Dietary Components in the Development of Leptin Resistance¹–³

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ABSTRACT

Classically, leptin resistance has been associated with increased body fat and circulating leptin levels, and the condition is believed to contribute to the onset and/or maintenance of obesity. Although a great deal is known about the central nervous system mechanisms mediating leptin resistance, considerably less is known about the role of diet in establishing and maintaining this altered hormonal state. An exciting new finding has recently been published demonstrating the existence of leptin resistance in normal-weight rats with lean leptin levels by feeding them a high-concentration-fructose diet. This finding has opened the possibility that specific macronutrients may be capable of inducing leptin resistance, independently of the amount of body fat or circulating leptin present in the treated animals. This review describes several lines of research that have recently emerged indicating that specific types of dietary sugars and fats are capable of inducing leptin resistance in experimental rodent models. The results further show that diet-induced leptin resistance is capable of increasing energy intake and elevating body weight gain under appropriate dietary challenges. It appears that biological mechanisms on multiple levels may underlie the dietary experimental rodent models. The results further show that diet-induced leptin resistance is capable of increasing energy intake and elevating body weight gain under appropriate dietary challenges. What is clear from the findings reviewed here is that diet-induced leptin resistance can occur in the absence of elevated circulating leptin levels and body weight, rendering it a potential cause and/or predisposing factor to excess body weight gain and obesity. Adv. Nutr. 4: 164–175, 2013.

Introduction

The term leptin resistance first appeared in the literature shortly after the discovery of the hormone itself (1), and since that time over 4500 articles citing the term have been published. Leptin resistance was originally defined as excess weight maintenance in the face of leptin levels that are higher than those associated with healthy weight (2,3).

The development of leptin resistance classically has been characterized by increased body fat and elevated circulating leptin levels (4), although, as shown below, onset of the effect does not appear to be limited solely to these conditions. More recently, leptin resistance has been redefined in terms of its specific action at peripheral and central cites (5) and in relation to its effects on cellular pathways (6). For purposes of this review, however, we will address leptin resistance as it is expressed in vivo in relation to its feeding and body weight–altering effects. Specifically, the impaired reduction in food consumption and body weight in response to peripheral or central administration of leptin is considered to reflect a state of leptin resistance. Behavioral manifestations such as excess intake of energy-dense or highly palatable diets will be considered evidence of apparent leptin resistance, in the absence of more definitive data.

At least 2 major biological mechanisms have been defined that potentially may mediate leptin resistance as expressed in vivo in relation to overall energy balance. The first was...
identified in early studies indicating that the blood-brain barrier (BBB)\(^8\) appears to limit the entry of leptin into the brain as circulating leptin levels increase (7,8). Support for this notion was gained when it was demonstrated that leptin is transported across the BBB by a saturable process and that the saturation occurs at relatively low levels of circulating leptin (9). This means that BBB transporter capacity, and not the secretory capacity of the adipose tissue, is the limiting factor in overcoming target tissue resistance. Thus, it has been proposed that obese individuals experience a relative deficit of leptin at central nervous system (CNS) sites responsible for mediating its feeding and body weight inhibitory effects (10). The second involves alterations in leptin receptor expression and second messenger signaling in the arcuate nucleus and other areas of the CNS, which act to inhibit leptin’s normal feeding and body weight regulatory signals (11). This primarily involves inhibition of the leptin receptor second messenger signal transducer and activator of transcription-3 (STAT 3) (12), which activates the leptin signaling pathway. The feeding of high-fat diets (HFDs) to rodents reliably induces obesity and leptin resistance (13), and use of this model has provided experimental evidence that these 2 leptin-resistance mechanisms develop independently, with BBB resistance being predominant in early and mild obesity (14,15). As adiposity and circulating leptin levels increase, the impairment in BBB leptin transport increasingly fails to translate increases in serum leptin into increases in CNS leptin. Thus, resistance at the CNS leptin receptor/second messenger level, developing in tandem, is increasingly uncompensated for.

Although a great deal is known about the CNS mechanisms associated with leptin resistance, much less is known about the effects of diet in establishing and maintaining this altered hormonal state. For example, it was demonstrated some time ago that consumption of an HFD by rats quickly induces a state of leptin resistance, before increases in leptin levels and body weight (16,17). This effect was observed in these studies within 3–5 d, clearly indicating that elevations of circulating leptin and adiposity may not be required for the induction of leptin resistance. However, detailed studies of the components of the HFD responsible for the effect were not pursued at the time. More recently, it was shown that prolonged maintenance of rats fed a high-fructose (HFr) diet, which did not elevate body weight or circulating leptin levels, nevertheless resulted in the expression of leptin resistance in response to both injected leptin and to feeding of an HFD (18). This finding was highly significant in that it implicated a single dietary component in the induction of leptin resistance, acting in the absence of developing obesity (19). After the appearance of this study, its authors and other investigators initiated a more systematic analysis of the role of dietary components in the development of leptin resistance, and their potential underlying metabolic and CNS mechanisms. This review presents these new findings, including the effects of type and form of dietary sugar and dietary TGs on feeding and body weight responses to leptin, and the effects of specific nutrients on BBB leptin transport. One notable characteristic of the dietary components described below, which we will return to at the conclusion of our review, is their ability to rapidly induce leptin resistance in animals of normal body weight and leptin levels. Although strain differences in response to HFDs and leptin effects are well known (4), the studies presented here were all conducted in Sprague-Dawley rats or CD-1 mice, strains that are highly susceptible to palatable high-energy diets and obesity. Thus, results can be directly compared within the studies presented here and compared with numerous published studies in which leptin resistance was induced by high-fat (HF) feeding in these rodent models.

Current status of knowledge

The role of fructose in promoting leptin resistance and overnutrition

There are numerous conflicting reports in the literature as to whether fructose feeding results in obesity in rats (18,20–23). Several factors likely contribute, including variations in experimental design, strain susceptibility to dietary fructose manipulations, and vendor husbandry. Another explanation for these discrepant results is that the palatability of the various diets depends on the concentrations and the ratio of fructose to fat. The hyperphagic and weight-gain response to low-fat diets with increasing concentrations of fructose was recently examined and compared with a non-purified diet (no simple sugars and 13% fat). Diets with either 20 or 40% of the energy provided by fructose induced hyperphagia and promoted weight gain (data not shown), whereas a 60% fructose diet did not (Fig. 1A), and remarkably, resulted in reduced weight gain (Fig. 1B) (18). These data suggest that for a fructose diet to induce weight gain, that diet must induce hyperphagia, and likely the hyperphagia is driven by increased palatability. Thus, with fructose-containing diets that are palatable and drive hyperphagia, it is difficult to separate the contribution of other weight-gain–induced impairments in energy homeostatic mechanisms from those induced by fructose consumption itself. The observation that the 60% fructose diet did not result in palatability-driven hyperphagia allowed us to examine whether dietary fructose disrupts energy-homeostatic processes through a mechanism independent of hyperphagia and obesity. Rats were fed the HFr (60% fructose, 13% fat, 18% protein; 15.1 kJ/g) or control purified diet (60% corn starch, 13% fat, 18% protein; 15.1 kJ/g) for 6 mo. There were no differences in body weight or food intake or serum leptin during the fructose feeding period compared with the control diet, although serum TGs were elevated with the

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\(^8\) Abbreviations used: ACC, acetyl CoA carboxylase; Akt, serine/threonine protein kinase; AMPK, adenosine monophosphate-activated kinase; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DIO, diet-induced obesity; ERK 1/2, extracellular signal–regulated kinases 1 and 2; GFAT, glutamine:fructose-6-phosphate amidotransferase; HBP, hexosamine biosynthetic pathway; HF, high-fat; HFD, high-fat diet; HFr, high-fructose; HSF, high-fructose/sugar-free; i.p., intraperitoneal(i.p.); IRS-1, insulin receptor substrate 1; O-GlcNAc, O-linked N-acetylglucosamine; OGt, O-GlcNAc transferase; O-linked, O-glycan linked; P38K, phosphatidlyinositol-3 kinase; SF, sugar-free; STAT 3, signal transducer and activator of transcription 3; UDP-GlcNAc, uridine 5’-diphospho-4-acetylglucosamine.
fructose diet (18). The rats were then challenged with leptin [intraperitoneally (i.p.), 0.6 mg/kg], and 24-h food consumption was assessed. The fructose-fed rats were unresponsive to the peripheral leptin challenge, whereas the nonpurified laboratory diet-fed rats showed an expected 25% decrease in food intake (Fig. 2A). Both groups of rats were then challenged with an obesogenic diet (60% fat, 7% sucrose). The leptin-resistant rats with prior fructose feeding demonstrated exacerbated energy consumption and weight gain compared with the control animals (Fig. 2B). These data demonstrate that fructose feeding, in the absence of weight gain, affects energy homeostatic regulatory pathways in such a way that predisposes animals to excessive energy consumption and weight gain with subsequent exposure to an obesogenic diet.

The observation that the combination of fructose feeding and a subsequent challenge with a HF/sugar-containing diet results in exacerbated weight gain led to a study examining the consequences of feeding diets high in both fructose and fat. Rats were fed a diet formulated with 30% fat and 40% fructose (HF/HFr) and compared with rats fed a matched HF diet without sugar [HF/sugar-free (SF)] or a nonpurified control diet for 70 d. Energy consumption was similar across groups, and weight gain was not different between the HF/HFr diet and the nonpurified control diet (24). Note that the HF/HFr diet did not induce hyperphagia over the nonpurified control diet, and this was in contrast to the hyperphagia described above with the 40% fructose/low-fat diet. This finding is most likely due to reduced palatability of the HF/HFr diet. Despite no difference in food consumption, weight gain was 25% less with the HF/SF diet compared with either the HF/HFr or nonpurified diets (24). Although the HF/SF diet did not induce weight gain, body fat measured by time-domain nuclear magnetic resonance indicated increased total adiposity, and this was accompanied by increased serum leptin and TG concentrations (24). As expected, rats fed the HF/HFr diet demonstrated an impaired response to peripheral leptin (0.6 mg/kg, i.p.) as assessed by 24-h food intake (Fig. 3A, second set of bars) or by the anorectic response to a central infusion of leptin (1.5 μg/d, administered intracerebroventricularly) (Fig. 3B, HF/HFr). Interestingly, those fed the HF/SF diet retained full leptin responsiveness to peripheral leptin injection and central leptin infusion (Fig. 3). Moreover, switching from the HF/HFr diet to the HF/SF diet reversed the leptin resistance (Fig. 3). The reversal of leptin resistance was not secondary to weight loss, because switching from the HF/HFr to the HF/SF diet did not decrease

Figure 1  Food intake (A) and change in BW (B) in rats either maintained by feeding the nonpurified diet (13% fat, 0% fructose) or a high-fructose diet (13% fat, 60% fructose). Values are means ± SE, n = 12. *P < 0.001 for difference in food consumption at d 1 by Student’s t test. BW, body weight.

Figure 2  Leptin responsiveness as measured by cumulative food intake 24 h after an intraperitoneal injection in rats of either saline or 0.6 mg/kg leptin after 6 mo of being fed either the nonpurified control or 60% fructose diet (A). Values are means ± SE, n = 6. *P < 0.04 by 2-way ANOVA. Change in body weight after introduction of a 60% HF/7% sucrose diet in rats either previously maintained by feeding the nonpurified diet (13% fat, 0% fructose) or the high-fructose diet (13% fat, 60% fructose) (B). Values are means ± SE, n = 5–6. Adapted from reference 18 with permission. HF, high-fat.
phosphorylation of AMPK, which in turn reduces phosphorylation of ACC. Reduced phosphorylated ACC results in elevated malonyl-CoA and decreased food consumption (26). This pathway is sensitive to central fructose injections (27). It was recently reported that consumption of an HF diet resulted in an impaired leptin-mediated decrease in phosphorylation in both AMPK and ACC (28), and this may underlie the reduced physiologic responses to leptin associated with dietary fructose. Moreover, it is suggested that this fructose impairment in the AMPK pathway, in and of itself, is not sufficient to promote obesity with a standard laboratory diet. An additional factor is necessary, for instance, a challenge with a HF/sugar or other palatable diet, or an additional insult, such as an impairment in the leptin-mediated STAT3 pathway. Because an HF/sugar-containing diet impairs leptin-mediated STAT3 phosphorylation in as few as 2 d (29), the HF/sugar challenge is the equivalent of an insult to the STAT3 leptin signaling pathway. Additional studies are necessary to test these speculations.

In summary, dietary fructose contributes to obesity through 2 mechanisms: 1) palatability-driven hyperphagia and weight gain and 2) impaired leptin responsiveness that exacerbates the palatable diet/overnutrition–induced weight gain. Hyperphagia-driven leptin resistance is associated with impaired STAT3 phosphorylation, whereas fructose-driven leptin resistance is reversible and may be associated with an impaired leptin-mediated decrease in AMPK phosphorylation.

**Sucrose-induced leptin resistance and its potential metabolic basis**

It is well established that mice and rats made obese by feeding of HF diets become resistant to both the peripheral and central administration of exogenous leptin (14,24,30). A majority of investigators induce diet-induced obesity (DIO) by using a purified HF/high-sucrose diet. Others have used a model in which the rats are offered a choice of nonpurified laboratory diet (Harlan Teklad Rodent Diet 8604; Harlan Laboratories), a 30% sucrose solution and lard (choice diet) (31,32). These animals increased their energy intake, rapidly gained body fat mass (33), and developed both central and peripheral leptin resistance after only 18 d of being fed the diet (J. Apolzan, unpublished observations). A recent study (31) tested which component of the choice diet was responsible for the development of leptin resistance by comparing rats offered the choice diet, the nonpurified laboratory diet plus 30% sucrose solution, or the nonpurified diet plus lard. Additional groups of rats were offered a purified low-fat diet (D12450B Research Diets, Inc.) or an HFD (D12492 Very High Fat Diet, Research Diets, Inc.) to allow comparison with the purified HFD used in many DIO studies. Rats offered the choice diet or nonpurified laboratory diet plus 30% sucrose solution were resistant to peripheral injections of leptin after 17 d of being fed the experimental diets, whereas all other groups of rats reduced their food intake in response to the leptin injection (31). Thus, leptin resistance developed only in those rats that were consuming a significant amount of fructose.

Collectively, these data suggest that diets containing fructose promote leptin resistance and obesity through different mechanisms than those of high-palatable diets that drive hyperphagia. This hypothesis was tested by examining central leptin signaling in rats fed a low-fat/HFr diet compared with the palatable, obesogenic HF/high-fructose diet that is associated with hyperphagia in the hypothalamus (25). In contrast, HFr diets that did not induce either hyperphagia or obesity did not impair hypothalamic phosphorylation of STAT3 (25). Leptin, in addition to stimulating STAT3 phosphorylation, also signals through the nutrient-sensing adenosine monophosphate-activated kinase/acetyl CoA carboxylase (AMPK/ACC) pathway by decreasing body weight, but the diet switch did halt the excessive weight gain (24).

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![Figure 3](https://academic.oup.com/advances/article-abstract/4/2/164/4591625/167)

**Figure 3** Leptin responsiveness as measured by cumulative food intake 24 h after an intraperitoneal injection of either saline or 0.6 mg/kg leptin at d 65 in rats fed the 30% HF diet without sugar (HF/SF), in rats fed the 30% fat and 40% fructose (HF/HFr) diet, or 18 d after some of the rats fed the HF/HFr diet were switched to the HF/SF diet (Switched) (A). Values are means ± SE, n = 6. *P < 0.03 for difference with leptin by paired t test. Body weight change in rats after a central infusion of ACSF or leptin (1.5 μg/d) in HF/HFr-fed rats, HF/SF-fed rats and rats switched from HF/HFr to HF/SF (B). *Body weight change in the HF/SF-fed and Switched rats was different between ACSF and leptin-treated rats beginning at d 3, P < 0.01. Values are means ± SE, n = 6. Adapted from reference 24 with permission. ACSF, artificial cerebrospinal fluid; HF/SF, high-fat/sugar-free; HF/HFr, high-fat/high-fructose.
proportion of their energy intake in the form of liquid sucrose. The leptin resistance in rats consuming liquid sucrose did not appear to be due to obesity because although they had 70% more adipose tissue than control rats, rats offered an HFD or nonpurified diet plus lard were equally fat but leptin responsive. In addition, it appeared to be important for the sucrose to be offered as a solution because rats fed the low-fat diet consumed the same amount of energy as sucrose as the choice rats, but the low-fat–fed rats remained leptin responsive (31). A subsequent study confirmed that rats consuming 50% of their energy intake from a 30% sucrose solution were leptin resistant after 25 d.

One possible explanation for the early onset of leptin resistance in the rats consuming liquid sucrose is a shift in substrate metabolism, which results in a modification of leptin signaling. The consumption of sucrose would lead to an increase in the amount of both glucose and fructose available for metabolism. These 2 substrates have the potential to drive the activity of all anabolic pathways in the liver, including the hexosamine biosynthetic pathway (HBP), which normally is responsible for only 1–3% of glucose metabolism but has been characterized as a nutrient-sensing pathway because its activity increases as more glucose becomes available (34). The consumption of sucrose also has the potential to increase delivery of glucose to other insulin- and leptin-responsive tissues where it can enter into the HBP. Increased glucose availability in muscle cells has been reported to stimulate HBP activity and lead to insulin resistance (35). In adipose tissue, increased HBP activity stimulates expression of lipogenic enzymes (36), which could contribute to the development of obesity. The rate-limiting enzyme of the HBP, glutamine:fructose-6-phosphate amidotransferase (GFAT), uses glutamine to form glucosamine-6-P from fructose-6-P. The end product of the pathway, uridine 5′-diphospho-N-acetylglucosamine (UDP-GlcNAc), is the substrate for the enzyme O-GlcNac transferase (OGT), which transfers 1 molecule of O-linked-N-acetylgalactosamine (O-GlcNac) onto serine or threonine residues of nuclear and cytoplasmic proteins (34). O-glycan linked (O-linked) glycosylation can increase or decrease bioactivity of proteins either by competing for a phosphorylation site or by glycosylating a site that modifies the activity or stability of the protein (37).

There is a significant literature demonstrating that increased activity of the HBP leads to insulin resistance (38). In vivo infusions of glucosamine, which enters the HBP independently of GFAT, rapidly increase activity of the HBP and inhibit whole-animal insulin-stimulated glucose clearance (39) due to glycosylation of the insulin receptor, insulin receptor substrate-1 (IRS-1), phosphatidylinositol-3 kinase (PI3K), and a serine/threonine protein kinase (Akt) found downstream in the insulin signaling pathway (40–43). Overexpression of OGT, which uses UDP-GlcNAc to modify proteins and transcription factors, results in insulin resistance (44), whereas insulin sensitivity is restored in cells treated with inhibitors of GFAT, the rate-limiting enzyme for entry of substrate into the HBP (45). Leptin signaling is dependent on activation of multiple proteins, including IRS-1, PI3K, extracellular signal–regulated kinases 1 and 2 (ERK 1/2), and STAT3, all of which are O-linked glycosylated in conditions of glucose excess (40–42). Glycosylation inhibits activation of IRS-1, PI3K, and ERK 1/2, but it is not known whether glycosylation changes activity of STAT3 (43). By contrast, glycosylation increases the activity of stimulatory protein 1 (46), a transcription factor that promotes leptin expression, accounting for HBP regulation of leptin production (47).

A series of studies has been initiated to determine whether consumption of a nonpurified diet plus 30% sucrose solution increases activity of the HBP in rats, and whether activation of the HBP pathway leads to leptin resistance. The first study tested whether sucrose consumption increased activity of the HBP. O-linked glycosylated proteins and GFAT were measured by Western blot in the liver from rats that had been fed a nonpurified laboratory diet plus 30% sucrose (sucrose rats) for 36 d and that had been injected i.p. with 2 mg/kg leptin or PBS 30 min before tissues were collected. There was no measurable increase in the total amount of glycosylated protein (Fig. 4A), but surprisingly, leptin treatment increased liver GFAT in rats fed a nonpurified diet and this effect was blocked in the sucrose rats (Fig. 4B). Although leptin was not expected to activate the HBP pathway, the loss of leptin response in the sucrose rats was consistent with the animals being leptin resistant. STAT3 also was measured by Western blot in blocks of hypothalamic (Fig. 4C) and brainstem tissue (Fig. 4D) as a marker of central leptin receptor activation in the sucrose rats. Results indicated that sucrose caused a significant stimulation of STAT3 phosphorylation in the brainstem of PBS-injected rats, with no significant additional effect of leptin in either the nonpurified diet–fed or sucrose-fed rats (Fig. 4D). STAT3 activation is required for leptin to inhibit food intake (48), and these data suggest that the failure of leptin to inhibit food intake or weight gain in sucrose-fed rats was because STAT3 was already maximally phosphorylated in the nonstimulated state.

A second study was conducted to test whether activation of the HBP would cause leptin resistance. Leptin responsiveness was measured in rats receiving chronic peripheral infusions of glucosamine, which enters the HBP below the rate-limiting step controlled by GFAT. In this study, 16 male Sprague-Dawley rats were adapted to the environment and fed nonpurified laboratory diet ad libitum. On each test day, the rats were food deprived for 9 h during the light period and then received an i.p. injection of either PBS or 2 mg leptin/kg. Food was returned to the cages 1 h after the injection, and intake and body weight were measured the next morning, 14 h after the leptin or PBS injections. Leptin responsiveness was tested in the rats twice, 5 d apart, with treatments switched between days so that each animal was tested with both leptin and PBS. The rats were then divided into 2 weight-matched groups, and each rat was fitted with an i.p. miniosmotic pump (Alzet Model 2002; Durect Corporation) that delivered either 10 or 20 μmol glucosamine/kg per 24 h. On d 2 of infusion, the rats were tested for leptin responsiveness with half of each treatment group injected with PBS and half with 2 mg/kg leptin. The test was
repeated on d 5, with the PBS and leptin groups switched so that each rat acted as its own control. Leptin significantly inhibited food intake and weight gain of rats tested before the pumps were implanted and in the rats infused with 10 μmol glucosamine/(kg · d) but not of those receiving the 20 μmol glucosamine/(kg · d) (Fig. 5A, B), indicating the development of leptin resistance. These data indicate that activation of the HBP can induce leptin resistance, but further work is needed to identify which aspects of leptin receptor signaling are modified.

The data from the 2 experiments described here indicate that consumption of a 30% sucrose solution causes the relatively rapid onset of leptin resistance and that activation of the HBP can also induce leptin resistance. Further studies are required to confirm that leptin resistance in rats consuming sucrose is due to O-linked glycosylation of signaling proteins, and whether this happens in only selected tissue or in all tissues that are leptin responsive. An additional issue is why consumption of sucrose in solution causes leptin resistance when sucrose consumed as part of a composite pelleted diet does not (33). In a previous study (31), we excluded adiposity as the exclusive cause of diet-induced leptin resistance in rats consuming a choice diet; however, animals that consume liquid sucrose are fatter than their controls, whereas rats fed a pelleted sucrose diet do not overeat, and it is likely that a state of positive energy balance is required for resistance to develop. The pattern of sucrose consumption may be important because sucrose is consumed simultaneously with all other components of a dry diet. By contrast, sucrose that is in solution will be consumed as a bolus, with the ingested sucrose isolated from other nutrients, and it is possible that this intake pattern stimulates pathways such as the HBP, which may then influence leptin signaling.

**The role of TG saturation in leptin resistance**

The potential role of dietary TGs in the onset of leptin resistance first came to light as a result of a study published by...
Banks et al. in 2004 (49), demonstrating that acute infusion or injection of rats with saturated TGs blocks leptin transport across the BBB, whereas the composite free fatty acids are without effect. Further support for this notion was seen in the results of a study published by Shapiro et al. (18), showing that HFr-fed rats, although maintaining normal body weights and leptin levels, were leptin resistant and characterized by significantly elevated circulating TGs. Although elevated TGs may not be the sole mechanism mediating leptin resistance in the case of HFr feeding (see above), this finding is consistent with the notion that elevated TGs may potentially contribute to the effect. A role for TG saturation in this phenomenon is suggested by reports showing that although an HFD containing saturated fats quickly induces obesity in rodents, this is not the case when an equal amount of polyunsaturated fats are substituted in the diet (50,51).

Accordingly, a series of studies was designed to test the hypothesis that leptin resistance can be induced in vivo by low-level administration of saturated TGs by gavage, whereas polyunsaturated TGs should not have this effect (52). The administration of emulsified TGs in small amounts by gavage to normal rats consuming a diet low in fat content permits precise control of the amount of TG administered, and avoids the elevated body fat and leptin levels induced by HFD feeding. Two equicaloric TG emulsions varying in saturation level were administered to rats by oral gavage for a single day (acute), and after a 2-wk washout period, for 11 consecutive days (subchronic). Diluted cream (majority saturated TGs) and fish oil (majority polyunsaturated TGs) emulsions containing 8.36 kJ/mL were administered to groups of adult male rats fed a nonpurified laboratory diet, whereas a third group received equimolar solutions of physiologic saline. In the acute protocol, fasted groups received 3.0 mL of their assigned emulsions or saline a total of 3 times over a 6-h period (20% of total daily energy intake), separated by 3-h intervals. This administration regimen highly significantly elevated circulating TGs in the animals, which was the purpose of the concentrated administration protocol. In the subchronic protocol, the amount of emulsion or saline administered was reduced to 1.0 mL twice daily (4.5% of total daily energy), which did not elevate circulating TGs significantly. Two hours after the final gavage in both the acute and subchronic studies, a leptin injection–feeding test was carried out (1.0 mg leptin/kg body weight i.p.), with feeding monitored at 8- and 24-h post–leptin injection. Feeding after the leptin injection was compared with an earlier feeding test in which physiologic saline only was injected into all groups. The saline and fish oil groups responded to injected leptin with significant inhibition of feeding at 8 and 24 h after both acute and subchronic gavage administration (Fig. 6A, B). In contrast, the cream group showed no effect of leptin on feeding at 8-h postinjection and no effect at 8- and 24-h postinjection after the acute and subchronic administration protocols, respectively. One may speculate that failure of the cream group to respond at 8 h under the acute condition was the result of elevated circulating TGs, which may have impaired leptin transport across the BBB, whereas the complete absence of response by this group at 8 and 24 h under the subchronic condition was the result of more lasting changes induced by TGs at some point in the leptin-response pathway, or in BBB endothelial membrane transport proteins (53). Further research will be required to determine if this is the case. No differences were seen before and after 11-d gavage treatment in

![Figure 5](https://academic.oup.com/advances/article-abstract/4/2/164/4591625/1644591625)

**Figure 5** Food intake (A) and weight gain (B) of rats during the 14 h after an intraperitoneal injection of PBS or 2 mg leptin/kg. Values are means ± SEM for 16 rats receiving 0 μmol glucosamine/kg per 24 h (not infused) and for 8 rats receiving either 10 or 20 μmol glucosamine/kg per 24 h. *Difference between PBS- and leptin-injected rats, P < 0.05.

![Figure 6](https://academic.oup.com/advances/article-abstract/4/2/164/4591625/1644591625)

**Figure 6** Mean 8- and 24-h food intakes of saline-, cream-, and fish oil–gavaged groups of rats after injection of saline or 1.0 mg/kg intraperitoneal leptin. Groups were tested after 1 d (A) and 11 d (B) of daily gavage administration as detailed in the text. The groups were fed nonpurified laboratory diet ad libitum throughout the gavage administration periods and injection testing. Values are means ± SEM, n = 6–7. **Different from saline injection condition: *P < 0.05, **P < 0.01.
body weight or TG or leptin levels of the groups, all of which remained at normal lean levels.

A second series of experiments with the same groups was then conducted to determine if impairments of leptin responsiveness induced by saturated TG administration could affect acute or chronic energy intake by the animals. The groups received 1.0 mL of their respective gavage solutions twice daily for a total of 12 consecutive days while maintained on a nonpurified laboratory diet and again on d 14 of the study. On d 13 and d 15, the groups were offered ad libitum access for 24 h to a purified HFD containing 45% and 60% of energy as fat, respectively (Research Diets, Inc.), in the absence of gavage administration. The cream group consumed significantly more of each HFD over the 24-h test periods than the saline group (Fig. 7). There were no differences in body weight or TG or leptin levels among the groups at this point. The groups were then returned to an ad libitum nonpurified laboratory diet and daily gavage administration for an additional 5 d before being offered continuous ad libitum access to the 45% HFD for an additional 12 d, in the absence of gavage administration. The cream group immediately consumed more of the HFD than did the saline group, with the difference in cumulative energy intake becoming significant at d 8 and d 12 (Table 1). A significant difference in body weight gain from that of the saline group was also detected in the cream group at d 8, and this effect persisted, although not significantly so, until d 12. With the exception of d 12 cumulative energy intake, significant effects were not seen at the same intervals in the fish oil group. It is important to note that this prolonged response to an HFD challenge by the cream group occurred after a prior period of saturated TG administration, implicating more long-term alterations in the leptin transport or response pathway than would be predicted on the basis of BBB transport alone. In summary, it appears that administration of extremely small amounts of saturated TG to normal-weight rats consuming a low-fat diet can induce resistance to the effects of injected leptin, and elevate feeding in response to an HFD, both acutely and in the long term. The findings support the hypothesis that TGs induce resistance to leptin transport at the BBB, and quite likely at other points in the leptin response pathway, with leptin level being the critical factor.

**Nutrients and leptin resistance at the BBB: TGs as an example**

If the level of leptin in the brain is assumed to convey information to the CNS regarding energy reserves, then nutrient status can be shown to modulate that information. This section concentrates on the modulation that occurs at the level of BBB, although modulation likely also occurs at the level of leptin synthesis and secretion and at the level of receptor/postreceptor activation. The principles underlying BBB modulation of leptin transport are that leptin crosses the BBB by way of a saturable transport system and that the transporter is, in turn, regulated (10). This regulation can increase the degree of leptin resistance and, at least in theory, increase the degree of leptin sensitivity that occurs because of BBB transport. Leptin resistance at the BBB has several causes. As a first cause, the saturable nature of the blood-to-brain transport of leptin limits the degree to which leptin can enter and accumulate within the CNS (7,54,55). At levels seen in thin animals, the relation between serum and cerebrospinal fluid (CSF) levels of leptin is near linear, but at levels seen in obese animals there is little increase in CSF leptin levels despite dramatic increases in serum leptin concentrations. The flattening of the serum-CSF curve begins at relatively low serum concentrations of leptin, perhaps at ~300 pmol/L (7,8,56). This means that the peripheral signal to brain as mediated by leptin is greatly attenuated in moderate obesity, and perhaps even at levels of adiposity generally thought to reflect ideal body weight. The signal conveyed to the brain by leptin is most proportional to peripheral events when serum leptin concentrations are low—that is, in lean animals.

Modulators of the leptin transporter are likely also involved as a second cause of leptin resistance. The leptin transport rate is modified by a number of factors, including adrenergics, insulin, glucose, and TGs (49,57–61). TGs may be major modulators of the blood-to-brain transfer of information between the gut and brain as mediated by gastrointestinal hormones. TGs inhibit leptin transport in a dose-dependent manner (49). They are apparently the mechanism by which fasting and starvation modulate leptin transport across the BBB, thus modifying the food-seeking behavior of the animal. In this scenario, serum TGs are elevated during starvation, with TGs inhibiting the anorectic signal to the brain as conveyed by leptin.

Leptin resistance at the CNS receptor level may also be influenced by blood-borne factors, including TGs. Recent
work has shown that serum from animals with DIO induces neuroinflammatory events as evaluated by a model of injury-induced gliosis (62). Leptin accounted for some of this reaction, possibly being mediated through astrocytic leptin receptors (63). Nutrients acting as informational molecules, exemplified by TGs, likely reinforce their influence on brain events by affecting the ability of other gastrointestinal hormones to cross the BBB. Both TGs and starvation stimulate the transport of insulin and ghrelin across the BBB (64,65). Thus, TGs by modulating the influx of gastrointestinal hormones into the brain may be one of a host of circulating factors that help to determine feeding behavior.

TGs may also affect other central actions of leptin in addition to that of feeding. Work has long indicated that hyperlipidemia is associated with cognitive impairments (66–68). TGs, the main lipid involved in the dyslipidemia of metabolic syndrome, in the serum and brain are correlated with cognitive behavior (69,70). Obese mice have learning and memory deficits that can be reversed when they are treated with gemfibrozil, a drug that selectively decreases TG concentrations (71). Furthermore, normal-weight mice given TGs directly into the brain have defects in memory. Some nutritional components may act indirectly to affect feeding, cognition, and other behaviors. For example, recent work has shown that feeding mice extra virgin olive oil can improve cognitive performance (72). Several mechanisms exist by which a diet rich in extra virgin olive oil could affect cognition, including that of affecting TGs. In conclusion, these results show that TGs exemplify what is probably a general phenomenon: the ability of nutrients to modify CNS activities by their ability to affect the blood-to-brain transport of hormones involved in gut-brain axes.

**Conclusions**

The studies reviewed above indicate that specific types of dietary sugars or fats are capable of inducing leptin resistance in the absence of elevated circulating leptin levels and body weight in experimental animals, and can do so relatively rapidly. Moreover, the above studies have identified potential new biological mechanisms that may mediate leptin resistance, including alterations in central and peripheral metabolism and/or leptin receptor signaling. Thus, in addition to the already demonstrated BBB and CNS second messenger mechanisms involved in the development and/or expression of leptin resistance, newly identified potential mechanisms described above include fructose-induced impairment of the AMPK/ACC pathway, increased activation of the HBP pathway with potential increases in O-linked glycosylation of signaling proteins, and long-term alterations of the leptin BBB transport pathway or associated membrane transport proteins by saturated TGs. The identification of these new mechanisms of leptin resistance represents a potential advance in our understanding of the biology of the phenomenon, although a great deal of further study is required to precisely define their role in the effect.

One of the longstanding issues regarding the role of leptin resistance in DIO is whether the effect participates in the onset of obesity or is the result of becoming obese, thereby simply acting, along with other influences, to maintain obesity. This issue has been addressed by several investigators, and hypotheses range from little or no role for leptin resistance (73), to a predisposing effect (74), to a primary role (16) in the onset of obesity. Bearing on this controversy is the issue of whether endogenous levels of leptin at normal body weight participate at all in the regulation of energy intake. Several investigators have suggested that circulating leptin at normal body weight has virtually no catabolic role in the control of energy balance, i.e., has no feeding inhibitory or thermogenic effects in calorically replete normal animals. In contrast, leptin’s absence during states of energy deficit, which signals the need to feed, constitutes the hormone’s primary effect (75,76). If this is the case, then the term leptin resistance is artifactual, since it is based on the predicted loss of leptin sensitivity as leptin levels achieve or exceed the normal range. Actually, there are quite convincing data that leptin does indeed participate in the daily control of energy intake, supplied by studies using leptin receptor antagonists (77–79). These studies show that administration of antagonists to leptin’s action results in significant dose-dependent increases in daily energy intake and weight gain in rodents fed nonpurified laboratory diets, or an HFD. Studies implicating leptin’s involvement in the control of 24-h human energy intake have also been reported (80,81).

In light of the above data, which offer support for the notion that leptin exerts a catabolic effect on energy balance at least within its normal circulating range, we propose the following role for diet-induced leptin resistance in relation to the development of obesity. Because specific dietary components induce leptin resistance rapidly at normal body weight and leptin levels, this effect has the potential to reduce the range within which the antiobesity effects of leptin are normally expressed. In this interpretation, diet-induced leptin.

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**Table 1.** Cumulative energy intake and BW gain of saline-, cream-, and fish oil–gavaged groups of rats during 12 d of ad libitum access to a 45% HFD after 18 d of saline, cream, or fish oil gavage

<table>
<thead>
<tr>
<th>Prior gavage condition</th>
<th>Cumulative energy intake</th>
<th>Δ BW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 1</td>
<td>d 4</td>
</tr>
<tr>
<td>Saline</td>
<td>748.1 ± 28.3</td>
<td>2656.4 ± 114.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>772.7 ± 18.7</td>
<td>2720.0 ± 81.5</td>
</tr>
<tr>
<td>Cream</td>
<td>814.0 ± 35.5</td>
<td>2872.1 ± 80.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = 6–7. No saline or oil emulsions were administered during the 12-d ad libitum HFD test period. * Different from saline: *P < 0.05, **P < 0.01.
resistance results in the loss of leptin’s catabolic effects at an even earlier point than would otherwise be the case in normal-weight animals consuming neutral (non-resistance-inducing) diets and gradually increasing their body weight. Diet-induced leptin-resistant animals, whether being maintained with or challenged with highly palatable diet items, immediately elevate overall energy intake and initiate a chain of events leading to the development of obesity. The earlier identified BBB and CNS resistance mechanisms reviewed above undoubtedly participate as leptin levels increase in response to increasing adiposity. Thus, diet-induced leptin resistance can either initiate the development of obesity, or enhance the predisposition to it, in normal, lean individuals. As such, it represents a newly identified risk factor for obesity inherent in the specific components of the diet itself. Note that this hypothesis is quite consistent with the increasing body of data implicating intake of sugar-containing beverages with increasing rates of obesity in developed societies (82,83) and the role of dietary fat intake, particularly saturated fat, in overeating and weight regain (84,85).

Of course, the weight regulatory system is quite complex, and it is clear that leptin acts in concert with other hunger/satiety hormones in coordinating energy balance. Nevertheless, the above evidence indicates that leptin plays an important role in this process.

In summary, the studies reviewed here show that specific dietary components can induce leptin resistance in relatively short periods at normal body weight and leptin levels. This interpretation suggests that diet-induced leptin resistance is capable of interfering with the catabolic effects on energy intake exerted by leptin at normal body weight levels and can be considered an initiating or predisposing factor for the development of obesity. This possibility is eminently testable in human studies, in which relatively small quantities of commonly used nutritional components can be administered experimentally over relatively short periods, followed by behavioral assessment to determine whether energy intake or preference is altered. These kinds of manipulations in humans would involve no changes in body fat, no administration of exogenous leptin, and only minor alterations in hormone and metabolite levels. Such studies offer the opportunity to learn a great deal more about the involvement of leptin resistance in determining excess energy intake under common feeding conditions, such as multi-item diet selection regimens characteristic of human feeding situations, and about its role in initiating and maintaining obesity.

Acknowledgments
All authors read and approved the final manuscript.

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