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Review

Adiponectin and Leptin in Relation to Insulin Sensitivity

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ABSTRACT

An increased amount of adipose tissue or its disproportionate distribution between central and peripheral body regions is related to the development of insulin resistance, type 2 diabetes mellitus, dyslipidemia, atherosclerosis, and coronary artery disease. Until recently, adipose tissue was regarded as a storage depot for lipids. It is now viewed as a hormonally active organ that plays a crucial metabolic role. The most important products of adipose tissue collectively referred to as adipocytokines, include adiponectin, leptin, tumor necrosis factor–alpha (TNF-α), interleukin-6 (IL-6), resistin, plasminogen-activating inhibitor–I (PAI-1), and angiotensinogen. These low and medium molecular weight proteins play an important role in the adipose tissue physiology and are believed to be a link between obesity, insulin resistance and endothelial dysfunction. This review describes the metabolic role of two of these proteins, adiponectin, and leptin in relation to insulin sensitivity.

INTRODUCTION

In the past decade, it has become better appreciated that the relationship between obesity and the metabolic syndrome is mediated by the release of several hormones from adipose tissue. Collectively, these hormones are called adipocytokines. In this article, we review the role of two of these adipocytokines—adiponectin and leptin—and describe how they may lead to insulin resistance and the metabolic syndrome.

ADIPONECTIN STRUCTURE AND SYNTHESIS

In 1995, Scherer et al.[1] reported the cDNA encoding the Acrp30 protein in mice (adipocyte complement–related protein). This same protein, later named "adiponectin," was identified from a human adipose tissue library and called apM1 (Adipose Most Abundant gene transcript).[2] In the same year, another group identified the protein in mice and termed it adipoQ.[3] Finally, Nanko et al.[4] isolated the human adiponectin protein from plasma.

Human adiponectin contains 244 amino acid residues and consists of a 20-residue signal sequence, an N-terminal region without homology to any known proteins, a collagen-like region, and a C-terminal globular domain. The three dimensional structure of its C-terminal globular domain is similar to that of tumor necrosis factor–alpha (TNF-α), even though there is no sequence homology at the primary structure level.[5] Adiponectin is abundant in plasma and

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accounts for 0.01% of total plasma proteins in humans.[6] It is possible that adiponectin is cleaved proteolytically after secretion. A smaller form of adiponectin containing the C-terminal domain has been detected in human plasma.[7] This cleavage product has a higher biological value than native adiponectin. Two adiponectin receptors (AdipoR1, AdipoR2) have been cloned. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. They serve as receptors for globular and full-length adiponectin, and mediate increased AMP kinase, PPAR-γ ligand activity, fatty acid oxidation, and glucose uptake by adiponectin.[8]

RELATION BETWEEN ADIPONECTIN AND INSULIN RESISTANCE

Experimental animal studies

Reduction in adiponectin gene expression in adipose tissue is associated with obesity and insulin resistance in some animal models. Hu et al.[3] found that levels of transcripts for adiponectin in white adipose tissue were lower in obese (ob/ob) mice than in wild-type mice.[3] These investigators hypothesized that feedback inhibition from other factors associated with obesity were responsible for the suppressed levels.[9] A prospective study in rhesus monkeys showed that decrease in plasma adiponectin level paralleled the development of insulin resistance and diabetes, as the animals became obese.[10] These studies suggest that diminishment of adiponectin levels are secondary to obesity and insulin resistance. On the other hand, administration of the globular region of adiponectin to experimental animals was accompanied with weight loss in mice consuming a high-fat, high-sucrose diet.[7] This observation suggests that low plasma adiponectin may be a primary cause of weight gain and thereby insulin resistance.

More recently, evidence for a primary role for adiponectin in the pathogenesis of insulin resistance comes from a mouse model lacking adiponectin. In these animals, the heterozygous adiponectin-deficient mice (adipo −/+) show mild insulin resistance, while the homozygous adiponectin mice (adipo −/−) show moderate insulin resistance with glucose intolerance and weight gain.[11] Feeding the Adipo −/− mice a high-fat, high-sucrose diet induces insulin resistance. This suggests that adiponectin deficiency is important in the pathogenesis of insulin resistance.[12] Other studies have shown that insulin resistance in lipoatrophic mice can be reversed by the combination of physiologic doses of adiponectin and leptin, but only partially by either alone.[13] Taking all these observations together, it is possible to conclude that reduction of adiponectin plays a primary role in the development of insulin resistance in murine models of adiposity and lipoatrophy. These data further suggest that replenishment of adiponectin might provide a novel treatment modality for both insulin resistance and type 2 diabetes.

Human studies

In clinical studies a relationship between adiponectin and fat mass has been observed.[6,14–16] Arita et al.[6] showed that mean plasma adiponectin level in obese patients is lower than in lean subjects. In a longitudinal study, it was reported that plasma adiponectin concentrations decreased with increasing adiposity in children 5 and 10 years of age.[14] In a study of normal weight and obese women, plasma adiponectin levels were negatively correlated with body mass index, body fat mass, serum leptin concentrations, fasting insulin level, and insulin resistance.[15] Adiponectin concentration is higher among women than men.[16]

Hotta et al.[17] have shown that plasma adiponectin levels are lower in those with type 2 diabetes than non-diabetic individuals. When their cohort was stratified by presence or absence of CAD, diabetic patients with CAD had lower adiponectin levels than diabetic patients without CAD. In that study, plasma adiponectin levels were negatively correlated with fasting plasma glucose, serum insulin, serum triglycerides, and body mass index, and positively correlated with HDL-cholesterol. In other words, there was an inverse relationship with the metabolic syndrome. The same group found that a 10% weight loss leads to significant increase in adiponectin level (40–60%) in both diabetic and non-diabetic patients. In a recent study by our

group,[18] we also found that 7% reduction in body weight, by combined caloric reduction and increased physical activity for 6 months, resulted in significant increase in plasma adiponectin level in obese type 2 diabetic patients with insulin resistance. Yang et al.[19] demonstrated that reduction of body weight after gastric bypass surgery was associated with a similar increase in plasma adiponectin level. From these studies, it is not clear if the increase in adiponectin is related to weight loss per se or to the improvement of insulin sensitivity after weight loss. In Pima Indians,[20] an ethnic group with a high prevalence of type 2 diabetes and insulin resistance, adiponectin levels have been demonstrated to correlate positively with insulin-stimulated glucose disposal rate (GDR) measured by a hyperinsulinemic-euglycemic clamp. Multivariate analysis demonstrated that hypoadiponectinemia is more strongly related to the degree of insulin resistance and hyperinsulinemia than to the degree of adiposity or glucose intolerance.[20] This observation has been confirmed by another study, which found that, when subjects who are discordant for insulin sensitivity are matched for BMI, age, and gender, insulin-sensitive subjects had twofold higher plasma adiponectin level.[21] A recent cross-sectional study by Stephan et al.[22] demonstrated that low fasting adiponectin concentrations are associated with low insulin stimulated skeletal muscle insulin receptor tyrosine phosphorylation, which is associated with insulin resistance and type 2 diabetes. Taken together, these studies suggest that insulin resistance and hyperinsulinemia are the major determinants of the hypoadiponectinemia in obesity and type 2 diabetes.

Given these results, it is logical to ask whether decreased adiponectin is a risk factor for type 2 diabetes independent of obesity. Longitudinal comparison between diabetic patients and BMI-matched non-diabetic Pima Indians show that increased adiponectin levels protect against later development of type 2 diabetes.[23] This observation suggests that decreased adiponectin by itself, not necessarily consequent to obesity, predisposes humans to type 2 diabetes. In a case control study, Spranger et al.[24] showed that increased concentrations of adiponectin are strongly and independently associated with reduced risk of type 2 diabetes. The Funagata study from Japan[25] also showed that decreased serum adiponectin is an independent risk factor for progression to type 2 diabetes. Another study showed that first-degree relatives of type 2 diabetic patients, with normal circulating levels of adiponectin, have reduced adiponectin mRNA expression in adipose tissue compared to controls.[26] From these human studies, we may conclude that adiponectin has a substantial role in the pathogenesis of type 2 diabetes and that it may be used in the future as an indicator of diabetes risk. Although adiponectin seems to be implicated in the development of insulin resistance, explicit mechanisms linking adiponectin to incident type 2 diabetes remained speculative. Genetic polymorphisms might be involved in the regulation of adiponectin plasma concentration, especially when you take into account the existing linkage in the region of the adiponectin gene with type 2 diabetes.[27,28] Some missense mutations in the globular domain were associated with low adiponectin levels and type 2 diabetes.[29]

RELATIONSHIP BETWEEN PPAR-GAMMA AGONISTS AND ADIPONECTIN

The peroxisome proliferator–activated nuclear receptor (PPAR-γ) is a key transcriptional factor that induces adipocyte differentiation and controls many adipocyte genes. PPAR-γ is not only a regulator of adipocyte function but also an insulin sensitizer. Because it is abundant in adipose tissue, it is thought that PPAR-γ in adipose tissue plays a crucial role in explaining insulin resistance. One mechanism by which it can improve insulin sensitization could be through increased adiponectin production and secretion. Several laboratory studies suggest that such is the case.

In differentiated 3T3-L1 adipocytes, the mRNA of adiponectin is increased by administration of rosiglitazone, a synthetic PPAR-γ agonist.[30] It has also been reported that adiponectin mRNA expression is normalized or even increased by thiazolidinediones in the adi-

pose tissue of obese mice.[31] In cultured 3T3-L1 adipocytes, incubation with troglitazone—another thiazolidinedione—enhances adiponectin mRNA expression and adiponectin secretion in a dose- and time-dependent fashion. Further, troglitazone reverses the suppressive effect of TNF-α on the expression of adiponectin in adipocytes.[31]

A recent study has shown that plasma levels of adiponectin are affected by PPAR-γ agonist in both lean and obese mice. Coombs et al.[32] showed that, in db/db mice, chronic treatment with PPAR-γ agonists induced a significant increase in plasma adiponectin levels. Similar effects were noted in a non-genetic type 2 diabetes model (fat-fed and low-dose streptozocin-treated mice). In contrast, treatment of these mice (db/db or fat fed mice) with metformin or a PPAR-α agonist did not affect plasma adiponectin levels.

Human studies have replicated these findings. In a group of mildly overweight subjects with glucose intolerance, administration of troglitazone for 12 weeks significantly increased plasma adiponectin level in a dose-dependent way.[31] Yu et al.[33] have also shown that treatment with troglitazone for 3 months resulted in increased adiponectin levels in diabetic subjects and in both lean and obese non-diabetic subjects. Baseline adiponectin levels were significantly lower in diabetic subject versus lean non-diabetic controls, but rose uniformly in all subjects after treatment, with no significant difference between the three groups. In a recent randomized double-blind placebo-controlled trial performed in 64 diabetic patients, rosiglitazone therapy for 6 months was accompanied by more than twofold increase in adiponectin level.[34] The increase in adiponectin induced by TZD's was not affected by co-administration of glyburide or metformin.[35]

ADIPONECTIN, INFLAMMATION AND ATHEROSCLEROSIS

Recent studies have established the fundamental role of inflammation in mediating all stages of atherosclerosis.[36] Adiponectin is involved in the modulation of inflammatory responses both by its anti-inflammatory effect and by its specific function in the blood vessel wall.[37] Adiponectin attenuates TNF-α–mediated inflammatory response,[38] probably through the activation of cAMP protein kinase A. It has been also shown that adiponectin inhibits some functions of mature macrophage, such as phagocytosis and cytokine production.[37] Strong inverse relationship between TNF-α and adiponectin was shown in nondiabetic subjects with varying degrees of obesity and insulin resistance.[39] Adiponectin also modulates endothelial function and has an inhibitory effect on proliferation of vascular smooth muscles induced by growth factors.[40,41] Low plasma concentrations of adiponectin are found in patients with coronary artery disease.[42] In tissue cultures, adiponectin attenuates adhesion of monocyte to endothelial cells by reducing the expression of adhesion molecules in endothelial cells. Adiponectin also suppresses lipid accumulation in monocyte-derived macrophages in the blood vessel wall through the suppression of macrophage scavenger receptor expression.[43] It has also been reported that adiponectin infiltrates rapidly into the subendothelial space when the endothelial barrier of the arterial wall is injured by balloon angioplasty.[44] The recent finding that adiponectin deficiency aggravates neointimal thickening, and that supplementation with adiponectin decreases neointimal thickening in mechanically injured arteries, suggests that increasing plasma adiponectin might be useful in preventing restenosis after vascular intervention.[45]

LEPTIN STRUCTURE AND SYNTHESIS

Leptin is a 16-kDa adipocyte-derived hormone that circulates in the serum in the free and bound form.[46] Positional cloning of ob/ob mouse model of obesity, which is deficient in leptin, led to the discovery of the leptin gene (ob gene).[47] The ob mRNA, encodes a 167–amino acid protein whose crystal structure suggests that it belongs to the cytokine family.[48,49] Analysis of the ob gene product also reveals a high degree of homology among species, showing that human leptin is 84% identical to mouse

leptin and 83% identical to rat leptin. Leptin exerts its effect on energy balance mainly by acting in the brain. Intravenous leptin injection activates neurons in the arcuate, venteromedial, and dorsomedial hypothalamic nuclei and in brainstem neuronal circuits implicated in the regulation of feeding behavior and energy balance.[50,51] Leptin acts either directly or by activating specific centers in the hypothalamus to decrease food intake, increase energy expenditure, influence glucose and fat metabolism, or alter neuroendocrine function.[52]

Leptin levels increase exponentially with increased fat mass.[52,53] Considine et al.[53] found that the mean serum leptin concentrations were 7.5 ng per milliliter in normal-weight subjects and 31.3 ng per milliliter in obese subjects. The *ob* mRNA content of adipocytes was twice as high in the obese subjects as in the normal-weight subjects. This observation suggests that obese persons are insensitive to endogenous leptin production, indicating the possible existence of a leptin resistant state. Heymsfield et al.[54] conducted a study in which varying amounts of leptin were administered to a group of obese subjects. Those who received the highest dose showed a significant and progressive reduction of body weight (7 kg) with no change in glycemic control or in insulin action over 24 weeks. Interestingly the average serum leptin concentration in this group was as high as 667 ng/mL, 30–40-fold higher than its level in the placebo and at baseline. This observation indicates that very high leptin levels are needed to overcome leptin resistance. Leptin transport to the brain appears to be a saturable carrier-mediated process, which may limit its entry into the central nervous system and may create resistance.[55]

Leptin mRNA is expressed predominantly by subcutaneous rather than visceral fat cells.[56] This suggests a role for leptin in modulating adipose tissue mass and distribution. After adjustment of BMI, women seem to have higher leptin levels than men.[57–59] This could be either related to the increased percentage peripheral body fat in women, or a result of stimulation of leptin production by estrogen/progesterone and/or by androgens.[60] The relationship between serum leptin level and the subcutaneous fat mass was also observed in Japanese type 2 diabetic subjects.[61]

ROLE OF LEPTIN IN REGULATING APPETITE

Leptin levels reflect the amount of stored fat and the degree of energy imbalance. Prolonged fasting decreases leptin levels, whereas overfeeding greatly increases levels.[62,63] Composition of the diet may also regulate leptin levels—specifically intake of macronutrients such as carbohydrates[64] and micronutrients such as zinc.[65] Regulation of leptin expression by nutrition is probably mediated in part by insulin. Leptin expression increases after peak insulin secretion during the feeding cycle.[66] Insulin stimulates leptin expression directly in isolated adipocytes *in vitro*[67] and increases leptin levels when injected into rodents.[66] In contrast, leptin is decreased in low insulin states, such as experimentally induced diabetes, and increases after insulin treatment.[68] In humans, leptin expression is also correlated with insulin levels and increases after insulin infusion for several days.[69,70] Segal et al.[71] studied the relationship between insulin sensitivity and serum leptin concentration in three groups of patients: lean insulin-sensitive, lean insulin-resistant, and obese insulin-resistant. They found that insulin resistance is associated with elevated plasma leptin levels independent of body fat mass. In a study from a multiethnic group in Mauritius,[72] the association between insulin resistance and leptin concentration was also observed after controlling for overall and central adiposity. In that study, serum leptin concentration increased across quartiles of fasting insulin irrespective of gender or body mass index (BMI). Leptin concentrations were also found to be high in first-degree relatives of patients with type 2 diabetes.[73] In a cross-sectional study of obese men, multiple regression analysis showed abdominal subcutaneous adipose tissue, glucose disposal rate (GDR) and BMI to explain most of the variability in serum leptin concentration.[74]

Tatti et al.[75] found that BMI, waist-to-hip ratio, and fasting plasma insulin were significantly related to leptin only in the nondiabetic

population, but not in diabetic patients. On the other hand, Wauters et al.[76] found that fat mass and gender are the main independent predictors of leptin concentration in type 2 diabetic patients, and that insulin secretion and the degree of insulin resistance contribute significantly to leptin levels. Fischer et al.[77] found that, when the influence of body fat mass or BMI were excluded, the correlations between leptin and insulin or insulin sensitivity remained significant in type 2 diabetic patients.

In patients with lipodystrophy, there is absence of adipose tissue, and leptin levels are very low. The low leptin levels correlate significantly with markers of insulin resistance. Leptin therapy in lipodystrophic patients was shown to improve glycemic control, improve insulin stimulated hepatic and peripheral glucose metabolism and to reduce hepatic and muscle triglyceride content, suggesting that leptin acts as a signal that contributes to regulation of total body sensitivity to insulin.[78]

LEPTIN, INFLAMMATION, AND THROMBOSIS

Leptin plays an important role in the inflammatory process. During the inflammatory reaction, plasma leptin is enhanced and may contribute to the anorexia and cachexia of infection. It also plays an important role in regulating the hypothalamo-pitutary-adrenocortical axis, in angiogenesis, and in regulation of the immune response.[79] It has been reported to induce proliferation, differentiation, and functional activation of hematopoietic cells. It can enhance the proliferation and phagocytic activity of macrophages.[80,81] Bullo et al.[82] studied the links between systemic inflammation and leptin expression. By dividing patients into tertiles of C-reactive protein (CRP), they found that TNF-α and leptin expression in adipose tissue were particularly high in the upper tertile. In another study, plasma leptin levels were found to correlate closely with inflammatory cytokine levels (TNF-α, IL-6) and also with acute phase proteins (CRP, alpha-1-antitrypsin).[83,84]

Piemonte et al.[85] prospectively studied the effect of fasting plasma leptin on cardiovascular mortality in diabetic, impaired glucose tolerance, and normal glucose tolerance subjects over seven years. They found that leptin was independently associated with cardiovascular mortality. One explanation of this association is the prothrombotic effect of leptin. Nakata et al.[86] found that higher concentration of leptin promotes adenosine diphosphate (ADP)–induced aggregation of human platelets via the long form of its receptor. At 50 ng/mL, human leptin induced phosphorylation of several platelet proteins on tyrosine residues, inducing in turn a thrombotic tendency. More recently, other investigators[87] raised the possibility that phospholipase C, protein kinase C, phospholipase A2, and calcium play a role in mediating the proaggregating action of leptin. Interestingly, Corsonello et al.[88] found that platelet aggregation response to leptin is blunted, but not completely abolished in overweight/obese subjects, thus suggesting that platelet may also represent a site of leptin resistance in human obesity.

LEPTIN AND INSULIN SENSITIZERS

Troglitazone was found to decrease leptin expression in adipocytes with no reduction in fat mass.[89] The glucocorticoid induced leptin secretion was also shown to be blocked by troglitazone in obese nondiabetic individuals.[90] There was no gender-related difference in the effect of troglitazone to inhibit dexamethasone-stimulated leptin release.[91] It was also noted that omental adipocytes may be more responsive to such hormonal regulation *in vivo* than are subcutaneous adipocytes.[92] In contrast, an earlier clinical study showed that troglitazone 200 mg twice daily for 12 weeks in obese patients was not associated with any change in fasting plasma leptin concentrations, despite a 40–50% reduction in fasting and postmeal plasma insulin concentrations.[93] This observation can be explained by the improvement in insulin sensitivity and reduction in plasma insulin concentrations after troglitazone therapy.[93]

CONCLUSION

We have so far discussed the roles of adiponectin and leptin on insulin resistance sepa-

rately. The question arises, which of these two fat-related cytokines has the greater impact or association with insulin resistance when looked at together. A recent study has tried to answer this question.[94] It examined the relationship between insulin resistance and both adiponectin and leptin in a large group of obese and lean non-diabetic subjects. After adjustment for age and gender, adiponectin negatively and leptin positively correlated with Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), BMI, waist, mean blood pressure (MBP), fasting plasma glucose, and insulin glucose disposal. In addition, adiponectin correlated negatively with total cholesterol/HDL ratio and triglycerides and positively with HDL, while leptin was not correlated. After further adjustment for BMI, similar correlations were present with the above parameters for adiponectin, but not for leptin. In other words, once the effects of overweight were accounted for, leptin was no longer related to factors associated with the insulin resistance syndrome (IRS). A subset of the obese patients was also evaluated after weight reduction. As expected, adiponectin increased while leptin decreased. IRS prevalence significantly decreased. After adjusting data for BMI reduction, the variation in adiponectin level, but not that of leptin, was associated with the persistence of IRS. These data confirm that leptin is a marker of the adipose tissue mass, but indicate that it does not play the major role in determining IRS. In contrast, adiponectin reduction may be a new component of IRS, independent of BMI.[94]

Another recent study looked at the relationship between adiponectin, leptin, and body fat distribution in relation to the metabolic parameter of the insulin resistance syndrome. Subjects were categorized into two groups: a subcutaneous fat dominant group (SFDG) and visceral fat dominant group (VFDG). The VFDG showed significantly lower adiponectin levels than the SFDG, but leptin levels did not differ between groups. The study found that adiponectin was more strongly related to visceral abdominal fat, while leptin was more closely related to subcutaneous fat content.[95]

As can be seen from this brief review, adiponectin and leptin are integrally related to the IRS. For now, many aspects of that association are unclear. The coming years should shed light on the relationships between them and offer the prospect for new treatments for overweight and the metabolic syndrome.

REFERENCES

1. Scherer PE, Williams S, Fogliano M, et al. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995;270:26746–26749.
2. Maeda K, Okubo K, Shimomura I, et al. cDNA cloning and expression of a novel adipose specific collagen like factor, apM1 (adipose most abundant gene transcript 1). *Biochem Biophys Res Commun* 1996; 221:286–289.
3. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose specific gene dysregulated in obesity. *J Biol Chem* 1996;271:10697–10703.
4. Nakano Y, Tobe T, Choi-Miura NH, et al. Isolation and characterization of GBP28, a novel gelatin binding protein purified from plasma. *J Biochem* 1996;120: 803–812.
5. Takahashi M, Arita Y, Yamagata K, et al. Genomic structure and mutations in adipose specific gene, adiponectin. *Int J Obes* 2000;24:861–868.
6. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999;257:79–83.
7. Freubis J, Tsao TS, Javorschi S, et al. Proteolytic clevage product of 30kDa adipocyte-complement related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001;98:2005–2010.
8. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003;423:762–769.
9. Nadler ST, Stoehr JP, Schueler KL, et al. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc Natl Acad Sci USA* 2000;97: 11371–11376.
10. Hotta K, Funahashi T, Bodkin NL, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001;50:1126–1133.
11. Kuboto N, Terauchi Y, Yamauchi T, et al. Disruption of adiponectin causes insulin resistance and neointimal proliferation. *J Biol Chem* 2002;277:25863–25866.
12. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002;8:731–737.
13. Yamauchi T, Kamon J, Waki H, et al. The fat derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001;7:941–946.

14. Stefan N, Bunt JC, Salbe AD, et al. Plasma adiponectin concentration in children: relations with obesity and insulinemia. *J Clin Endocrinol Metab* 2002; 87:4652–4656.
15. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal weight and obese women. *Eur J Endocrinol* 2002;147:173–180.
16. Nishizawa H, Shimomura I, Kishida K, et al. Androgens decrease plasma adiponectin, an insulin sensitizing adipocyte derived protein. *Diabetes* 2002;51: 2734–2741.
17. Hotta K, Funahashi T, Arita Y, et al. Plasma concentration of a novel adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;20:1595–1599.
18. Monzillo LU, Hamdy O, Horton ES, et al. Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obes Res* 2003;11:1048–1054.
19. Yang WS, Lee WJ, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 2001;86:3815–3819.
20. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001;86:1930–1935.
21. Abbasi F, Chu JW, Mclaughlin T, et al. Obesity versus insulin resistance in modulation of plasma adiponectin concentration. *Diabetes* 2002;52(suppl 1):A81.
22. Stephen N, Vozarova B, Funahashi T, et al. Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation and low plasma concentration precedes a decrease in whole body insulin sensitivity in humans. *Diabetes* 2002 51:1884–1888.
23. Lindsay RS, Funahashi T, Hanson RL, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002;360:57–58.
24. Spanger J, Kroke A, Mohlig M, et al. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003;361:226–28.
25. Daimon M, Oizumi T, Saitoh T, et al. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese population. *Diabetes Care* 2003;26:2015–2020.
26. Lihn AS, Ostergard T, Nyholm B, et al. Adiponectin mRNA expression in subcutaneous adipose tissue is reduced in first-degree relatives of type 2 diabetic patients. *Am J Physiol Endocrinol Metab* 2003;284: E443–E448.
27. Hara K, Boutin P, Mori Y, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 2002;51:536–540.
28. Menzaghi C, Ercolino T, Di Paola R, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 2002;51:2306–2312.
29. Kondo H, Shimomura I, Matsukawa Y, et al. Association of adiponectin mutation with type 2 diabetes. A candidate gene for the insulin resistance syndrome. *Diabetes* 2002;51:2325–2328.
30. Lin WS, Chang HM, Tai TY, et al. Effect of thiazolidinedione on gene expression in NIH3T3-L1 adipocytes. *Diabetes* 1999;48(suppl 1):A-217.
31. Maeda N, Takahashi M, Funahashi T, et al. PPAR gamma ligands increase expression and plasma concentrations of adiponectin, an adipose derived protein. *Diabetes* 2001;50:2094–2099.
32. Coombs TP, Wagner JA, Berger J, et al. Induction of adipocyte complement-related protein of 30 kDa by PPAR-gamma agonists: a potential mechanism of insulin sensitization. *Endocrinology* 2002;143:998–1007.
33. Yu JG, Javorschi H, Henever AL, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 2002;51:2968–2974.
34. Yang WS, Jeng CY, Wu TJ, et al. Synthetic peroxisome proliferator-activated receptor gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care* 2002;25: 376–380.
35. Schubert UK, Kaplan RC, Rood J, et al. The serum adiponectin response to rosiglitazone in type 2 diabetes is not affected by co-administration of glyburide or metformin. *Diabetes* 2003;52(suppl 1):A543.
36. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–1143.
37. Yokota T, Oritani K, Takahashi I, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulated the growth of myelmonocytic progenitors and the functions of macrophages. *Blood* 2000;96:1723–1732.
38. Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-Kappa B signaling through a camp dependent pathway. *Circulation* 2000;102:1296–1301.
39. Kern PA, DiGregorio GB, Lu T, et al. Adiponectin expression from human adipose tissue: relationship to obesity, insulin resistance and TNF alpha expression. *Diabetes* 2003;52(suppl 1):A329.
40. Aldahi W, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. *Curr Diab Rep* 2003; Aug 3:293–298.
41. Matsuzawa Y, Funahashi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derives bioactive substances. *Ann N Y Acad Sci* 1999;892:146–154.
42. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules. *Circulation* 1999; 100:2473–2476.
43. Ouchi N, Kihara S, Arita Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 2001;103:1057–1106.

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44. Okamoto Y, Arita Y, Nishida M, et al. An adipocyte derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 2000;32:47–50.
45. Matsuda M, Shimomura I, Sata M, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *J Biol Chem* 2002; 277:37487–37491.
46. Mantzoros CS. The role of leptin in human obesity and disease. *Ann Intern Med* 1999;130:671–680.
47. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–432.
48. Zhang F, Babinski MB, Beals JM, et al. Crystal structure of the obese protein leptin-E100. *Nature* 1997; 387:206–209.
49. Madej T, Boguski MS, Bryant SH. Threading analysis suggests that the obese gene product may be a helical cytokine. *FEBS Lett* 1995;373:13–18.
50. Elmquist JK, Ahima RS, Maratos-Flier E, et al. Leptin activates neurons in the ventrobasal hypothalamus and brainstem. *Endocrinology* 1997;839–842.
51. Elmquist JK, Ahima RS, Elias CS, et al. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci USA* 1998;741–748.
52. Lonnqvist F, Arner P, Nordfors L, et al. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nat Med* 1995;1:950–993.
53. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;334: 292–295.
54. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant Leptin for weight loss in obese and lean adults: a randomized control dose escalation trial. *JAMA* 1999;282:1568–1575.
55. Caro JF, Kolazynski JW, Nyce MR, et al. Decreased cerebrospinal fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996; 348:159–161.
56. Montague CT, Prins JB, Sanders L, et al. Depot and sex specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes* 1997;46:342–347.
57. Hickey MS, Houmard JA, Considine RV, et al. Gender-dependent effects of exercise training on serum leptin levels in humans. *Am J Physiol* 1997;272:E562–E566.
58. Saad MF, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 1997;82:579–584.
59. Ostlund RE Jr, Wang JW, Klein S, et al. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996;81:3909–3913.
60. Shimizu H, Shimomura Y, Nakanihi Y, et al. Estrogen increases *in vivo* leptin production in rats and human subjects. *J Endocrinol* 1997;154:285–292.
61. Okumura T, Taniguchi A, Nagasaka S, et al. Relationship of regional adiposity to serum leptin level in nonobese Japanese type 2 diabetic male patients. *Diabetes Metab* 2003;29:15–18.
62. Kolaczynski JW, Considine RV, Ohannesian J, et al. Responses of leptin to short-term and refeeding in humans. *Diabetes* 1996;45:1511–1515.
63. Kolaczynski JW, Ohannesian JP, Considine RV, et al. Response of leptin to short term and prolonged overfeeding in humans. *J Clin Endocrinol Metab* 1996;81: 4162–4165.
64. Jenkins AB, Markovic TP, Fleury A, et al. Carbohydrate intake and short term regulation of leptin in humans. *Diabetologia* 1997;40:348–351.
65. Mantzoros CS, Prasad AS, Beck F, et al. Zinc may regulate serum leptin concentrations in humans. *J Am Coll Nutr* 1998;17:270–275.
66. Saladin R, Dvos P, Guerro-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. *Nature* 1995;377:527–529.
67. Rentsch J, Chiesi M. Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Lett* 1996;379:55–59.
68. MacDougald OA, Hwang CS, Fan H, et al. Regulated expression of the obese gene product in white adipose tissue and 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 1995;92:9034–9037.
69. Kolaczynski JW, Nyce MR, Considine RV, et al. Acute and Chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. *Diabetes* 1996; 45:699–701.
70. Boden G, Chen X, Kolacynski JPM. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *J Clin Invest* 1997;100:1107–1113.
71. Segal KR, Landt M, Klien S. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. *Diabetes* 1996;45:988–991.
72. Zimmet PZ, Collins VR, de Courten MP, et al. Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean of Mauritius. *Int J Obes Relat Metab Disord* 1998;22:171–177.
73. Nyholm B, Fisker S, Lund S, et al. Increased circulating leptin concentration in insulin-resistant first-degree relatives of patients with non-insulin dependent diabetes mellitus: relationship to body composition and insulin sensitivity but not to family history of NIDDM. *Eur J Endocrinol* 1997;136:173–179.
74. Johannsson G, Karlsson C, Lonn L, et al. Serum leptin concentration and insulin sensitivity in men with abdominal obesity. *Obes Res* 1998;6:416–421.
75. Tatti P, Masselli L, Buonanno A, et al. Leptin levels in diabetic and nondiabetic subjects. *Endocrine* 2001;15: 305–308.
76. Wauters M, Considine RV, Yudkin JS, et al. Leptin levels in type 2 diabetes: associations with measures of insulin resistance and insulin secretion. *Horm Metab Res* 2003;35:92–96.
77. Fischer S, Hanefeld M, Haffner SM, et al. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. *Acta Diabetol* 2002;39:105–110.

AU: For refs. 50 & 51, please provide vol. no.

78. Peterson KF, Oral EA, Dufour S, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 2002;109:1345–1350.
79. Barbier M, Cherbut C, Aube AC, et al. Elevated plasma leptin concentrations in early stages of experimental intestinal inflammation in rats. *Gut* 1998; 43:783–790.
80. Loffreda S, Yang SQ, Lin HZ, et al. Leptin regulates proinflammatory immune responses. *FASEB J* 1998; 12:57–65.
81. Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell Immunol* 1999;194:6–11.
82. Bullo M, Garcai-Lorda P, Megias I, et al. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obes Res* 2002;11:525–531.
83. Maruna P, Gurulich R, Frasko R, et al. Serum leptin levels in septic men correlate well with C-reactive protein and TNF-alpha but not with BMI. *Physiol Res* 2001;50:589–594.
84. Macchi M, Pieroni L, Bruckert E, et al. Systemic low-grade inflammation is related to both circulating and adipose tissue TNF alpha, leptin and IL-6 levels in obese women. *Int J Obes Relat Metab Disord* 2004;28: 993–997.
85. Piemonti L, Calori G, Mercalli A, et al. Fasting plasma leptin, tumor necrosis factor–alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. *Diabetes Care* 2003;26:2883–2889.
86. Nakata M, Yada T, Soejima N, et al. Leptin promotes aggregation of human platelets via the long form of its receptor. *Diabetes* 1999;48:426–429.
87. Corsonello A, Malara A, De Domenico D, et al. Identifying pathways involved in leptin-dependent aggregation of human platelets. *Int J Obes Relat Metab Disord* 2004;28:979–984.
88. Corsonello A, Perticone F, Malara A, et al. Leptin-dependent platelet aggregation in healthy, overweight and obese subjects. *Int J Obes Relat Metab Disord* 2003;27:566–573.
89. Watanabe S, Takeuchi Y, Fukumoto S, et al. Decrease in serum leptin by troglitazone is associated with preventing bone loss in type 2 diabetic patients. *J Bone Miner Metab* 2003;21:166–171.
90. Willi SM, Kennedy A, Wallace P, et al. Troglitazone antagonizes metabolic effects of glucocorticoids in humans: effects on glucose tolerance, insulin sensitivity, suppression of free fatty acids, and leptin. *Diabetes* 2002;51:2895–2902.
91. Williams LB, Fawcett RL, Waechter AS, et al. Leptin production in adipocytes from morbidly obese subjects: stimulation by dexamethasone, inhibition with troglitazone, and influence of gender. *J Clin Endocrinol Metab* 2000;85:2678–2684.
92. Nolan JJ, Olefsky JM, Nyce MR, et al. Effect of troglitazone on leptin production. Studies *in vitro* and in human subjects. *Diabetes* 1996;45:1276–1278.
93. Baratta R, Amato S, Patane G, et al. Adiponectin and leptin: relationship with the insulin resistance syndrome. *Diabetes* 2003;52:A81.
94. Park KG, Park KS, Kim MJ, et al. Relationship between serum adiponectin and leptin concentrations and body fat distribution. *Diabetes Res Clin Pract* 2004;63:135–142.

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