leptin receptors were shown in experimentation with mice. It modulates the immune response to atherosclerosis, which is a predisposing factor in patients with obesity. In addition to its effect on the hypothalamus, leptin acts directly on

- cells of the liver and skeletal muscle where it stimulates the oxidation of fatty acids in the mitochondria. This reduces the storage of fat in those tissues (but not in adipose tissue).
- T cells where it enhances the production of Th1 cells promoting inflammation. Mice without leptin are protected from autoimmune disease (which may account for the reports that restricting food intake helps humans with rheumatoid arthritis).

Mutations in the gene for leptin, or in its receptor, are rarely found in obese people. The results of trials of *recombinant leptin* in obese humans who do not have mutations in both their leptin genes so far has not shown any great benefit in weight reduction.

Lipodystrophy

Lipodystrophy is the term given for a condition (very rare) in which the person cannot manufacture adipose tissue. With no fat cells, these people do not make leptin, but of course cannot become obese as a result. They do, however, suffer some problems — most often Type II diabetes (NIDDM). Treatment with recombinant leptin helps them.

Resistin

Resistin causes tissues — especially the liver — to be less sensitive to the action of insulin, which is the hallmark of Non Insulin-Dependent Diabetes Mellitus (NIDDM) ("Type 2" diabetes). Blood glucose levels rise because of increased glycogenolysis and gluconeogenesis in the liver.

In humans, resistin is primarily a product of macrophages, not fat cells. Nevertheless, there is a strong association in humans between elevated levels of resistin, obesity, and Type 2 diabetes (over 80% of the people with NIDDM are obese).

Retinol Binding Protein 4 (RBP4)

This protein (of ~180 amino acids) is responsible for the transport of retinol (vitamin A) in the blood.

When it is secreted in elevated amounts by fat cells, it

- suppresses glucose uptake by skeletal muscle;
- enhances glucose release by the liver.

These actions counteract those of insulin. Elevated levels of RBP4 occur in humans with Type 2 diabetes mellitus (NIDDM).

The World Health Organization has now classified obesity as a disease. It is often said that obesity is the biggest health problem facing the developed world today. It causes health problems such as

hypertension, type II diabetes, heart attacks and strokes, elevated cholesterol and many more. Obesity is said to lead to 30,000 premature deaths each year and it is shortening the lives of people by an average of nine years.

Redux, in the 1980's which sold 2 million prescriptions within the first 6 months of its launch in the US and which went on to be sold in 65 countries suppressed the appetite. However it was eventually taken off the market because of the effects it had on the heart. There are now only 2 drugs on the market in the UK- Xenical, which slows absorption of fat and Reductil, which suppresses the appetite. Both of these are prescription drugs, can have side affects and can only reduce weight slightly.

Several hormones are responsible for our eating habits. For example; Leptin and alpha-MSH are both appetite represent. Cannaboids, neuropeptide Y, ghrelin and anandamid are all feeding stimulants.

One of the hormones being researched for this reducing weight is Leptin. Leptin is an appetite suppressant. It stops you eating too much as well as makes you more active so you burn off more energy. The amount of Leptin found in people increases as their body fat increases. There is also a higher concentration of mRNA in fat from obese compared to thin subjects. Leptin acts on receptors in the hypothalamus of the where the theory is that as you get fatter you also get less sensitive to the affects of Leptin.

Leptin works on the body in the following ways;

- counteracts the effects of neuropeptide Y (feeding stimulant secreted by cells in the gut wall and in the hypothalamus);
- counteracts the affects of anandamid (another feeding stimulant that binds to the same receptors as THC the active ingredient of marijuana)
- promotes the effects of alpha-MSH a appetite suppressant resulting in inhibition of food intake

- it also stimulates secretion of reproductive hormones such as gonadotrophin-releasing hormone and thus luteinizing the follicle stimulating hormone from the anterior pituitary.
- it raises the temperature of the subject so energy expenditure is increased

In rare cases the gene that produces leptin or its receptors mutates. This can cause severe obesity and diabetes in certain individuals as well as in certain cases failure to reach puberty. However, it has been observed that most people who are obese do not have a defective ob gene.

It was previously reported that consumption of high-fat meals, which produce smaller postprandial glucose and insulin responses than equicaloric high-carbohydrate meals, reduces 24-h circulating leptin concentrations in humans. This reduction of leptin concentrations is likely as the result of decreased insulin-mediated glucose metabolism in adipose tissue. Because insulin and leptin function as key signals conveying information on energy intake and body fat stores to the central nervous system (CNS) for the long-term regulation of food intake and energy homeostasis, it is possible that reduced insulin and leptin production contributes to increased energy intake, weight gain, and obesity in humans consuming high-fat diets. In contrast, high-carbohydrate, low-fat diets are known to induce weight loss, even when consumed *ad libitum*.

However, not all types of dietary carbohydrate are likely to have the same effect on these signals of peripheral energy status. Fructose, unlike glucose, does not stimulate insulin secretion from pancreatic ß-cells. In rhesus monkeys, an 8-h iv fructose infusion resulted in markedly reduced insulin secretion and did not increase circulating leptin concentrations compared with infusion of the same amount of glucose, which increased plasma leptin levels by more than 50% above baseline fasting levels. Thus, similar to fat, fructose does not increase insulin-mediated glucose metabolism or circulating leptin levels. Even a relative deficit in leptin production has been shown to

be associated with increased body adiposity in humans. In addition, it has recently been reported that an augmentation of the proportional amplitude (nadir to peak) of the 24-h diurnal pattern of circulating leptin concentrations was predictive of the extent of weight and body fat loss during a 12-wk *ad libitum* low-fat diet (15% of energy). Therefore, it is important to determine the effects of dietary fructose on meal-associated insulin secretion and the diurnal pattern of leptin production in humans. This is particularly relevant in light of the fact that per capita fructose consumption has increased during the past three decades within the same time frame as a marked increase in the prevalence of obesity.

Other factors, including a number of gastrointestinal hormones that are known to influence food intake and glucose homeostasis, could potentially contribute to a metabolic profile promoting increased body adiposity after fructose ingestion. Ghrelin, a recently discovered enteric hormone, is a potent orexigen and may play a role in the regulation of food intake and nutrient selection. Circulating ghrelin concentrations have not been examined in response to changes of dietary carbohydrate composition in human subjects, and the influence of fructose on ghrelin secretion is not known. In contrast, the acute effects of fructose on the two primary incretin hormones (insulinotropic peptides), glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), have been investigated. Typically, these hormones are released when glucose is ingested, but although acute fructose consumption has also been reported to stimulate GLP-1 release, GIP secretion is unaffected by fructose. However, circulating levels of these hormones have not been examined over the course of a day in which glucose and fructose are consumed in the context of mixed nutrient meals.

Circulating glucose, insulin, and leptin concentrations was examined as well as ghrelin, GLP-1 (the active form of GLP-1), and GIP over a 24-h period on 2 separate days during which the subjects consumed three isocaloric (between treatments) mixed nutrient meals accompanied by either glucose-sweetened [high glucose (HGl)] or

fructose-sweetened [high fructose (HFr)] beverages. Furthermore, because the hepatic metabolism of fructose is considered to favor lipogenesis and triglyceride (TG) synthesis (20), plasma TG and free fatty acid (FFA) levels were also measured.

The major aim of this study was to compare effects of fructose and glucose on endocrine signals involved in the regulation of body adiposity and energy metabolism, as well as glucose and lipid metabolism. In a previous study in normal-weight women, we reported that high-fat, low-carbohydrate meals produced smaller excursions of plasma insulin and glucose than low-fat, high-carbohydrate meals, resulting in reduced circulating leptin concentrations over a 24-h period. The results of the present study, also in young, normal-weight women, indicate that like fat, consuming fructose with a mixed meal results in substantially smaller postprandial plasma glucose and insulin excursions and attenuated circulating leptin profiles when compared with glucose.

Fructose does not stimulate insulin secretion, presumably because pancreatic ß-cells have low levels of the fructose transporter, glucose transporter 5. Thus, iv fructose infusion only marginally increases circulating insulin concentrations, and ingested fructose is ineffective in eliciting postprandial insulin secretion. When the effects of iv glucose and fructose infusion were compared in monkeys, plasma leptin concentrations were increased by 4 h after the start of the glucose infusion. In contrast, leptin did not increase during infusion of the same amount of fructose. We have reported that leptin production by adipocytes is regulated by insulin-mediated glucose metabolism. Accordingly, the reduction of circulating leptin in response to fructose infusion is likely the result of the smaller insulin and glucose excursions leading to less insulin-induced glucose utilization by adipose tissue. Thus, in the present study, as predicted, consumption of three high fructose meals resulted in reductions in the amplitude of the diurnal leptin pattern (peak – nadir) and of circulating leptin levels over 24 h when compared with HGl meals. The diurnal pattern of the circulating leptin may be an important

determinant of its biological effects because the proportional amplitude (percentage change) of leptin was predictive of the loss of weight and body fat during a 12-wk *ad libitum* low-fat diet.

The smaller postprandial excursions of circulating glucose and insulin after consumption of fructose beverages with meals may also contribute to the attenuated suppression of ghrelin secretion after subjects consumed HFr beverages, compared with HGl beverages with meals. Insulin and glucose have been shown to decrease circulating ghrelin in rodents and humans. Ghrelin has been the focus of considerable attention due to its potent effects to stimulate food intake in animals and humans. The suppression of ghrelin after meal ingestion is blunted in obese subjects compared with normal-weight subjects, and circulating ghrelin is markedly elevated in patients with Prader-Willi syndrome, a genetic disorder characterized by marked hyperphagia and obesity. Plasma ghrelin levels increase after diet-induced weight loss but remain dramatically reduced in patients after weight loss induced by gastric bypass surgery. The relative elevation of plasma ghrelin after fructose ingestion in the present study suggests that a failure of fructose to suppress ghrelin, along with reduced insulin and leptin, could contribute to decreased satiety and increased food intake during long-term fructose consumption.

In this study, we observed delayed and reduced GIP responses and prolonged GLP-1 responses when HFr beverages were consumed with each meal. Other investigators have reported that ingestion of fructose by itself does not stimulate GIP release. It is unlikely that the decreased GIP responses are due to delayed gastric emptying because fructose ingestion increases gastric emptying. We hypothesize that although consumption of the HGl beverages with a meal provides a rapid direct stimulus for GIP release, GIP release after the consumption of the HFr beverages with a mixed meal is dependent on delivery of the other nutrients in the meal to the intestine, resulting in an attenuated/delayed GIP response.

Although the effects of glucose and fructose in isolation on GLP-1 release have been reported, the effects of consuming the two sugars in combination with mixed meals over a 24-h period have not been compared. The results indicate that fructose ingestion prolonged the postprandial release of GLP-1. Given that we specifically measured the intact peptide, which is rapidly metabolized by dipeptidyl peptidase IV, it is likely that the prolonged GLP-1 responses are due to increased secretion rather than reduced clearance. It does not appear likely that either the observed delay in GIP release or increased GLP-1 release makes a major contribution as incretins to the differences in insulin secretion after glucose and fructose consumption because the temporal patterns of the hormones do not coincide with the insulin responses. Although GLP-1 responses to meals could potentially lead to decreased food intake, perhaps by delaying gastric emptying, the magnitude of difference is relatively small.

Ingestion of HFr beverages with meals resulted in elevated TG levels compared with HGl beverages. The increase in postprandial TG levels after fructose ingestion is likely to reflect differences in the hepatic metabolism of fructose and glucose. Fructose is phosphorylated by fructokinase to fructose-1-phosphate. Unlike the glycolytic metabolism of glucose via phosphofructokinase, fructokinase is not subject to feedback inhibition by cytosolic citrate and ATP. Thus, in contrast to glucose, when large amounts of fructose are ingested, the glycolytic pathway becomes saturated with the fructose carbon, and TG production is facilitated from increased carbon flux into both the glycerol and the acyl portions of TG. Decreased TG clearance could also contribute to the increased TG after fructose ingestion. The observed decrease in circulating leptin levels could be an additional mechanism by which fructose influences TG levels because leptin is known to promote fat use. Other investigators have reported that TG increases during more long-term consumption of diets high in fructose or sucrose compared with HGl or starch diets. We have reported that the effect of fructose to increase postprandial TG persists during 10 wk of fructose

consumption and that plasma level of the atherogenic lipoprotein, Apo-B, are also elevated after long-term fructose, but not glucose consumption. Thus, it is possible that the type of carbohydrate, specifically the fructose contained in added sugars, contributes to the known effect of high-carbohydrate diets to raise TG levels. Furthermore, existing hyperlipidemia and/or insulin resistance may predispose individuals to greater postprandial hypertriglyceridemia after consuming fructose.

The two main sources of fructose in the U.S. diet are sucrose, which consists of 50% fructose, and HFr-fructose corn syrup (HFCS), typically containing 55% fructose. It is estimated that fructose consumption has increased by at least 26% over the past three decades, primarily due to the increased use of HFCS in soft drinks and other beverages. The average per capita data for added fructose in 1997 from the combined use of sucrose and HFCS was 81 g/d. Individuals in the 90th percentile of fructose intake are estimated to consume between 1.5 and 2.5 times the mean intake. In the present study, the amount of fructose consumed was 45 g at each meal, which is approximately the same amount of fructose as that found in 670 ml of soft drink. Because this is equivalent to approximately 1.5 times the average intake, it is likely that a significant portion of the population is consuming comparable amounts of fructose.

The prevalence of obesity in the U.S. population has increased over the same time period as the increase in fructose consumption. Results of the present study indicate that fructose appears to behave more like fat than like other carbohydrates with respect to insulin secretion, leptin production, and postprandial TG levels. Furthermore, fructose, unlike glucose does not cross the blood-brain barrier and could potentially contribute to increased energy intake because it does not trigger CNS glucose sensors involved in the regulation of food intake. Increased fructose consumption, along with consumption of larger portions of high-fat foods and inactivity, may be a contributing factor to the increased incidence of obesity. Studies in humans have reported weight gain during prolonged *ad libitum* consumption of

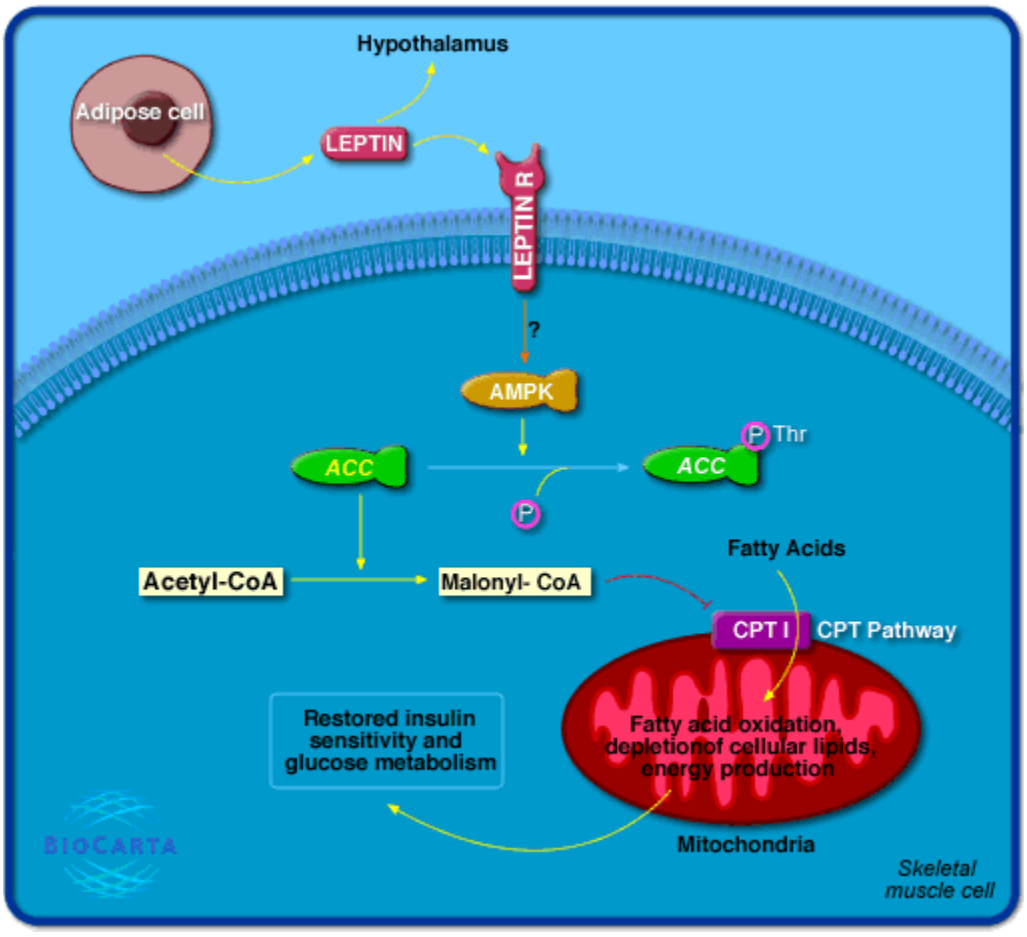
fructose, In addition, fructose ingestion leads to increased rates of *de novo* lipogenesis compared with eucaloric glucose ingestion. Children who consume more than 265 ml (9 oz) of soda per day have a 15% higher energy intake than those who do not regularly consume soft drinks, and for each sugar-sweetened beverage consumed, both body mass index and the frequency of obesity in children are increased.

We found that ingestion of meals with HFr beverages resulted in subsequent increases of hunger and fat intake compared with the same meals with HGl beverages, but only in a subset of subjects with a psychological profile of dietary restraint. These data suggest that certain individuals are more susceptible to the effects of the endocrine/metabolic profile elicited by fructose ingestion. Many obese individuals exhibit a high degree of dietary restraint. Thus, these individuals remain obese despite their efforts to curtail caloric and fat intake through dieting. Furthermore, children even as young as 5 yr can exhibit restrained eating behavior. These results demonstrate one of the difficulties in identifying the physiological mechanisms regulating body weight in humans, *i.e.* that psychological attitude toward food impact eating behavior. Although the limited number of subjects in the present study precludes any definitive conclusions, the results provide groundwork for future investigations. The finding that ingestion of fructose beverages with mixed nutrient meals is associated with a subsequent increase of food intake contrasts with two other studies that reported decreased caloric intake after a fructose preload compared with glucose preload. However, in those experiments, the effects of the fructose on food intake were only evident when the sugar was ingested alone and not in combination with a mixed meal. Furthermore, unlike the present study, which examined the effects of repeated fructose ingestion over 24 h, previous studies only measured acute responses.

In summary, consuming HFr beverages with meals results in lower circulating insulin and leptin concentrations and higher ghrelin and TG levels compared with consumption of HGl beverages. Because insulin, leptin, and possibly ghrelin function as key signals to the CNS

in the long-term regulation of energy balance, prolonged consumption of diets high in energy from fructose could lead to increased caloric intake and contribute to weight gain and obesity. The sustained elevation of plasma TG levels after fructose ingestion suggests that chronic fructose consumption could contribute to atherogenesis and cardiovascular disease. Additional studies are needed to investigate the effects of prolonged fructose consumption on the endocrine signals regulating energy homeostasis, insulin action, and lipid metabolism, as well as its long-term effects on appetite and energy intake.

Reversal of Insulin Resistance by Leptin



The insulin resistance of type II diabetes appears to be caused in part by the presence of high levels of lipids in cells such as skeletal muscle where this would not normally be found. The presence of excess lipid stores in skeletal muscle cells interferes with energy metabolism, impairing glucose oxidation and insulin response. Skeletal muscle is one of the primary glucose-consuming tissues, giving it a central role in insulin resistance. The increased risk of diabetes associated with obesity may be caused by increased lipid deposits in skeletal muscle and liver, creating insulin resistance.

Leptin is a peptide hormone secreted by adipose tissue that has been associated with many processes. One of the target tissues of leptin is the hypothalamus where it can act to regulate feeding behavior and metabolism. Another leptin target is skeletal muscle. Activation of leptin signaling in skeletal muscle activates the AMP-activated protein kinase (AMP-kinase), known to play a key role in signaling in response to nutrients throughout evolution. AMPK phosphorylates and inactivates the enzyme ACC, acetyl-CoA carboxylase. ACC catalyzes the production of malonyl-CoA from acetyl-CoA. Malonyl-CoA in turn is an inhibitor of the import of fatty acids into mitochondria by carnitine palmitoyl-transferase I for oxidation and energy production. In the presence of leptin, AMPK is activated, ACC is inhibited, and malonyl-CoA levels fall, increasing the oxidation of fatty acids and reducing the lipid content of cells. The reduced lipid content in skeletal muscle allows insulin signaling and glucose consumption to return to their normal levels, reducing insulin resistance.

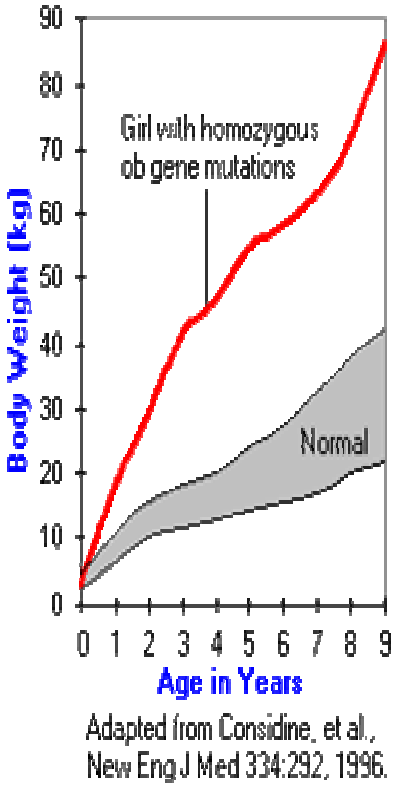
Parasite-induced anorexia: leptin, insulin and corticosterone responses to infection with the nematode, *Nippostrongylus brasiliensis*

The nematode parasite, *Nippostrongylus brasiliensis*, induces biphasic anorexia in its rat host. The mechanisms, underlying this anorexia and its possible advantages to the host or parasite are unknown. We have investigated the effect of acute (12–24 h) and chronic (2–17

days) infections on plasma concentrations of leptin, insulin and corticosterone, and on hypothalamic expression of neuropeptide Y, galanin and corticotrophin-releasing factor genes. Plasma leptin was elevated in infected rats relative to uninfected *ad libitum*-fed controls and pair-fed controls in 12 h infections initiated at dark onset and in infections of 2 days duration. At other times prior to parasite expulsion, plasma leptin in infected and pair-fed rats was lower than that of uninfected *ad libitum*-fed controls, reflecting the existing state of negative energy balance. Elevated plasma leptin concentrations in infected rats at day 2 post-infection were accompanied by reduced neuropeptide Y gene expression in the hypothalamic arcuate nucleus compared with both *ad libitum* control and pair-fed animals, and by lowered corticotrophin-releasing factor gene expression in the paraventricular nucleus relative to pair-feds. Twelve hour infections were characterized by a substantial increase in plasma corticosterone that was independent of reduced food intake, and in 12 h infections initiated at dark onset, where plasma leptin was elevated; there was also increased plasma insulin concentration in infected rats. In longer infections, differences between the groups in plasma insulin and corticosterone concentration were only observed at day 4 post-infection. In summary, perturbations to leptin, insulin and corticosterone signals early in infection may have a causative role and might feed back onto hypothalamic gene expression, whereas subsequent changes in these parameters are more likely to be secondary to negative energy balance.

Disease States

Mice with inactivating mutations in the gene encoding leptin or its receptor have indistinguishable, recessive phenotypes of obesity, with roughly three times the body weight and five times the fat mass of normal mice. They also manifest diabetes, and show cold intolerance, depressed immune function and infertility.



Adapted from Considine, et al.,
New Eng J Med 334:292, 1996.

Blood concentrations of leptin are usually increased in obese humans, suggesting that they are in some way insensitive to leptin, rather than suffering from leptin deficiency. Mutations in ob or db genes appear to be a very rare cause of morbid obesity in humans, but both have been described. The effect of such mutations on body weight is dramatic, as shown here. The figure to the right depicts the growth curve for a young girl found to have homozygous inactivating mutations of the ob gene, contrasted to normal children (2nd to 98th percentiles).

Will ob protein be useful for treating human obesity? Perhaps, but considerable work remains to be done to characterize its effects and, as described above, it appears that frank deficiencies in leptin secretion are a rare cause of human obesity. Leptin therapy will require either frequent injections or genetic therapy, precluding its use for trivial purposes.

Several strains of laboratory mice are homozygous for single-gene mutations that causes them to become grossly obese.

These fall into two classes:

- *ob/ob* = mutations in the gene for the protein hormone leptin

  When *ob/ob* mice are treated with injections of leptin they lose their excess fat and return to normal body weight.

- *db/db* = mutations in the gene that encodes the receptor for leptin

Study of these animals has led to an understanding of the action of leptin in humans.

Human leptin is a protein of 167 amino acids. It is manufactured in fat cells (adipose tissue), and the level of circulating leptin is directly proportional to the total amount of fat in the body.

Leptin acts on receptors in the hypothalamus of the brain where it:

- counteracts the effects of neuropeptide Y (a potent feeding stimulant secreted by cells in the gut and in the hypothalamus);
- counteracts the effects of anandamide (another potent feeding stimulant that binds to the same receptors as THC, the active ingredient of marijuana)
- promotes the synthesis of α-MSH, an appetite suppressant;
- the result: inhibition of food intake.

This inhibition is long-term, in contrast to

- the rapid inhibition of eating by Cholecystokinin (CCK) and
- the slower suppression of hunger between meals mediated by PPY

The absence of a functional hormone (or its receptor) leads to uncontrolled food intake and resulting obesity.

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- The secretion of gonadotropin-releasing hormone (GnRH).

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- The secretion of gonadotropin-releasing hormone (GnRH). Women who are very thin from limited food intake or intense physical training may cease to menstruate because of their lack of leptin-secreting fat cells. Treating them with recombinant human leptin can sometimes restore normal menstruation.
- Stimulating the sympathetic nervous system to modulate the balance between the formation and breakdown of bone.

In addition to its effect on the hypothalamus, leptin acts directly on

- The cells of the liver and skeletal muscle where it stimulates the oxidation of fatty acids in the mitochondria. This reduces the storage of fat in those tissues (but not in adipose tissue).
- T cells where it enhances the production of Th1 cells promoting inflammation. Mice without leptin are protected from autoimmune disease (which may account for the reports that restricting food intake helps humans with rheumatoid arthritis).

Mutations in the gene for leptin, or in its receptor, are rarely found in obese people.

The rare cases:

- Extreme obesity in five members of two families that are homozygous for mutations (frame shift in one family, missense in the other) in their leptin gene; i.e., they are like *ob/ob* mice.
- Extreme obesity among three members of a family that are homozygous for mutations in their leptin receptor gene; i.e., they are like *db/db* mice.
- Only moderate obesity in people who are heterozygous (one

mutant and one normal) for their leptin genes.

Recombinant human leptin is now available, and trials are underway to see if it can reduce obesity in humans as it does in *ob/ob* mice.

The 16 September 1999 issue of The New England Journal of Medicine reports the results of a year-long trial of recombinant human leptin in a 9-year-old girl who is homozygous for a frameshift mutation in her leptin genes. The findings:

- She began the trial weighing 208 pounds (94.4 kg), of which 123 lbs (55.9 kg) was fat (adipose tissue).
- She was given daily injections of recombinant leptin for one year.
- At the end of that time,
- She had lost 36 lbs (16.4 kg), most of it fat.
- Her appetite and thus food intake had decreased.
- Her immune system made anti-leptin antibodies but these did not seem to interfere with the action of the hormone.

The results of trials of recombinant leptin in obese humans that do **not** have mutations in **both** their leptin genes so far has not shown any great benefit in weight reduction. (All the heterozygous individuals so far identified have declined to be tested.)

- Women who are very thin from limited food intake or intense physical training may cease to menstruate because of their lack of leptin-secreting fat cells. Treating them with recombinant human leptin can sometimes restore normal menstruation.
- Stimulating the sympathetic nervous system to modulate the balance between the formation and breakdown of bone.

The World Health Organization has now classified obesity as a disease. It is often said that obesity is the biggest health problem facing the developed world today. It causes health problems such as hypertension, type II diabetes, heart attacks and strokes, elevated

cholesterol and many more. Obesity is said to lead to 30,000 premature deaths each year and it is shortening the lives of people by an average of nine years.

Redux, in the 1980's which sold 2 million prescriptions within the first 6 months of its launch in the US and which went on to be sold in 65 countries suppressed the appetite. However it was eventually taken off the market because of the effects it had on the heart. There are now only 2 drugs on the market in the UK- Xenical, which slows absorption of fat and Reductil, which suppresses the appetite. Both of these are prescription drugs, can have side affects and can only reduce weight slightly.

Several hormones are responsible for our eating habits. For example; Leptin and alpha-MSH are both appetite represent. Cannaboids, neuropeptide Y, ghrelin and anandamid are all feeding stimulants.

One of the hormones being researched for this reducing weight is Leptin. Leptin is an appetite suppressant. It stops you eating too much as well as makes you more active so you burn off more energy.

It is produced by a specific gene found in fat cells called the obese (ob) gene. Small amounts of leptin are also secreted by cells in the epithelium, stomach and placenta. The amount of Leptin found in people increases as their body fat increases. There is also a higher concentration of mRNA in fat from obese compared to thin subjects. Leptin acts on receptors in the hypothalamus of the where it: The theory is that as you get fatter you also get less sensitive to the affects of Leptin. Leptin works on the body in the following ways;

- counteracts the effects of neuropeptide Y(feeding stimulant secreted by cells in the gut wall and in the hypothalamus);
- counteracts the affects of anandamid(another feeding stimulant that binds to the same receptors as THC the active ingredient of marijuana)
- promotes the effects of alpha-MSH a appetite represent;
- resulting in inhibition of food intake

- it also stimulates secretion of reproductive hormones such as gonadotrophin-releasing hormone and thus leutenizing and follicle stimulating hormone from the anterior pituitary.
- it raises the temperature of the subject so energy expenditure is increased

Leptin also acts directly on the cells of the liver and skeletal muscles where it stimulates the oxidation of fatty acids in the mitochondria. This reduces the storage of fat in those tissues (but not in adipose [fat] tissue). Leptin receptors are also present in T lymphocytes.

In rare cases the gene that produces leptin or its receptors mutates. This can cause severe obesity and diabetes in certain individuals as well as in certain cases failure to reach puberty. However, most people who are obese, do not have a defective ob gene.

Leptin of humans has 146 amino acid sequences containing one disulphide bond. Its molecular weight is around 16 kDa. Leptin has 67% sequence identity among diverse species.

Leptin is a four-helix bundle with one very short strand segment and two relatively long interconnected loops. This is consistent with a classification as a cytokine four-helix bundle.

Fructose and leptin

Previous studies indicate that leptin secretion is regulated by insulin-mediated glucose metabolism. Because fructose, unlike glucose, does not stimulate insulin secretion, we hypothesized those meals high in fructose would result in lower leptin concentrations than meals containing the same amount of glucose. Blood samples were collected every 30–60 min for 24 h from 12 normal-weight women on 2 randomized days during which the subjects consumed three meals containing 55, 30, and 15% of total kilocalories as carbohydrate, fat, and protein, respectively, with 30% of kilocalories as either a fructose-sweetened [high fructose (HFr)] or glucose-sweetened [high glucose (HGl)] beverage. Meals were isocaloric in the two treatments.

Postprandial glycemic excursions were reduced by 66 ± 12% and insulin responses were 65 ± 5% lower (both $P < 0.001$) during HFr consumption. The area under the curve for leptin during the first 12 h (–33 ± 7%; $P < 0.005$), the entire 24 h (–21 ± 8%; $P < 0.02$), and the diurnal amplitude (peak – nadir) (24 ± 6%; $P < 0.0025$) were reduced on the HFr day compared with the HGl day. In addition, circulating levels of the orexigenic gastroenteric hormone, ghrelin, were suppressed by approximately 30% 1–2 h after ingestion of each HGl meal ($P < 0.01$), but postprandial suppression of ghrelin was significantly less pronounced after HFr meals ($P < 0.05$ *vs.* HGl). Consumption of HFr meals produced a rapid and prolonged elevation of plasma triglycerides compared with the HGl day ($P < 0.005$). Because insulin and leptin, and possibly ghrelin, function as key signals to the central nervous system in the long-term regulation of energy balance, decreases of circulating insulin and leptin and increased ghrelin concentrations, as demonstrated in this study, could lead to increased caloric intake and ultimately contribute to weight gain and obesity **during chronic consumption of diets high in fructose.**

The two main sources of fructose in the U.S. diet are sucrose, which consists of 50% fructose, and HFr-fructose corn syrup (HFCS), typically containing 55% fructose. It is estimated that fructose consumption has increased by at least 26% over the past three decades, primarily due to the increased use of HFCS in soft drinks and other beverages. The average per capita disappearance data for added fructose in 1997 from the combined use of sucrose and HFCS was 81 g/d. Individuals in the 90th percentile of fructose intake are estimated to consume between 1.5 and 2.5 times the mean intake. In the present study, the amount of fructose consumed was 45 g at each meal, which is approximately the same amount of fructose as that found in 670 ml (24 oz) of soft drink. Because this is equivalent to approximately 1.5 times the average intake, it is likely that a significant portion of the population is consuming comparable amounts of fructose.

The prevalence of obesity in the U.S. population has increased over the same time period as the increase in fructose consumption. Results of the present study indicate that fructose appears to behave more like fat than like other carbohydrates with respect to insulin secretion, leptin production, and postprandial TG levels. Furthermore, fructose, unlike glucose does not cross the blood-brain barrier and could potentially contribute to increased energy intake because it does not trigger CNS glucose sensors involved in the regulation of food intake. Increased fructose consumption, along with consumption of larger portions of high-fat foods and inactivity, may be a contributing factor to the increased incidence of obesity. Studies in humans have reported weight gain during prolonged *ad libitum* consumption of fructose, In addition, fructose ingestion leads to increased rates of *de novo* lipogenesis compared with eucaloric glucose ingestion. **Children who consume more than 265 ml (9 oz) of soda per day have a 15% higher energy intake than those who do not regularly consume soft drinks, and for each sugar-sweetened beverage consumed, both body mass index and the frequency of obesity in children are increased.**

Consuming HFr beverages with meals results in lower circulating insulin and leptin concentrations and higher ghrelin and TG levels compared with consumption of HGl beverages. Because insulin, leptin, and possibly ghrelin function as key signals to the CNS in the long-term regulation of energy balance, prolonged consumption of diets high in energy from fructose could lead to increased caloric intake and contribute to weight gain and obesity. The sustained elevation of plasma TG levels after fructose ingestion suggests that chronic fructose consumption could contribute to atherogenesis and cardiovascular disease. Additional studies are needed to investigate the effects of prolonged fructose consumption on the endocrine signals regulating energy homeostasis, insulin action, and lipid metabolism, as well as its long-term effects on appetite and energy intake.