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Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin

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Adipose tissue performs complex metabolic and endocrine functions. This review will focus on the recent literature on the biology and actions of three adipocyte hormones involved in the control of energy homeostasis and insulin action, leptin, acylation-stimulating protein, and adiponectin, and mechanisms regulating their production. Results from studies of individuals with absolute leptin deficiency (or receptor defects), and more recently partial leptin deficiency, reveal leptin's critical role in the normal regulation of appetite and body adiposity in humans. The primary biological role of leptin appears to be adaptation to low energy intake rather than a brake on overconsumption and obesity. Leptin production is mainly regulated by insulin-induced changes of adipocyte metabolism. Consumption of fat and fructose, which do not initiate insulin secretion, results in lower circulating leptin levels, a consequence which may lead to overeating and weight gain in individuals or populations consuming diets high in energy derived from these macronutrients. Acylation-stimulating protein acts as a paracrine signal to increase the efficiency of triacylglycerol synthesis in adipocytes, an action that results in more rapid postprandial lipid clearance. Genetic knockout of acylation-stimulating protein leads to reduced body fat, obesity resistance and improved insulin sensitivity in mice. The primary regulator of acylation-stimulating protein production appears to be circulating dietary lipid packaged as chylomicrons. Adiponectin increases insulin sensitivity, perhaps by increasing tissue fat oxidation resulting in reduced circulating fatty acid levels and reduced intramyocellular or liver triglyceride content. Adiponectin and leptin together normalize insulin action in severely insulin-resistant animals that have very low levels of adiponectin and leptin due to lipoatrophy. Leptin also improves insulin resistance and reduces hyperlipidemia in lipoatrophic humans. Adiponectin production is stimulated by agonists of peroxisome proliferator-activated receptor-gamma; an action may contribute to the insulin-sensitizing effects of this class of compounds. The production of all three hormones is influenced by nutritional status. These adipocyte hormones, the pathways controlling their production, and their receptors represent promising targets for managing obesity, hyperlipidemia, and insulin resistance. Curr Opin Lipidol 13:51–59.

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Abbreviations

ASP	acylation stimulating protein
BMI	body mass index
CNS	central nervous system
DGAT	diacylglycerol acyltransferase
PPAR	peroxisome proliferator-activated receptor
TNFα	tumor necrosis factor-alpha

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Introduction

In the 7 years since the discovery of leptin it has become abundantly clear that adipose tissue, long considered to be a generally passive repository for stored triglycerides, carries out a large number of intricate metabolic, paracrine, and endocrine functions. Moreover, there is an increasing body of evidence that adipose tissue has a pivotal role, in concert with central nervous system (CNS) mechanisms [1,2••], not only in the regulation of energy homeostasis, but also of whole-body insulin action. Recent experiments, in which a number of non-secreted adipocyte proteins are either knocked out or overexpressed in mice, have demonstrated that glucose transporters (GLUT4 [3]), key metabolic enzymes (diacylglycerol acyltransferase [4], acetyl-CoA carboxylase [5], hormone sensitive lipase [6]), protein coating lipid droplets (perilipin [7,8]), and transcription factors (Hmgic [9], FOXC2 [10]) are involved in the regulation of energy metabolism, body adiposity, and insulin sensitivity.

Hormones and cytokines produced by adipose tissue have wide-ranging effects on food intake, energy expenditure, and carbohydrate and lipid metabolism. These secreted factors include tumor necrosis factor-alpha (TNFα), interleukin-6, plasminogen activator inhibitor 1, angiotensin II, leptin, acylation stimulating protein (ASP), and adiponectin (also known as Acrp30, AdipoQ, apM1) (see [11,12]). One novel adipocyte hormone, resistin, is postulated to be a key factor connecting obesity with insulin resistance since its expression and circulating levels are increased in obese, insulin-resistant mice and inhibited by insulin-sensitizing peroxisome proliferator-activated receptor (PPAR) agonists [13]. Although some subsequent reports examining resistin gene expression in rodents [14] and humans [15] have questioned its importance, it is nonetheless evident that substances produced by adipose tissue are required for normal body weight regulation and glucose homeostasis. For example,

humans and animals that lack adipose tissue due to congenital or acquired lipoatrophy are severely insulin resistant [16,17], a condition that can be reversed in a dose-dependent manner by transplantation of adipose tissue in fat-deficient animals [18]. Replacing leptin in either lipoatrophic patients or mouse models reduces insulin resistance, but does not fully restore normal insulin sensitivity [19••,20]. Similarly, administering leptin to leptin-deficient *ob/ob* mice acutely reduces their hyperglycemia beyond the reduction observed in pair-fed animals, but does not normalize circulating insulin levels [21]. Thus while leptin clearly has a role in insulin resistance in this model, factor(s) other than leptin are also involved in coupling insulin resistance to the obese state. This review will focus primarily on the literature investigating the biology of leptin and two other adipocyte hormones, ASP and adiponectin, that have recently been implicated in regulating energy homeostasis and insulin action. Emphasis will be given to the nutritional regulation of these hormones, the known and likely mechanisms controlling their production, and their roles in glucose homeostasis.

Role of leptin in energy homeostasis

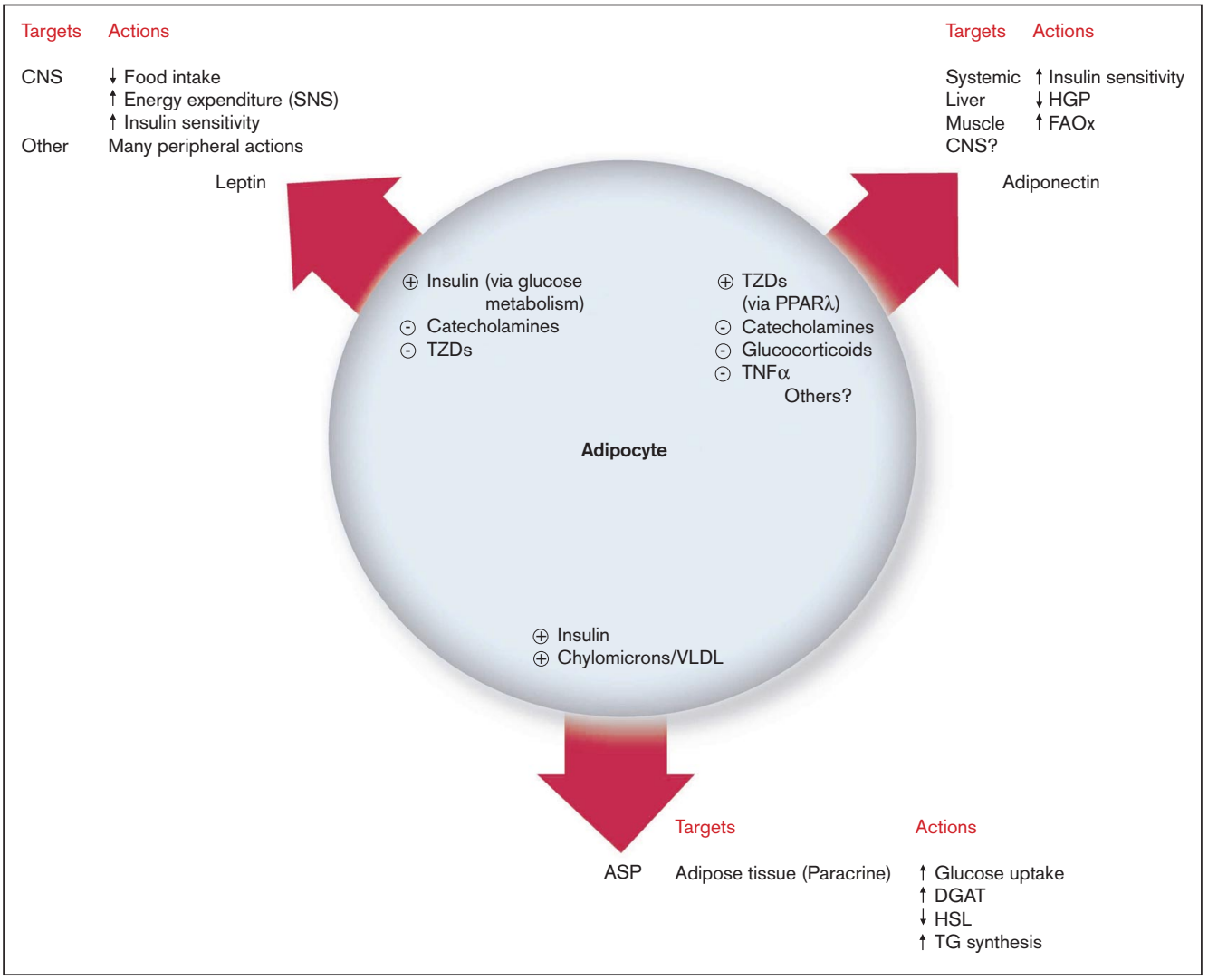
A large body of evidence indicates that leptin, along with insulin, has actions within the central nervous system to inhibit food intake and activate thermogenesis [12,22•]. Leptin and insulin function as critical signals to the brain in the long-term regulation of energy homeostasis and body adiposity, and do so in part by interacting with short-term signals of satiety emanating from the gastrointestinal tract such as cholecystokinin [23,24•]. Interestingly, the CNS effects of insulin and leptin to inhibit food intake may share a common signaling pathway through phosphatidylinositol 3-kinase [25••]. Leptin also has significant effects on hepatic insulin action and peripheral glucose utilization that appear, for the most part, to be mediated through CNS mechanisms [26,27]. Humans and animals with mutations that result in an absolute deficiency in the ability to produce leptin [28] or with genetic defects in the leptin receptor [29] are markedly hyperphagic and morbidly obese. The administration of low doses of leptin to individuals with genetic leptin deficiency diminishes hyperphagia and induces weight loss that consists primarily of excess body fat [30]. In contrast, administration of long-acting forms of leptin to humans without leptin deficiency induces only modest and variable weight loss [31] or no weight loss [32]. In other recent clinical studies, leptin administration improved insulin resistance and hyperlipidemia in patients with lipoatrophy [20], produced a moderate suppression of appetite in obese volunteers [33], but did not affect cardiovascular or autonomic parameters in lean volunteers [34]. The observation that leptin levels are elevated in proportion to body adiposity in nearly all obese individuals has led to the generally accepted idea that most obese individuals are leptin resistant [35]. Resistance to the actions of leptin could be caused by decreased leptin transport into the CNS [36,37] or to reduced signaling distal to the leptin receptor [38,39]. It has also been suggested that apparent resistance to leptin in obese individuals may reflect a primary biological role of leptin, when decreased, to serve as a signal of reduced energy intake and lowered body fat stores [24•,40]. In contrast, excess leptin appears to have less physiological significance. In agreement with this hypothesis, a relative deficit in the ability to produce leptin has a biologically significant impact in humans. It was recently reported that the heterozygous relatives of patients with absolute leptin deficiency have a high rate of obesity (body mass index, BMI >30 kg/m^2), relatively low leptin levels for their BMI, and a greater degree of body adiposity (% body fat) than would be predicted by their BMI compared with matched controls or their homozygous wild-type relatives [41••]. This particular subset of obese individuals may be more responsive to leptin therapy than unselected obese individuals.

Although fasting leptin levels are, in general, proportional to body fat mass, circulating leptin concentration decreases acutely in response to fasting or restriction of energy intake to a much larger extent than would be expected for smaller reductions of adiposity [12]. This adiposity-independent decline of leptin would presumably help to ensure that increased energy intake is triggered well before body energy stores are compromised. Accordingly, the physiological and behavioral consequences of changes in circulating leptin concentrations are more pronounced when leptin levels are declining than when they are elevated. In animal studies, decreases in leptin mediate changes in food intake [42,43], food-seeking behavior [44], energy expenditure [45] and neuroendocrine function [46], whereas increases in leptin associated with overfeeding or obesity appear to have substantially less of a biological impact. In humans, the reduction in plasma leptin levels during prolonged consumption of a moderately energy-restricted diet are correlated with increased sensations of hunger [47], suggesting a role for leptin in the regulation of appetite in humans when leptin production is decreased. Thus, the major role of leptin in regulating food intake and energy expenditure appears to be in the adaptation to reduced energy intake and body fat stores rather than as a restraint to limit energy intake and obesity [24•,40,46].

Nutritional regulation of leptin production

As discussed above, circulating leptin is regulated independently of changes in body adiposity by recent energy intake. There are considerable data to support the idea that changes in insulin and glucose are the major mediators of the effects of energy intake on leptin

Figure 1. Actions of leptin, acylation stimulating protein and adiponectin



Leptin acts within the central nervous system (CNS) to inhibit food intake and increase energy expenditure, perhaps via its effects to activate the sympathetic nervous system (SNS). Leptin also increases insulin sensitivity, an effect that may be largely mediated via CNS mechanisms. Leptin receptors are also found in numerous peripheral tissues where it exerts diverse effects. Leptin secretion is primarily mediated by changes in adipocyte glucose metabolism driven by increases or decreases in meal-induced insulin secretion. Catecholamines and thiazolidenediones (TZDs) have been reported to inhibit leptin production, however the physiological role of these mechanisms has not been definitively established. Acylation stimulating protein (ASP) increases triglyceride (TG) synthesis by increasing adipocyte glucose uptake, activating diacylglycerol acyltransferase (DGAT), and inhibiting hormone sensitive lipase (HSL). ASP deficiency results in obesity resistance and increased insulin sensitivity. ASP production is stimulated by insulin and by the presence of chylomicrons/VLDL following meals. Adiponectin increases insulin sensitivity and lowers glucose levels, possibly by decreasing hepatic production (HGP) and increasing fatty acid oxidation (FAOx) and lowering intramyocellular lipid content. Adiponectin could effect insulin action via CNS mechanisms. Adiponectin production is stimulated by TZDs (via peroxisome proliferator-activated receptor gamma, PPARγ) and inhibited by catecholamines, glucocorticoids, and TNFα.

production by adipose [12,24•]. Results from in-vitro experiments in isolated adipocytes demonstrate that the effect of insulin to increase glucose metabolism, rather than activation of insulin signal transduction *per se*, mediates the stimulatory effects of insulin on leptin gene expression and leptin secretion [48]. Increased glucose metabolism also mediates the effects of insulin to stimulate the transcriptional activity of the leptin promoter [49]. Recent data suggest that increased glucose metabolism mediates the effects of glucose and insulin infusion to increase circulating leptin concentrations in humans [50•]. Other studies indicate that anaerobic glucose utilization is insufficient to increase leptin secretion [51] and suggest that oxidative mito-

chondrial metabolism to CO_2 is necessary to increase leptin production [52]. Accordingly, the effects of fasting and feeding to regulate circulating leptin concentrations, independently of changes in body adiposity, are probably secondary to changes of aerobic glucose metabolism in adipose tissue attributable to transitional changes of ambient glucose and insulin levels.

In humans consuming meals on a regular schedule, circulating leptin concentrations display a diurnal rhythm, with a nocturnal peak shortly after midnight and a mid-morning trough typically seen between 10.00 am and 12.00 noon [53]. The timing of the diurnal pattern is contingent on the times that meals are consumed [54]. In fasting individuals, circulating leptin levels decline and remain low [55,56] until 4–6 h after a meal is eaten [54] or glucose and insulin are infused [56]. Thus, the diurnal pattern of circulating leptin concentrations is not an actual circadian rhythm, as are the diurnal rhythms of plasma cortisol and growth hormone levels, but is a consequence of the dynamics of daily energy intake. Although administration of exogenous glucocorticoids can increase circulating leptin concentrations, endogenous glucocorticoids are not likely to have more than a modulatory role in the regulation of leptin production because during energy restriction, when leptin levels are markedly decreased, cortisol levels are increased [57]. In addition, the diurnal pattern of circulating leptin is present in patients with adrenal insufficiency (Addison's disease) and its timing is not affected by altering the diurnal cortisol rhythm [58,59].

Since insulin and its effects on adipocyte glucose metabolism appear to regulate leptin production, we hypothesized that alterations in dietary macronutrient intake and its effects on postprandial glycemic responses and insulin secretion would have a major influence on leptin and its diurnal pattern. This hypothesis was supported by the results of a study comparing circulating leptin concentrations during consumption of high fat/low carbohydrate versus high carbohydrate/low fat meals. When high fat meals were consumed, both the amplitude of the nocturnal leptin peak and 24 h circulating leptin levels were reduced, with the largest differences (40–60%) observed 4–6 h after each meal [60]. In contrast to glucose, another major source of dietary carbohydrate, fructose, does not directly stimulate insulin secretion. In a recent study postprandial glucose and insulin responses were reduced and circulating leptin levels were lowered by 30% over a 24 h period in human volunteers following consumption of fructose-sweetened beverages, compared with isocaloric glucose-sweetened beverages, with three meals [61]. The reductions in leptin production after dietary fat or fructose consumption, along with reduced insulin secretion, could contribute to the recognized effects of high fat diets to promote increased food intake, weight gain, and obesity, and suggest that diets containing a high percentage of energy derived from fructose could have similar effects.

Acylation stimulating protein

ASP is produced by adipocytes as a result of an interaction of complement factor C3, factor B, and adipsin (factor D) resulting in the formation of the C3 derivative, C3a-des-Arg or ASP. ASP increases the efficiency of triacylglycerol synthesis in adipocytes via its paracrine/autocrine actions to stimulate adipocyte glucose uptake, activate diacylglycerol acyltransferase (DGAT), and inhibit the activity of hormone sensitive lipase [62,63•]. Mice that are genetically deficient in C3, and therefore unable to synthesize ASP, have delayed postprandial lipid clearance [64] and exogenous ASP administration increases triglyceride and free fatty acid clearance in normal and obese mice [65,66], suggesting a role in postprandial lipid disposition. Evidence that ASP may be involved in regulating human lipid metabolism is provided by a recent report that circulating ASP levels are influenced by genes that also affect total cholesterol, LDL, and triglycerides [67]. ASP deficiency, however, may also have a major impact on energy homeostasis and insulin action. ASP/C3 knockout mice, when compared with wild-type animals, are hyperphagic, yet have significantly reduced adipose tissue depots and are resistant to weight gain induced by feeding a high fat diet [68••]. Recent data indicate that these animals have elevated energy expenditure [69]. C3/ASP knockout animals also have reduced fasting insulin levels and improved glucose tolerance [60,64]. In humans, circulating ASP levels were inversely correlated with glucose disposal during a euglycemic clamp [70] and C3 was shown to be inversely related to insulin sensitivity as assessed by a clamp or by fasting insulin concentrations, independently of body adiposity [71]. Interestingly, mice deficient in DGAT, another adipocyte protein involved in triacylglycerol synthesis that is regulated by ASP, have a similar lean, obesity-resistant phenotype [4]. In summary, ASP promotes storage of energy as fat whereas interfering with ASP (or DGAT) production attenuates lipid storage and leads to obesity resistance and improved insulin sensitivity.

Regulation of acylation stimulating protein production

Levels of ASP, and its precursor, C3, are increased in obese humans [63•,72] and are reduced after fasting or weight loss [73]. Plasma ASP concentrations were recently shown to increase in patients with type-2 diabetes following sulfonylurea treatment to improve glycemic control [74]. Although systemic circulating ASP does not change after an oral fat administration in humans [70], increased postprandial ASP release can be

measured in venous plasma from subcutaneous adipose tissue with peak release 4–5 h after meals [75]. Interestingly, this time period is similar to that for peak increases in circulating leptin after meals, which are known to be regulated by meal-induced insulin secretion (see above). The changes in ASP production in response to fasting and food ingestion could be mediated by insulin, which has been shown to increase ASP secretion in isolated adipocytes at fairly high concentrations [76]. An effect of circulating lipids to stimulate postprandial ASP production, however, is likely to be more important, since incubation of isolated human adipocytes with chylomicrons potently stimulates ASP release [76,77], an effect that appears to require de-novo protein synthesis [78]. Retinoic acid has also recently been reported to increase ASP production from adipocytes *in vitro* [78]. Additional work is needed to more fully understand the nutritional regulation of ASP production and its underlying mechanisms.

Adiponectin/ACRP30/adipoQ

Another adipocyte protein that is of considerable interest with regard to the regulation of energy balance and insulin action is adiponectin. This large molecular weight protein, which is structurally related to collagen and TNFα, was identified by several laboratories in the mid-1990s and hence has multiple names (complement-related protein 30 (ACRP30) [79], adipose most abundant gene transcript (apM1), adiponectin [80], adipoQ [81], and gelatin-binding protein (gdp28) [82]). For the purpose of simplicity and because the majority of the papers cited refer to the protein as adiponectin, this designation will be used here. Circulating adiponectin concentrations are decreased in obese individuals [83] and the reduction was proposed to have a role in the pathogenesis of atherosclerosis and cardiovascular disease associated with obesity and other components of the metabolic syndrome [84,85]. This idea is supported by reports that adiponectin has effects considered to be protective against cardiovascular disease [86,87] and that genes influencing circulating adiponectin concentrations exhibit pleiotropic genetic effects on serum HDL and triglyceride levels [88•].

Recent evidence has also suggested an important role for adiponectin in the regulation of insulin action and energy homeostasis and low levels of adiponectin have been proposed to be a critical link between obesity and insulin resistance [89]. Circulating adiponectin levels [90] and adiponectin gene expression in adipose tissue [91] are reduced in patients with type 2 diabetes. Circulating adiponectin levels in humans are negatively correlated with fasting insulin concentrations and positively correlated with insulin sensitivity, as assessed by glucose disposal during euglycemic, hyperinsulinemic clamps and the relationship between adiponectin and insulin action is independent of body adiposity [92••]. Furthermore, a decline in circulating adiponectin levels coincides with the onset of insulin resistance and the development of type 2 diabetes levels in obese rhesus monkeys [93••], a model of adult-onset obesity that exhibits a similar progression of the insulin resistance syndrome observed in humans [94]. Lastly, a genome-wide scan examining the loci influencing six traits associated with obesity and insulin resistance reported a quantitative trait locus on chromosome 3 in the region of the adiponectin gene with LOD scores of 2.4–3.5 [95••].

Actions of adiponectin

Administration of the globular region of adiponectin, gAcrp30, to mice has a number of interesting actions including induction of weight loss in animals consuming a high fat, high sucrose diet without decreasing food intake, an effect that was associated with reductions in circulating fatty acids and increased fatty acid oxidation in muscle [96••]. Recombinant adiponectin reduces serum glucose in normal mice and in mouse models of diabetes, without stimulating insulin secretion, although in contrast to the aforementioned study, the full-length protein was required to lower glucose [97••]. Adiponectin also enhances insulin suppression of glucose production by isolated hepatocytes, suggesting it may lower glucose by acting directly on the liver. In addition, adiponectin improves glucose tolerance in insulin-resistant *db/db* mice and reduces insulin resistance associated with low adiponectin levels in mice with either lipoatrophy or obesity-induced insulin resistance, although complete reversal of insulin resistance in lipoatrophic animals required co-administration of leptin [19••]. The improvements in insulin sensitivity were associated with decreased triglyceride content of muscle and liver and increased fatty acid oxidation in muscle, and accompanied by increased expression of genes for proteins involved in fatty acid transport and utilization [19••]. Although the site and mechanism of adiponectin's actions on whole-body glucose metabolism remain unknown, and the receptor(s) has not been identified to date, available data suggest that adiponectin reduces hepatic glucose production and increases muscle glucose utilization, perhaps by increasing fat oxidation [96••] and thereby reducing circulating free fatty acid (FFA) levels and intramyocellular lipid accumulation [98]. Additional effects in the CNS cannot be excluded since the CNS has been shown to have a significant role in the regulation of insulin sensitivity [1,2••].

Regulation of adiponectin

As discussed above, circulating adiponectin concentrations are reduced in obese mice [19••,82] humans [84,92••] and rhesus monkeys [93••]. This observation is in marked contrast to the increased levels of many other adipocyte derived hormones (such as leptin, TNFα,

plasminogen activator inhibitor 1, and ASP) in obese animals and humans. Conversely, weight loss increases circulating adiponectin concentrations in nondiabetic and diabetic humans [90,94]. Serum adiponectin levels also increase during fasting in mice [96••]. Similar to leptin [57,99], circulating adiponectin concentrations are higher in women than in men, and there is a significant gender difference in the adiposity-independent response of circulating adiponectin during acute energy restriction (P.J. Havel, unpublished observation).

Although there is little information available on the mechanisms regulating adiponectin production, several studies have reported that thiazolidenedione agonists of PPARγ increase both adiponectin gene expression and circulating adiponectin levels in animals, humans, and *in vitro* [19••,96••,100••]. This effect of PPARγ agonists suggests that increased adiponectin production is a mechanism by which this class of compounds acts in adipose tissue to increase whole-body insulin sensitivity [101]. Interestingly, subjects with severe insulin resistant diabetes due to dominant negative mutations that inactivate PPARγ are not obese [102], but have very low circulating adiponectin levels (S.R. O'Rahilly, personal communication).

Adiponectin expression is increased with markers of adipocyte differentiation and its secretion is enhanced by the calcium ionophore, ionomycin, and inhibited by cAMP analogs [103]. β-Adrenergic agonists, activators of adenylate cyclase [104], TNFα [100••,103], and glucocorticoids [105] are also reported to inhibit adiponectin gene expression and secretion, suggesting that decreased adiponectin production could play a role in catecholamine, TNFα, or glucocortcoid-induced insulin resistance. Clearly, additional work is needed to understand the physiological mechanisms involved in regulating the production of this potentially very important adipocyte hormone and its paradoxical reduction in obesity, which appears to lead to insulin resistance and type 2 diabetes. For example, the role of body fat distribution is of interest, since centrally distributed, intra-abdominal body fat is more closely associated with insulin resistance than subcutaneous fat [106], suggesting that there may be fat depot-specific differences in adiponectin production.

Conclusions

A large number of proteins produced by adipose tissue, both intracellular and secreted, function in concert with the CNS, liver, and muscle in the coordination of energy homeostasis and fuel metabolism. Among these proteins, alterations in the production of the hormones, leptin, ASP, and adiponectin appear to have substantial effects on both body adiposity and insulin sensitivity. The processes involved in regulating energy homeostasis and intermediary lipid and carbohydrate metabolism are inextricably linked by common neuroendocrine mediators, including leptin, ASP, and adiponectin. The production of all three adipocyte hormones appears to be regulated by nutritional status, that is, feeding, fasting, and weight loss. A more complete understanding of the molecular and biochemical pathways regulating the biosynthesis of these hormones and their precise mechanisms of action is likely to lead to new approaches for managing obesity, insulin resistance, and type 2 diabetes.

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