Peripheral Signals Conveying Metabolic Information to the Brain: Short-Term and Long-Term Regulation of Food Intake and Energy Homeostasis

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Numerous peripheral signals contribute to the regulation of food intake and energy homeostasis. Mechano- and chemoreceptors signaling the presence and energy density of food in the gastrointestinal (GI) tract contribute to satiety in the immediate postprandial period. Changes in circulating glucose concentrations appear to elicit meal initiation and termination by regulating activity of specific hypothalamic neurons that respond to glucose. Other nutrients (e.g., amino acids and fatty acids) and GI peptide hormones, most notably cholecystokinin, are also involved in short-term regulation of food intake. However, the energy density of food and short-term hormonal signals by themselves are insufficient to produce sustained changes in energy balance and body adiposity. Rather, these signals interact with long-term regulators (i.e., insulin, leptin, and possibly the orexigenic gastric peptide, ghrelin) to maintain energy homeostasis. Insulin and leptin are transported into the brain where they modulate expression of hypothalamic neuropeptides known to regulate feeding behavior and body weight. Circulating insulin and leptin concentrations are proportional to body fat content; however, their secretion and circulating levels are also influenced by recent energy intake and dietary macronutrient content. Insulin and leptin concentrations decrease during fasting and energy-restricted diets, independent of body fat changes, ensuring that feeding is triggered before body energy stores become depleted. Dietary fat and fructose do not stimulate insulin secretion and leptin production. Therefore, attenuated production of insulin and leptin could lead to increased energy intake and contribute to weight gain and obesity during long-term consumption of diets high in fat and/or fructose. Transcription of the leptin gene and leptin secretion are regulated by insulin-mediated increases of glucose utilization and appear to require aerobic metabolism of glucose beyond pyruvate. Other adipocyte-derived hormones and proteins that regulate adipocyte metabolism, including acylation stimulating protein, adiponectin, diacylglycerol acyltransferase, and perilipin, are likely to have significant roles in energy homeostasis. [Exp Biol Med Vol. 226(11):963–977, 2001]

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There is considerable evidence that body weight and body fat content are well regulated. In adult animals and humans, body weight tends to remain within a relatively narrow range, despite large day-to-day fluctuations in the amount of food consumed. Although major changes of body adiposity can be induced in humans and animals by restricting energy intake or by overfeeding, body weight and adiposity return very close to baseline levels when *ad libitum* feeding is resumed (reviewed in Refs. 1–3). Investigations of the regulation of food intake in surgically joined parabiotic animals in which circulating substances can move between animals supported the hypothesis that a humorally transported factor or factors is involved in regulating feeding behavior. For example, when one member of a pair of parabiotic rodents is overfed via stomach tube, the other member of the pair reduces its voluntary food intake (4, 5). The potential humoral signals identified by such experiments were postulated to be either nutrients, nutrient metabolites, or hormones.

Although there are many peripheral signals that can contribute to feeding behavior and body weight regulation, it is important to recognize that short-term and long-term food intake and energy balance are regulated through distinct, but interacting, mechanisms. In this context, some signals (e.g., nutrients and gastrointestinal [GI] hormones) act primarily as determinants of satiety to limit the size of individual meals (see Fig. 1 for overview). These short-term signals have a markedly different function than the long-term regulators of energy homeostasis that are activated in proportion to both body adipose stores and to the amount of energy consumed over a more prolonged period of time. Insulin and leptin are two such long-term signals. These hormones regulate food intake and energy expenditure to

Short-term Signals Regulating Feeding

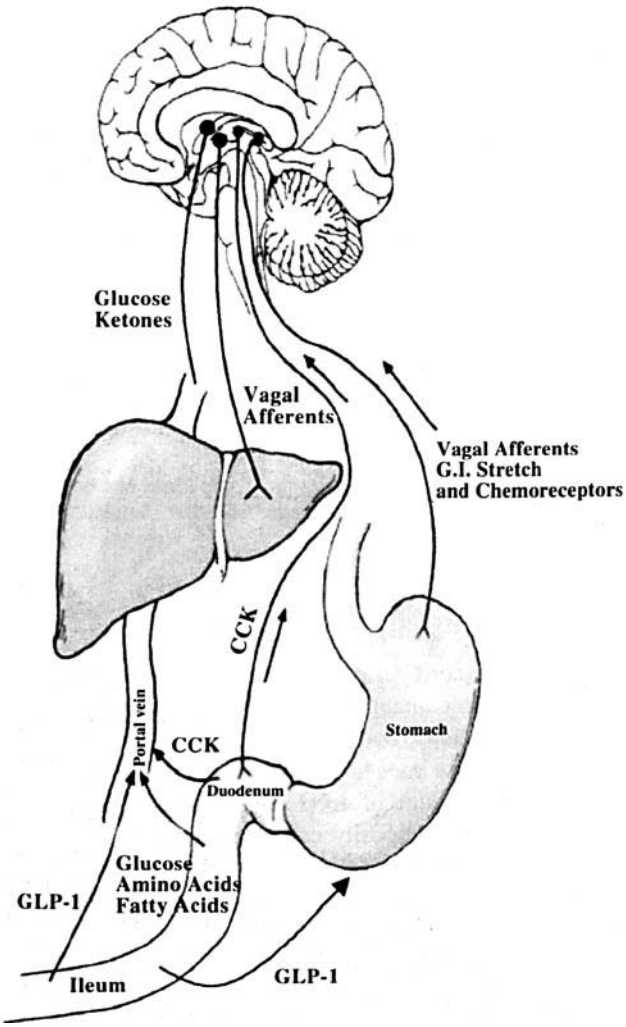

Figure 1. Short-term signals regulating food intake. Signals from the GI tract and the liver are involved in short-term regulation of feeding. Afferent signals travel in vagal nerve fibers from stretch receptors, and chemoreceptors activated by the presence of nutrients in the stomach and proximal small intestine are involved in meal termination. Nutrients arriving via the portal vein may also trigger vagal afferent signals from the liver. Glucose can modulate food intake by acting on glucose-responsive neurons in the CNS. Ketones appear to decrease appetite. In response to nutrient stimulation, the proximal intestine releases cholecystokinin (CCK), which reaches the liver via the portal vein and the CNS via the systemic circulation; CCK may act on CCK-A receptors at both sites to inhibit food intake. Endocrine L cells in the terminal small intestine (ileum) release glucagon-like peptide-1 (GLP-1), which inhibits feeding, most likely at a hepatic site or by inhibiting gastric emptying. The short-term signals by themselves do not produce sustained alterations in energy intake and body adiposity.

ensure that energy homeostasis is maintained and that body weight and adiposity remain relatively constant (see Fig. 2 for overview). In contrast, short-term signals are not primary determinants of body adiposity since they can be overridden by long-term regulatory signals. Nonetheless, the short-term and long-term mechanisms need to function in concert to integrate energy intake and energy expenditure to ensure that energy balance is maintained.

Nutrients

Glucose. A glucostatic hypothesis for the regulation of feeding behavior was proposed by Mayer nearly 50 years ago (6). This hypothesis has recently been revisited and reviewed (7). Hypoglycemia or inhibition of glucose metabolism with the glucose analog 2 deoxy-D-glucose increases food intake in animals (8) and increases hunger sensations and food intake in humans (9). This effect of hypoglycemia or 2-deoxy-D-glucose has been termed glucoprivic feeding. In 1969, Oomura and colleagues (10) reported that populations of neurons in the ventromedial and in the lateral hypothalamus increase their firing rates in response to the application of glucose. These glucose-sensitive/responsive neurons are likely to be responsible for the effects of glucoprivation to induce feeding. Other investigators have suggested that glucoprivic feeding may be regulated by receptors in the hindbrain (11) or in the liver (12). However, destruction of specific hypothalamic neurons by gold-thioglucose administration in mice results in a classic hypothalamic obesity syndrome associated with hyperphagia, and blocks the effect of glucose to suppress food intake (13), suggesting a key role for hypothalamic glucose-responsive neurons in regulating feeding behavior. Interestingly, there appear to be similarities in the metabolic events that activate glucose-responsive neurons in the hypothalamus in those that are involved in glucose-induced insulin secretion in β cells (14), and in the mechanisms by which insulin-mediated glucose metabolism stimulates leptin production by adipocytes (see below).

Although it is clear that an acute marked lowering of circulating glucose concentrations will trigger sensations of hunger, and that infusing glucose decreases food intake in baboons (15) and increases sensations of satiety in humans (16), the role of more subtle physiological changes of glucose in the regulation of feeding behavior has been difficult to characterize. Some studies have suggested that changes of substrate utilization/oxidation are involved in glucose-induced satiety (17). In fact, Mayer (6) proposed that arteriovenous glucose gradients, reflecting increased glucose utilization, were more important than absolute blood glucose concentrations. In these studies, smaller arteriovenous differences were associated with increased hunger, whereas larger arteriovenous differences were associated with satiety.

In addition to actions that limit food intake, changes of glucose or glucose utilization may also be involved in initiation of feeding. In a series of experiments, Campfield and Smith (18) demonstrated that small (10–15 mg/dl) transient decreases of blood glucose preceded spontaneous meal feeding in rats, and that blocking these decreases of circulating glucose prevented meal initiation. In contrast, admin-

istration of another hexose, fructose, does not block initiation of feeding (19). The declines of glucose are preceded by a spike of plasma insulin concentrations, and meal feeding can be induced by mimicking the naturally occurring glucose declines by administering small amounts of insulin. Small decreases of blood glucose have also been observed prior to spontaneous meal consumption in human subjects, suggesting that a similar mechanism may be operative in primates (20). Thus, circulating glucose and changes of glucose metabolism are clearly important in the regulation of food intake; however, the glucostatic and glucodynamic models of food intake regulation are not sufficient by themselves to explain the complex regulation of feeding behavior.

Protein (Amino Acids). There is some evidence in favor of a role for dietary protein intake or increased circulating amino acids in the regulation of food intake. A relationship between fluctuations in serum amino acids and appetite in humans was originally suggested by Mellinkoff *et al.* (21) in 1956 and was recently revisited (22). Dietary protein does induce satiety in the short term (23), and consumption of protein-deficient diets leads to increased appetite for protein-containing foods (24). Although the mechanisms underlying this phenomenon are not well understood, administration of amino acids such as phenylalanine and tryptophan that are precursors to monoamine neurotransmitters suppresses food intake in humans (25). The ratio of plasma tryptophan to other amino acids may influence brain serotonin levels, which are known to have an inhibitory influence on food intake (26). Furthermore, deficiencies of certain amino acids in the diet of rats lead to rapid reduction of food intake that is mediated via specific pathways in the brain (27). Amino acids may influence food intake either via direct actions within the CNS or via receptors located in the liver or portal vein (28).

Fat (Triglycerides, Fatty Acids, and Apolipoproteins). The intravenous infusion of lipid substrates such as Intralipid, along with heparin to release lipoprotein lipase and hydrolyze triglyceride to fatty acids and glycerol, decreases food intake in baboons (15). These data suggest that an increase of circulating lipids, in the absence of GI absorption, regulates feeding. However, it is unclear how much concomitant increases of ketones, resulting from enhanced delivery of fatty acids to the liver, might contribute to the effect of lipid infusion on food intake, since ketones can be used as a metabolic substrate by the CNS and are known to inhibit feeding (29). In addition to the inhibitory effects of fat administration, reducing fatty acid utilization with inhibitors of fat oxidation, mecaptoacetate or methyl palmoxirate, stimulates food intake in animals, a phenomenon known as lipoprivic feeding (30). Like inhibition of glucose metabolism, inhibition of lipid metabolism increases expression of the orexigenic neuropeptide melanin-concentrating hormone in the lateral hypothalamus. However, unlike glucoprivation, it does not increase expression of neuropeptide Y (NPY) or agouti-related peptide in the arcuate nucleus (31), both of which are orexigens. Friedman (32) has proposed that the rate of fatty acid oxidation and its effect on adenylate charge in the liver are involved in regulating food intake.

Transport mechanisms (33) and enzymes for fat oxidation (34) and fat synthesis (35) are also present in the brain, and administration of inhibitors of fat synthesis produces a centrally mediated inhibition of food intake in rodents (35, 36). Another lipid-related product potentially involved in the regulation of food intake is the apolipoprotein, Apo AIV. The production of Apo AIV in the intestine is stimulated by fat absorption, and administration of Apo AIV inhibits food intake (reviewed in Ref. 37). This apolipoprotein is also produced in the hypothalamus, and Apo AIV of central origin may also have a role in food intake regulation (38). Despite the potential for fat and fat metabolism to inhibit food intake, there is abundant evidence that consumption of diets high in energy from fat leads to increased energy intake, weight gain, and obesity in animals and humans (39–42).

Metabolites. In addition to glucose, amino acids, and fatty acids, a number of other metabolic products have effects on food intake. Lactate (43), pyruvate (43), and ketones (29) inhibit feeding in animals. Postprandial circulating lactate concentrations are increased in proportion to the carbohydrate content of meals (44), and could therefore contribute to the short-term inhibition of food intake during carbohydrate consumption. Increased circulating ketones generated in response to moderate or short-term energy restriction do not appear to affect appetite or food intake, since appetite increases dramatically at the same time ketones are increasing. In contrast, the severe ketonemia that occurs during prolonged energy restriction (i.e., starvation) could inhibit hunger occurring under this extreme condition. Similarly, the marked ketonemia accompanying consumption of high fat, very low carbohydrate diets may contribute to the decreased energy intake and weight loss that have been reported to occur during consumption of some popular weight loss diets (45).

Signals from the GI Tract

Mechano- and Chemoreceptors. GI chemoreceptors respond to the nutrient products of digestion (sugars, fatty acids, amino acids, and peptides). In addition, entry of food into the stomach and proximal small intestine activates stretch and mechano-receptors. Signals from these GI receptors are transmitted via vagal afferent nerves to the hindbrain where integration of this visceral input occurs. This provides a pathway whereby the physical and chemical properties of food can have a major role in short-term regulation of food intake by limiting the size of a single meal. These types of signals may also affect energy intake in a subsequent meal (reviewed in Ref. 46). For example, consumption of a large volume meal with a low nutrient/energy density can decrease subsequent food intake over a limited

period of time, usually 1 day. However, when the energy density of food is decreased over a more prolonged period of time by dilution with non-nutritive ingredients and the macronutrient proportions of the diets remain the same, more frequent but smaller meals are consumed such that energy intake remains relatively constant (47, 48). Thus, volume detection does not appear to have a major role in the long-term regulation of energy balance and body adiposity (reviewed in Ref. 48).

Hormones. Numerous GI hormones have been implicated in food intake regulation. With the exception of one of the more recently discovered GI hormones, ghrelin, these peptides uniformly inhibit food intake (reviewed in Ref. 50). It is important to consider that many of these GI peptides and their receptors are also present in regions of the CNS involved in regulating feeding behavior. Most GI peptides that inhibit feeding when administered peripherally also do so, but at much lower doses, when administered directly into the brain. Thus, it has often been unclear if the primary target for these GI peptides is in the periphery, in the CNS, or in both. It is likely that the peripheral and CNS production and actions of GI peptides represent parallel pathways in the modulation of feeding behavior.

CCK. There is a large body of data from studies investigating the role of CCK in food intake regulation (reviewed in Refs. 51 and 52). CCK is released from endocrine cells localized in the mucosal layer of the proximal small intestine. This release is primarily stimulated by dietary fat and by amino acids and small peptides released during protein digestion. Administration of exogenous CCK was first shown to decrease meal size in rats in 1973 (53) and subsequently in nonhuman primates (54, 55) and in humans (56, 57). CCK inhibits food intake by activation of the CCKA receptor subtype (58, 59). Antagonists of CCKA receptor signaling increase meal size in monkeys (60). One animal model of obesity, the Otsuka Long-Evans Tokushima fatty rat, exhibits increased energy intake and has a defect in the CCKA receptor (61). However, other genes appear to be involved in the pathophysiology of obesity and diabetes in this animal model (62). In addition, these rats have defects in CCK-induced insulin secretion, which could also contribute to their obese phenotype (63). CCK is likely to transmit vagal afferent signals to the hindbrain by acting on receptors located in the pylorus and liver. Because CCK is also a potent inhibitor of gastric emptying (65, 65), some of its effects to limit food intake may be indirectly mediated by the retention of food in the stomach.

CCK is also produced in the CNS, is localized in several brain areas involved in food intake regulation (reviewed in Ref. 66), and is released from hypothalamic neurons during feeding (67, 68). Administration of CCK into the cerebral ventricles inhibits food intake in nonhuman primates (69) at doses that are ineffective when given peripherally (70). CCKA receptor-specific agonists reduce food intake (71), suggesting that the central effects of CCK also involve CCKA receptors.

Although CCK reduces meal size, long-term peripheral administration of CCK does not reduce overall energy intake or induce sustained weight loss (72). In a study conducted by West and Woods (73), repeated administration of CCK when meals were initiated decreased meal size in rats; however, the number of meals eaten increased and after an initial period of adaptation, overall energy intake was unaffected and weight loss was minimal. Thus, although CCK functions as a short-term signal to inhibit food intake by inducing satiety and decreasing meal size, over time these short-term actions appear to be compensated for by reduced input from long-term regulators of energy balance (e.g., insulin and leptin), which decrease as a consequence of the CCK-induced reduction of energy intake.

GLP-1. GLP-1 is secreted by the endocrine L cells in the ileum (74) in response to the entry of nutrients into the small intestine. The GLP-1 response to food ingestion may be at least partially mediated via parasympathetic vagal activation (75); however, inhibition of GLP-1 by cholinergic blockade may also be a result of reduced GI motility. GLP-1, along with GIP, can contribute to nutrient-induced insulin secretion (76), and it is therefore considered to be an 'incretin' hor mone. Intravenous administration of GLP-1 has satiating actions in humans (77, 78), with inhibition of gastric emptying (78, 79) being a likely contributor to this effect. However, GLP-1 could also inhibit food intake by binding to GLP-1 receptors on afferent nerves in the liver and/or GI tract and activating vagal afferent nerves to the CNS. Intraventricular administration of GLP-1 inhibits food intake (80, 81), and central administration of GLP-1 antagonists increases feeding in rodents (80, 82), suggesting a role for endogenous GLP-1 in the regulation of food intake. However, since GLP-1 is produced in both the periphery and in hypothalamic neurons, the extent to which GLP-1 from each of these sources participates in the physiological regulation of feeding behavior is unclear.

Gastrin-releasing polypeptide (GRP)/bombesin. GRP, a peptide produced by endocrine cells in the gastric mucosa, is the mammalian homologue of a peptide (bombesin) first isolated from glands in the skin of amphibians (reviewed in Ref. 83). GRP not only regulates secretion of gastrin, but its peripheral administration (and that of bombesin) inhibits food intake in animals (84), and its intravenous infusion reduces appetite and food intake in humans (85). Because GRP/bombesin also potently delays gastric emptying (86), the extent to which these effects on GI motility contribute to the reduction of food intake is uncertain. However, intracerebroventricular injection of bombesin at a dose that has no peripheral effects decreases the size of meals in free-feeding baboons (87), suggesting that GRP-related peptides have a role in the central regulation of food intake.

Ghrelin. Ghrelin, a recently discovered peptide hor-

mone that is structurally related to motilin, is produced by the stomach (88). It was first identified based on its stimulation of growth hormone secretion via a growth hormone secretagogue receptor in animals and humans (reviewed in Refs. 89 and 90), Ghrelin is also produced in the hypothalamus (91). Circulating ghrelin concentrations increase during fasting (92), are reduced by the presence of nutrients in the stomach (92), and are lower in obese versus lean human subjects (93). In contrast to the anorexigenic effects of other GI peptide hormones, peripheral or central administration of ghrelin increases food intake in rodents, whereas administration of antibodies against ghrelin inhibits feeding (92, 94, 95). Central ghrelin administration also increases hypothalamic expression of the NPY (95). Daily peripheral ghrelin administration induces a progressive increase in body weight in rats that could result from a chronic decrease of fat oxidation as indicated by an increased respiratory quotient (92). Because ghrelin-induced increases of adiposity appear to be sustained over 1 week of treatment, there is potential for ghrelin to have a role in long-term body weight regulation.

Other GI peptides. In addition to those discussed above, a large number of other peptides produced by the stomach, intestine, and endocrine pancreas inhibit food intake (reviewed in Refs. 96 and 97). Glucagon and pancreatic polypeptide are secreted during food ingestion from pancreatic islet endocrine A and F cells, respectively (98). Although peripheral or central administration of glucagon (99, 100) or peripheral administration of pancreatic polypeptide (101) can inhibit feeding in rodents, a physiological role for these pancreatic hormones in food intake regulation has not been established. Enterostatin, a pentapeptide that results from trypsin-induced cleavage of procolipase, is produced in response to dietary fat and appears to selectively inhibit fat intake. Chronic administration of enterostatin produces weight loss in animals (reviewed in Refs. 102 and 103). Central or peripheral administration of somatostatin reduces food intake in animals (104) and produces satiety in humans (105). Amylin, which is cosecreted with insulin from pancreatic β cells and a related peptide, calcitonin gene-related peptide, inhibit food intake after peripheral administration in rats (106, 107). Gastric inhibitory polypeptide, also known as glucose-dependent insulinotropic polypeptide (GIP), is secreted from intestinal endocrine K cells after glucose administration or ingestion of high carbohydrate meals (76). Administration of a somatostatin analog, octreotide, in order to inhibit GIP and GLP-1 secretion prevents the subsequent decrease of food intake and increase of hunger ratings normally seen after intraduodenal administration of carbohydrate in humans (108). However, since somatostatin itself has effects on gastric emptying and food intake the extent to which decreased GIP and GLP-1 secretion mediate these effects is uncertain.

In summary, although many GI peptide hormones can potently inhibit food intake, their physiological role in food intake regulation, with the exception of CCK and possibly ghrelin, has yet to be definitively established.

Other Endocrine Regulators of Food Intake and Energy Balance

Cytokines. Peripheral or central administration of a number of cytokines, including IL-6 and TNF-α, inhibit food intake (reviewed in Ref. 109). Although cytokines are certainly involved in the anorexia associated with infection and cancer (110, 111), their role in regulating food intake under physiological conditions (i.e., in the absence of neoplasia or inflammation) is not well understood. Cytokines may also influence food intake indirectly via actions on insulin sensitivity (112) or leptin production (113, 114).

Glucocorticoids. Although glucocorticoids are primarily catabolic in the periphery, they have anabolic effects in the CNS (reviewed in Ref. 115) where they act to increase food intake (116). The changes in hypothalamic neuropeptide systems that inhibit food intake in response to insulin and leptin are for the most part opposed by glucocorticoids, and this is likely to mediate their central orexigenic effects (reviewed in Ref. 117). Glucocorticoid deficiency (Addison's Disease) is associated with anorexia. Although glucocorticoid administration and endogenous overproduction of glucocorticoids (Cushing's syndrome) are associated with hyperphagia (118), the concurrent hyperinsulinemia and hyperleptinemia (119), as well as the peripheral catabolic actions of glucocorticoids, are likely to prevent overt obesity in most Cushing's patients. Glucocorticoids probably interact with insulin and leptin in the long-term regulation of energy intake and body adiposity. For example, adrenalectomy increases sensitivity to the effect of central insulin administration to reduce food intake (120), whereas chronic glucocorticoid administration appears to impair CNS insulin transport (121). Hyperphagia in animals with leptin deficiency or leptin receptor defects is attenuated by adrenalectomy (reviewed in Ref. 122), suggesting important interactions between the effects of leptin deficiency/resistance and the hypothalamic-pituitary-adrenal axis.

Thyroid Hormones. The mechanisms by which thyroid hormones influence feeding behavior are not well understood. Food intake is increased by thyroid administration or endogenous hyperthyroidism; however the increase is likely to be mediated by thyroid hormone perturbations of energy expenditure. That is, thyroid hormones induce a marked stimulation of basal metabolic rate, creating a state of negative energy balance associated with loss of body fat and reduced circulating leptin and insulin, which would lead to increased energy intake. In contrast, hypothyroidism decreases basal metabolic rate and leads to weight gain and reduced food intake. Nonetheless, hypothyroidism does not cause marked obesity, perhaps because weight gain is limited by increased insulin and leptin. In addition, the removal of feedback inhibition of hypothalamic thyrotropin-releasing hormone by the reduced levels of thyroxine oc-

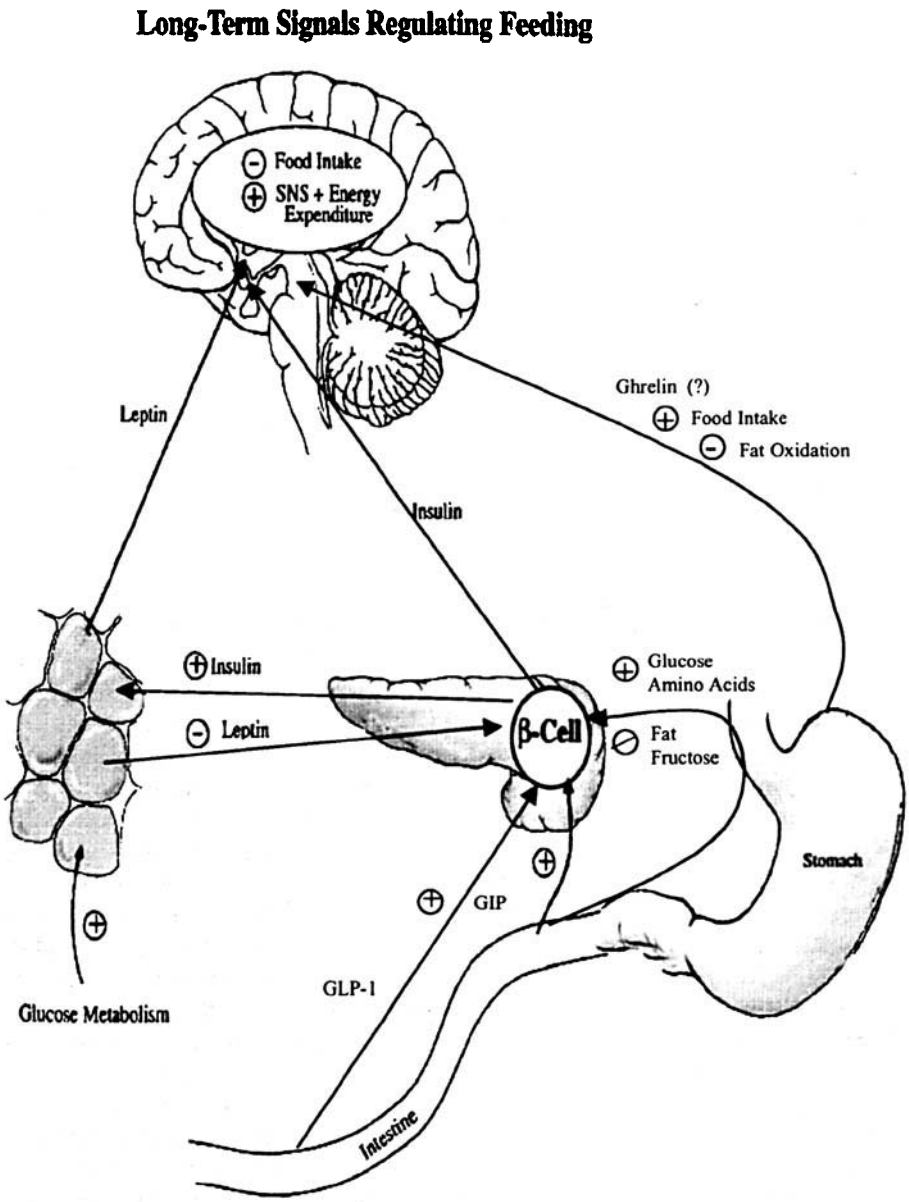

Figure 2. Long-term signals regulating food intake and energy homeostasis. Insulin and leptin are the two most important long-term regulators of food intake and energy balance. Both insulin and leptin act in the CNS to inhibit food intake and to increase energy expenditure, most likely by activating the sympathetic nervous system (SNS). Insulin is secreted from β cells in the endocrine pancreas in response to circulating nutrients (glucose and amino acids) and to the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1, which are released during meal ingestion and absorption. Insulin can also act indirectly by stimulating leptin production from adipose tissue via increased glucose metabolism. In contrast, dietary fat and fructose do not stimulate insulin secretion and therefore do not increase leptin production. There is also evidence that leptin can inhibit insulin secretion from the pancreas. The gastric hormone ghrelin increases food intake and decreases fat oxidation in rodents and may have an anabolic role in long-term food intake regulation. The long-term signals interact with the short-term signals in the regulation of energy homeostasis and appear to set sensitivity to the satiety-producing effects of short-term signal such as CCK.

curring in hypothyroidism could limit weight gain because central administration of thyrotropin-releasing hormone suppresses food intake (123).

Growth Hormone/IGF Axis. The hypothalamic growth hormone-insulin-like growth factor (IGF) axis has important effects on energy balance and nutrient partitioning. Administration of exogenous growth hormone is associated with increased food intake, and central administration of IGF-I, but not IGF-II, inhibits feeding (124). The extent to which this effect is mediated by binding to insulin receptors is unclear. Both growth hormone and IGFs can exert negative feedback inhibition on the somatotrophic axis via growth hormone releasing hormone (GHRH) in the hypothalamus. Since intraventricular administration of GHRH agonists (125) or injection of GHRH into the hypothalamic ventromedial nucleus (126) increases food intake in rats, it is possible that peripheral growth hormone and IGF could modulate feeding behavior through hypothalamic GHRH.

Long-Term Regulation of Food Intake and Energy Balance

Insulin. It was first proposed by Woods and colleagues (127) in the early 1970s that insulin is a long-term regulator of food intake, energy balance, and body adiposity. Since that time, much additional evidence has been generated in support of this hypothesis (128, 129). Insulin secretion from islet β cells of the endocrine pancreas is stimulated by food ingestion. This is a coordinated effect mediated via activation of the parasympathetic nerves innervating the pancreas, the direct effect of incoming nutrients, specifically glucose and amino acids, and the stimulation by incretin hormones such as GIP and GLP-1 (76),

Both fasting plasma insulin levels and insulin responses to meal ingestion are correlated with body adiposity. Accordingly, over a 24-hr period, overall insulin secretion and the concentrations of insulin in the systemic circulation are proportional to both body fat content and to recent carbohydrate and protein intake (44). Dietary fat does not stimulate insulin secretion (44), although the presence of some fatty acids appears to be necessary for the full insulin secretory response to glucose (130).

Insulin receptors have been identified in a number of brain regions implicated in the regulation of feeding behavior, including the arcuate nucleus of the hypothalamus (reviewed in Ref. 131). Although CNS neurons do not produce insulin, this hormone is transported into the brain by a receptor-mediated mechanism that is saturated at high insulin concentrations (132). Insulin transport into the CNS is not rapid, occurring over a period of hours after circulating insulin concentrations increase, consistent with a role for insulin in the long-term regulation of body adiposity rather than as a short-term satiety signal. In 1979, Woods, Porte and colleagues (123) reported that continuous infusion of insulin into the cerebral ventricles of free-feeding baboons induced a sustained suppression of food intake over a period of 20 days. This inhibition of food intake was accompanied by progressive reduction of body weight.

Insulin's effects to decrease food intake involve interactions with several hypothalamic neuropeptides that are also involved in the regulation of feeding behavior by leptin, including the NPY and melanocortin ligands and their receptors (134, 135). In an experiment designed to examine the role of insulin in the brain in the presence of insulin deficiency, insulin was infused into the cerebral ventricles of insulin-deficient diabetic rats at a rate low enough so that circulating insulin or glucose levels were unaffected. The marked increase of food intake known as diabetic hyperphagia was reduced by 50% by insulin infusion, indicating that central insulin deficiency contributed to, but was not solely responsible for, the hyperphagia in this model of diabetes (136). The importance of central insulin signaling in regulating energy balance was reinforced by the recent report that mice with a neuron-specific genetic knockout of the insulin receptor exhibit increased food intake and have larger adipose stores (137). In addition to inhibiting food intake, insulin increases sympathetic neural activity and energy expenditure (138, 139). Thus, insulin can modulate energy balance by inhibiting energy intake and by increasing thermogenesis. An important interaction of insulin as a long-term signal in regulating energy balance with the short-term satiety signal CCK has been demonstrated. Administration of insulin into the CNS of baboons at a dose that by itself did not significantly decrease food intake allowed a subthreshold dose of either intravenous or intraventricular CCK to inhibit feeding (140, 141). In these studies, energy intake decreased by more than 50% with the combination of insulin plus CCK, suggesting that sensitivity to the effect of CCK to induce satiety is enhanced by insulin's action in the brain.

As reviewed above, there is considerable evidence supporting the view that insulin signaling in the brain limits food intake and that over the long term, insulin secretion functions as a negative feedback signal of recent energy intake and body adiposity. However, because insulin also has peripheral anabolic effects that increase lipid synthesis and storage, a misconception that insulin causes weight gain and obesity has evolved. The idea that insulin promotes obesity has led to the promulgation of a number of popular diets that propose to induce weight loss by avoidance of foods that stimulate insulin secretion. These assertions fail to differentiate between insulin responses to meals in which circulating insulin concentrations rapidly rise and then return to basal levels within a short period of time and the chronic hyperinsulinemia that results from β cell compensation for insulin resistance. In fact, in human subjects, increased insulin secretion in response to glucose is predictive of a smaller degree of subsequent weight gain, rather than a factor leading to greater weight gain and obesity (142). In addition, after feeding, insulin is preferentially transported into the hypothalamus compared with other brain areas, and hypothalamic insulin content is increased after high carbohydrate meals, but not after high fat meals (143). This is likely due to the much smaller circulating insulin responses when high fat meals are consumed (44). Lastly, chronic consumption of a high fat diet in dogs impairs brain insulin transport, and the impairment is predictive of weight gain in response to the high fat feeding (144). Together, these effects of reduced insulin secretion and reduced insulin transport into the CNS could contribute to the increased energy intake and obesity observed in animals and humans consuming high fat diets (39–42).

Leptin. *History of leptin.* In 1953, Kennedy (145) proposed that body weight is regulated over a prolonged period of time by a humoral factor produced by adipocytes in proportion to the amount of lipid stored in adipose tissue. The elegant parabiosis experiments conducted by Coleman and coworkers indicated that the genetically obese *ob/ob* mouse failed to produce a factor that inhibits food intake. In contrast, the similarly obese *db/db* mouse appeared to produce such a substance, but did not respond with decreased food intake (146, 147). In a study designed to identify factors produced by adipose tissue from chronically overfed macaques, Wilson and colleagues (148) utilized subtraction cloning to identify cDNA segments coding for overexpressed RNA transcripts. Although a cDNA sequence coding for a transcript with enhanced expression was found, the protein was not identified by this experiment. Leptin was discovered in 1994, when the gene responsible for obesity in the *ob/ob* mouse was positionally cloned (149). This gene is expressed primarily in adipose tissue and codes for a 16-kDa protein now known as leptin. Soon after the leptin receptor gene was cloned in 1995 (150), mutations in this gene were identified in genetically obese *db/db* mice and

fa/fa rats (151, 152). There are several subtypes of the leptin receptor, with the long form (OB-Rb), which utilizes the JAK-STAT signal transduction pathway, appearing to be essential for leptin's central inhibition of food intake (reviewed in Ref. 153).

Leptin action and role in human energy balance. Administration of leptin acutely decreases food intake and induces weight loss in rodents (reviewed in Refs. 154 and 155). Leptin has also been implicated in the regulation of energy expenditure since its administration induces greater weight loss than can be explained solely by the reduction of food intake (156, 157). This enhanced energy expenditure is mediated via activation of the sympathetic nervous system (158, 159). Central administration of leptin into the third ventricle of rhesus monkeys increases circulating levels of the sympathetic neurotransmitter, norepinephrine, within 30 min, and produces a slower onset reduction of food intake that is sustained over more than 24 hr (160).

Humans with mutations causing complete leptin deficiency (161, 162) or with defects in the leptin receptor (163) exhibit marked hyperphagia and severe obesity. Small doses of leptin reduce hyperphagia and cause weight loss comprised exclusively of body fat in leptin-deficient patients (164), whereas administration of leptin to humans without leptin deficiency induces only modest and variable weight loss (165, 166). The observation that leptin levels are elevated in the vast majority of obese individuals has led to the hypothesis that most obese subjects are resistant to the actions of leptin (154). Leptin resistance could result from decreased leptin transport into the CNS (167, 168) or to impaired signaling downstream of the leptin receptor (169, 170). Together, these observations are consistent with the hypothesis that the biological impact of leptin is more pronounced when leptin levels are decreasing than when circulating leptin concentrations are elevated. Supporting this view is the observation that when endogenous leptin levels were chronically decreased in women during prolonged consumption of a moderately energy-restricted diet, their increased sensations of hunger correlated with reduction of plasma leptin levels (171). These results suggest a role for leptin in the regulation of appetite in humans when leptin production is decreased.

An important interaction between leptin as a long-term regulator of energy balance and the short-term satiety signal CCK has recently been demonstrated (172). In this experiment, a dose of peripheral CCK that reduced short-term food intake by more than 50% in fed rats was ineffective at reducing meal size in rats in which circulating leptin levels were reduced by fasting for 48 hr. However, when the fall of leptin in fasted rats was prevented by administering leptin at a low rate in order to match leptin levels in the *ad libitum*-fed animals, the ability of CCK to decrease food intake in these fasted animals was restored. Synergistic actions of leptin and CCK on food intake have been shown in several other studies (173–177), providing evidence for integration of long-term and short-term signals regulating energy balance.

Regulation of leptin production. Circulating leptin concentrations are highly correlated with indices of body fat content in humans (178–180) and in animals (178, 181, 182). There is a gender difference in circulating leptin levels, with 3- to 4-fold higher concentrations in women than in men with a comparable body mass index (183, 184). This difference persists after correction for greater body adiposity in women and does not appear to be explained by an effect of female reproductive hormones; i.e., plasma leptin concentrations do not differ between pre- and postmenopausal women, and hormone replacement therapy does not alter the relationship between leptin and body adiposity (184). It is possible that the gender difference results from inhibition by androgens and/or differences in body fat distribution between men and women.

Despite the close correlation between circulating leptin concentrations and body adiposity, plasma leptin levels decrease independently of modest changes of body fat content during short-term periods of fasting (185, 186) or during restriction of energy intake (171, 187), and they increase after refeeding (186, 188) or during overfeeding (189). These acute, adiposity-independent decreases of leptin production in response to an energy deficit would be expected to promote increased energy intake and energy conservation before body fat stores become significantly depleted. A number of studies have demonstrated that insulin and glucose can modulate leptin secretion. Insulin increases leptin gene expression and leptin secretion *in vitro* and *in vivo* (reviewed in Ref. 155). For example, glucose infusion increases plasma leptin levels in rhesus monkeys (190), and infusion of insulin at rates producing supraphysiological (191) or physiological (192) insulin levels increases circulating leptin concentrations in humans after several hours of infusion. The decrease of plasma leptin during fasting in humans is prevented by infusing glucose at a low rate sufficient to prevent decreases of plasma glucose and insulin. (185). Several studies have shown that the reduced circulating leptin levels observed in response to energy restriction are well correlated with decreases of plasma glucose (171, 187, 193). Circulating leptin levels decline quickly after the onset of hyperglycemia when insulin-deficient diabetes is induced in rodents with the β cell toxin streptozotocin; also, leptin levels are restored with insulin treatment that normalizes plasma glucose (194, 195). Interestingly, when the fall of leptin in diabetic rats is prevented by infusing leptin at a low rate via a chronically implanted osmotic minipump, the hyperphagia characteristic of untreated insulin-deficient diabetes does not develop (196). These results indicate that low leptin levels make an important contribution to diabetic hyperphagia. In another study, preventing the decrease of leptin during fasting in rats impaired the induction of the dopamine receptor-mediated food-seeking behavior known as conditioned place preference, providing further evidence that decreased leptin has a significant biological impact on feeding behavior (197).

The mechanisms by which insulin and glucose regulate

leptin production by adipose tissue are the focus of considerable research. A series of *in vitro* studies conducted in isolated adipocytes demonstrated that when glucose transport or glycolysis is blocked, insulin-stimulated activation of leptin gene expression and leptin secretion are inhibited in proportion to the impairment of glucose utilization (198). This inhibition of leptin expression and secretion occurs despite the presence of insulin in the culture media at concentrations at the high end of the physiological range (198), demonstrating that insulin-mediated glucose metabolism, and not insulin *per se*, stimulates leptin production. Furthermore, insulin-induced activation of the transcriptional activity of the leptin promoter is blocked by inhibition of glucose utilization (199). Other experiments have shown that anaerobic metabolism of glucose to lactate does not stimulate leptin secretion (200) and suggest that glucose must be aerobically metabolized to CO_2 in the mitochondria in order to increase the production of leptin by adipose tissue (201). These results indicate that adipocyte glucose utilization is an important determinant of insulin-mediated leptin gene transcription, mRNA expression, and leptin protein secretion. The effects of energy restriction and refeeding to respectively decrease and increase circulating leptin concentrations independent of adiposity are likely to be a consequence of altered oxidative glucose metabolism in adipose tissue, resulting from fluctuations of insulin secretion and glycemia.

Circulating leptin concentrations exhibit a diurnal pattern with a pronounced nocturnal peak (202). This diurnal pattern is not observed if the subjects do not eat (185, 192), and in fact, leptin concentrations fall and remain low until 4–6 hr after a meal is consumed (44). The timing of the nocturnal peak is dependent on when meals are eaten (203). Accordingly, the diurnal leptin pattern is not a true circadian rhythm, unlike the diurnal patterns of cortisol and growth hormone secretion. Although administration of high doses of exogenous glucocorticoids can increase circulating leptin concentrations (204, 205), a major role for endogenous glucocorticoids as positive regulators of leptin production does not appear likely. Glucocorticoid levels increase during energy restriction and in unregulated diabetes when circulating leptin concentrations are markedly reduced (187, 195). Furthermore, the 24-hr leptin pattern is still present in subjects with an absent 24-hr cortisol rhythm due to adrenal insufficiency and it is unaffected by cortisol infusions designed to mimic or reverse the timing of the normal circadian cortisol rhythm (206). Nonetheless, it is possible that cortisol and growth hormone can modulate the diurnal leptin pattern via their effects on insulin sensitivity (207, 208). However, the most important determinant of the both the diurnal leptin pattern and the influence of energy intake on circulating leptin concentrations in humans appears to be the effect of insulin to stimulate glucose metabolism in adipose tissue (155, 209).

If insulin and adipocyte glucose metabolism regulate leptin production *in vivo*, then it would be reasonable to propose that consumption of high fat meals that induce less insulin secretion and smaller glucose excursions would lead to lower leptin production and reduced circulating leptin concentrations. In several early studies that examined only morning fasting leptin concentrations, no effect of dietary fat content was observed (180, 186). However, in a study examining circulating leptin concentrations over a 24-hr period, consumption of high fat meals resulted in significant reductions in leptin levels, with the largest occurring 4–6 hr after each meal when compared with the same subjects consuming high carbohydrate meals (44). Since both the amplitude of the nocturnal peak of leptin and overall leptin production are significantly reduced by consumption of high fat meals, this decrease, along with reduced insulin secretion, could contribute to the well-known effects of high fat diets to promote increased energy intake, weight gain, and obesity in humans and animals (39–42).

Although glucose infusion or ingestion of carbohydrate meals with high glucose content enhance insulin secretion and consequently leptin production, another major source of dietary carbohydrate, fructose, does not directly stimulate insulin secretion from pancreatic β cells (210). Accordingly, intravenous infusion of fructose does not increase insulin secretion or circulating leptin concentrations in rhesus monkeys, whereas leptin levels increase progressively during infusion of a comparable amount of glucose (190). In human subjects, insulin responses were reduced and over a 24-hr period, circulating leptin levels were lower when a fructose-sweetened, rather than an isocaloric glucose-sweetened, beverage was consumed with each meal (211). Because insulin and leptin function as long-term regulators of energy balance, consumption of diets with a high percentage of energy derived from fructose could lead to increased energy intake and obesity (212).

Other Adipocyte Factors Involved in the Regulation of Energy Balance and Insulin Action

In addition to leptin, a number of other factors secreted by adipocytes or that regulate adipocyte metabolism have been implicated in the regulation of energy balance. For example, acylation stimulating protein (ASP), which is produced by adipocytes as a result of interaction of complement factor C3, factor B, and adipsin, has a role in increasing the efficiency of triacylglycerol synthesis (reviewed in Refs. 213 and 214). Mice that lack the ability to synthesize ASP have delayed postprandial lipid clearance (215). However, ASP deficiency also has a major impact on energy balance and insulin action. Despite increased energy intake, C3/ASP knockout mice have significantly reduced adipose tissue depots compared with wild-type animals fed either chow or high fat diet, indicating that these animals have elevated energy expenditure (216). In addition, the knockout animals have reduced fasting insulin levels and improved glucose tolerance (215, 216). Interestingly, mice with a knockout of another protein involved in triacylglycerol synthesis, the enzyme diacylglycerol acyltransferase,

are also lean and resistant to diet-induced obesity (217). Enhancement of adipocyte lipolysis can have a similar effect on energy balance. This is indicated by the finding that mice with a knockout of the gene for perilipin and hence an elevated activity of hormone-sensitive lipase are hyperphagic and lean with smaller adipocytes and that the absence of perilipin reverses obesity even in leptin receptor-deficient *db/db* mice (218, 219).

Another adipocyte protein that is of considerable interest with regard to the regulation of energy balance and insulin action is adiponectin (220, 221), which is also known as gACRP30, a cleavage product of adipocyte complement-related protein 30 (222), and as adipoQ (223). Circulating adiponectin levels are reduced in obese humans (221) and rhesus monkeys (224). In humans, adiponectin levels are negatively correlated with fasting insulin concentrations and positively correlated with insulin sensitivity (225), and the decline of adiponectin in rhesus monkeys coincides with the onset of insulin resistance (224). Administration of adiponectin/gACRP30 induces weight loss in mice consuming a high fat, high sucrose diet without decreasing food intake, an effect that is associated with increased fatty acid oxidation in muscle (226). Fatty acid oxidation is also increased and associated with a lean phenotype in acetyl-coenzyme A carboxylase 2 knockout mice (227). Interfering with adipocyte development appears to have a similar effect, as shown by the observation that mice lacking a protein involved in adipogenesis, Hmgic, are resistant to diet-induced obesity, and the absence of Hmgic reduces obesity in leptin-deficient (*ob/ob*) mice (228). Another novel adipocyte factor, resistin, is reported to be downregulated by PPARγ and increased in obese, insulin-resistant animals and therefore has been implicated in the insulin resistance of obesity (229). However, a recently published study has reported that resistin expression is decreased in several obese rodent models and increased by insulin-sensitizing PPARγ agonists (230), bringing into question the hypothesis that insulin resistance in obesity is mediated by resistin.

Summary

Short-term signals are primarily from the GI tract (e.g., CCK and GI stretch receptors) and are involved in promoting sensations of satiety that lead to meal termination. These short-term signals by themselves are not sufficient to regulate energy balance and body adiposity. The long-term signals insulin and leptin are produced and circulate in proportion to recent energy intake and body adiposity. Together, the short- and long-term signals interact to regulate energy balance in that insulin and leptin appear to determine the sensitivity of the brain to the satiety-producing effects of the short-term signals from the GI tract. In addition to the critical role of leptin in regulating food intake and energy expenditure, a number of other adipocyte hormones and proteins have recently been implicated in the regulation of energy balance and insulin action.

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