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Regulating adiponectin: of flax and flux

Published online: 12 May 2005
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Abbreviations ALA: α -linoleic acid · *c*: *cis* · CLA: conjugated linoleic acid · EFA: essential fatty acids · *t*: *trans*

Adiponectin, a novel adipocyte-specific protein, is now known to play an important role in a broad range of metabolic and cardiovascular processes related to the development of insulin resistance and atherosclerosis [1]. Although produced exclusively by adipocytes, circulating concentrations are paradoxically lower in obese individuals, particularly in the presence of abdominal obesity. Low levels of adiponectin are also associated with insulin resistance and type 2 diabetes. Adiponectin appears to increase insulin sensitivity via the primary mechanisms of increased fatty acid oxidation and inhibition of hepatic glucose production. It may also have important effects on a variety of cytokines and other molecules that can negatively influence glucose metabolism and vascular endothelial function.

Weight loss has been shown to increase adiponectin levels [2]. An increase in adiponectin levels has also been observed following treatment with thiazolidinediones [3], blockade of the renin–angiotensin system [4], and the use of the cannabinoid type 1 receptor antagonist rimonabant [5]. In contrast, adiponectin levels are reduced by proinflammatory cytokines, especially TNF- α [6]. Other factors and clinical situations reported to influence adiponectin levels are noted in Table 1.

In this issue of *Diabetologia*, two mediators of adiponectin down-regulation are examined, both of which significantly expand our understanding of how this important molecule is regulated. The first study, by Poirier et al. [7], examines the effect of conjugated linoleic acid (CLA)

on adiponectin and leptin levels in the mouse. CLA is a mixture of different isomers of linoleic acid, an ω -6 fatty acid found primarily in beef, lamb and dairy products, and serves as a substrate for arachidonic acid synthesis. Non-lipidologists often confuse linoleic acid with α -linolenic acid (ALA; Fig. 1), an ω -3 fatty acid found in flax-seed, walnuts, redcurrant and blackcurrant seeds, soy beans, canola oil and leafy vegetables. ALA is an important source of eicosapentaenoic and docosahexaenoic acids, both of which can also be obtained directly from fish oils. CLA and ALA are polyunsaturated fatty acids that cannot be made by the body and must be provided in the diet, hence the term essential fatty acids (EFA). The daily dietary requirements for EFA are 1–2% of dietary calories for adults and 3% for infants, with a suggested ratio of 10:1 for ω -6 : ω -3 fatty acids. However, pharmacological doses of EFA are often commercially promoted as dietary supplements for a variety of health reasons, ranging from weight loss and improvements in cardiovascular risk factors to anti-aging remedies and treatments for inflammatory disease, multiple sclerosis and cancer.

The interest in CLA as a weight-loss agent largely stems from studies using CLA mixtures containing different amounts of the *trans*-10,*cis*-12 CLA (*t*10,*c*12-CLA) and *c*9,*t*11-CLA isomers, which have shown promising effects on body weight and fat deposition [8]. Studies in mice fed CLA mixtures with different ratios of *c*9,*t*11-CLA and *t*10,*c*12-CLA suggest that the *t*10,*c*12-CLA isomer may be the form that is most important in fat deposition [9]. Studies in humans are less clear, but there is some evidence indicating that *t*10,*c*12-CLA is also the anti-adipogenic isomer of CLA [10]. Concerns have been raised following the observation that, in mice, *t*10,*c*12-CLA can produce profound insulin resistance, lipodystrophy, hepatic steatosis and frank diabetes [11]. The *t*10,*c*12-CLA isomer, but not a CLA mixture, also significantly increased insulin resistance, fasting glucose and dyslipidaemia in abdominally obese men [12]. These side effects may be a direct consequence of decreased preadipocyte differentiation and proliferation, decreased lipogenesis, or increased lipolysis and fat oxidation [13]. It has previously been suggested that

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Table 1 Conditions and mediators known to regulate adiponectin levels

Down-regulation	Up-regulation
Abdominal obesity	Weight loss
Type 2 diabetes	Type 1 diabetes
Polycystic ovary syndrome	Kidney failure
Hyperinsulinaemia	Anorexia nervosa
TNF- α	Leptin
Glucocorticoids	Thiazolidinediones
Testosterone	Renin–angiotensin blockade
β -receptor agonists	Cannabinoid type 1 receptor antagonists
Conjugated linoleic acid	

decreased preadipocyte recruitment and differentiation may be an important risk factor for the development of insulin resistance and type 2 diabetes [14].

In their study, Poirier et al. [7] found that mice fed an equal mixture of *t*10,*c*12-CLA and *c*9,*t*11-CLA showed a 70% decrease in adiponectin concentration at day 2, reaching 3% of baseline by day 10. Leptin concentrations also decreased to about 50% of baseline. These changes were accompanied by an increase in plasma insulin levels and a substantial increase in hepatic lipid content, starting at day 6. Moreover, the CLA-enriched diet resulted in frank lipotrophy, with a loss of around 70% of adipose tissue mass by day 28. A concomitant 3-fold increase in beta cell mass most likely reflected the compensatory changes necessary to meet the increased insulin demand induced by profound insulin resistance. The fact that the marked reduction in adiponectin levels was observed prior to any detectable change in fat mass points to the key role of adiponectin suppression by CLA in the chain of events. Although the precise mechanism by which CLA suppresses adiponectin secretion is unclear, the importance of this study lies in the clear demonstration of the potentially deleterious metabolic effects of mixed isomer CLA supplementation, which, if reproduced in humans, would certainly raise the question of the safety of CLA supplements, which are widely promoted on the Internet, drug stores and health shops for weight management and other putative benefits. Clearly, studies on the effects of CLA on adiponectin in humans are now warranted.

Approaching the issue of adiponectin regulation from an entirely different angle, Hattori et al. [15] explored the mechanism of angiotensin-II-induced suppression of adiponectin in rats. These studies were prompted by previous observations that angiotensin II inhibits adipogenic differentiation [16] and that blocking the renin–angiotensin system not only prevents type 2 diabetes [17], but also

Fig. 1 Molecular structure of linoleic and linolenic acid

increases plasma levels of adiponectin [4]. Angiotensin II is a powerful inducer of reactive oxygen species [18], whereas angiotensin type 1 receptor blockade can reduce oxidative stress [19, 20]. It is therefore not surprising that Hattori et al. chose to examine whether or not increased oxidative stress could play a role in the angiotensin II-induced suppression of adiponectin production.

Reactive oxygen species include highly reactive moieties, such as superoxide anions, hydrogen peroxide and hydroxyl radicals, that are formed as by-products of cellular respiration as electrons passing 'down' the mitochondrial respiratory chain leak away from the main path and directly reduce oxygen molecules. Thus, as a rule, increased mitochondrial oxygen flux leads to the increased formation of reactive oxygen species. Reactive oxygen species can also be synthesised by dedicated enzymes present in phagocytic cells, such as NAD(P)H oxidase or myeloperoxidase, that form an important part of essential cellular defence mechanisms. Cells possess a variety of defences to protect themselves against the toxic effects of the increased formation of reactive oxygen species, including the enzymes superoxide dismutase and catalase. Antioxidants such as α -tocopherol (vitamin E), uric acid and vitamin C may also serve as scavengers to neutralise reactive oxygen species. Because of the potentially toxic effects of reactive oxygen species on cells of the vascular endothelium, smooth muscle and skeletal muscle, as well as pancreatic beta cells, the increased formation of reactive oxygen species has been implicated in a wide range of cardiovascular and metabolic disorders.

In their study, Hattori et al. [15] demonstrated that the angiotensin-II-induced reduction in adiponectin production could be fully prevented by addition of tempol, a superoxide dismutase mimetic, or tetrahydrobiopterin, a powerful reducing agent. They also showed that angiotensin II infusion can lead to a 6-fold increase in vascular superoxide production in aortic rings and induce a marked up-regulation of NAD(P)H oxidase in adipose tissue. Importantly, adiponectin expression in cultured 3T3-L1 adipocytes was

Fig. 2 Changes from baseline to 12 weeks in urinary excretion of 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}; F₂-isoprostanes), a marker of non-enzymatic lipid peroxidation and an indirect marker of oxidative stress, in obese men supplemented with CLA (white bar), the *t*10,*c*12-CLA isomer (grey bar) or placebo (black bar). * $p < 0.0001$ vs placebo; ** $p < 0.0001$ vs placebo; *** $p < 0.05$ vs CLA. Adapted with permission from [21]

down-regulated in response to the glucose oxidase-induced formation of H_2O_2 . Taken together, these data lend strong support to the notion that the increased formation of reactive oxygen species, commonly observed in hypertension, obesity, insulin resistance and type 2 diabetes, may be a key determinant of the hypoadiponectinaemia associated with these conditions.

Interestingly, the findings of Hattori et al. [15] regarding the inhibition of adiponectin formation by reactive oxygen species may be linked to the observations of Poirier et al. [7] regarding the deleterious effects of CLA. In obese men, supplementation with *tl0,c12*-CLA resulted in a 6-fold increase in the urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ (F_2 -isoprostanes), an in vivo marker of non-enzymatic lipid peroxidation resulting from increased oxidative stress (Fig. 2) [21]. This observation suggests that CLA may also markedly increase oxidative stress. Indeed, CLA has previously been implicated in the increased production of reactive oxygen species mediated by the protein kinase C-NAD(P)H oxidase pathway [22]. Thus, it is tempting to speculate that angiotensin II and *tl0,c12*-CLA both down-regulate adiponectin production via a common pathway involving the increased formation of reactive oxygen species.

Whether or not dietary or pharmacological inhibition of the formation of reactive oxygen species can be used to ameliorate hypoadiponectinaemia in humans remains to be seen. The current observations pertaining to CLA supplementation also raise the interesting issue of whether other essential fatty acids, such as ALA, derived from flax-seeds or other sources, can affect adiponectin expression. Indeed, recent evidence in mice suggests that the administration of ω -3 fatty acid may ameliorate the CLA-mediated down-regulation of adiponectin in a dose-dependent manner [23]. Clearly, a better understanding of the factors regulating adiponectin formation will have important consequences for the prevention of the cardiovascular and metabolic complications associated with obesity.

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