Acute Regulation of Adiponectin by Free Fatty Acids

Elizabeth L. Bernstein, Polyxeni Koutkia, Karin Ljungquist, Jeff Breu, Bridget Canavan, and Steven Grinspoon

Little is known about the acute regulation of adiponectin in humans. In animal studies, adiponectin increases the clearance of free fatty acids (FFA) from the circulation by increasing skeletal uptake and oxidation of lipid, thereby regulating the FFA concentration. However, it is unknown if FFA regulate adiponectin. The aim of the present study was to investigate the effect of an acute reduction in free fatty acids on adiponectin concentration in healthy subjects. Ten normal male subjects were admitted for 2 inpatient visits and randomized to receive either acipimox (500 mg orally at 2 AM and again at 6 AM) or placebo on the first visit and vice versa on the second visit. Adiponectin, FFA, insulin and glucose were measured at 7:45 AM. FFA concentrations were significantly lower after acipimox than placebo administration (0.08 ± 0.02 mEq/L *v* 0.35 ± 0.53 mEq/L, *P* < .05). Adiponectin concentrations were also significantly lower after acipimox than placebo administration (7.4 ± 1.2 μg/mL *v* 10.3 ± 1.7 μg/mL, *P* < .05). The change in FFA between acipimox and placebo correlated significantly with the change in adiponectin (*r* = 0.66, *P* < .05), eg, the larger the reduction in FFA in response to acipimox, the larger the reduction in adiponectin. These results suggest that acute lowering of FFA is associated with decreased adiponectin concentrations.

ADIPONECTIN, a recently recognized adipocyte-derived cytokine, is the most abundant gene product in adipose tissue and is present in high levels in the plasma. It is thought to play an important role in both glucose and lipid metabolism. Unlike other adipocytokines, adiponectin is found to be paradoxically low in patients with obesity,[1] type II diabetes,[2] and coronary artery disease.[3] Plasma adiponectin concentrations correlate with insulin sensitivity[1,4,5] and predict the development of type II diabetes.[6] Replacement of recombinant adiponectin in diabetic rats and adiponectin knock-out mice improves insulin sensitivity.[7,8] In animal studies, adiponectin increases clearance of free fatty acids (FFA) from the blood, likely by increasing skeletal muscle uptake of FFA[8] and by increasing FFA oxidation.[7,9] Studies in humans have reported a negative association between adiponectin and triglyceride levels[2,10] and have demonstrated that reduced adiponectin concentration is a marker of increased intramyocellular lipid concentration in obesity.[11]

Although animal models have elucidated some of the metabolic effects of adiponectin, little is known about its acute regulation. Adiponectin concentrations increase chronically with weight loss, either through changes in lifestyle[12] or gastric bypass,[13] and in response to peroxisome proliferator–activated receptor gamma (PPARγ) administration.[14,15] In contrast, insulin acutely lowers adiponectin concentrations during the euglycemic hyperinsulinemic clamp procedure.[16] Because adiponectin regulates metabolism of FFA, we hypothesized that FFA may in turn regulate adiponectin. Our data demonstrate that acute lowering of FFA by acipimox is associated with decreased adiponectin concentrations.

From the Massachusetts General Hospital Program in Nutritional Metabolism and Neuroendocrine Unit, Harvard Medical School, Boston, MA; and the General Clinical Research Center Massachusetts Institute of Technology, Cambridge, MA.

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Address reprint requests to Steven Grinspoon, MD, Program in Nutritional Metabolism, LON 207, 55 Fruit St, Massachusetts General Hospital, Boston, MA 02114.

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MATERIALS AND METHODS

Subjects

Ten male healthy subjects were enrolled in the study between October 2001 and July 2003. All subjects had a waist-to-hip ratio (WHR) less than 0.95. Subjects with diabetes mellitus, defined by fasting blood glucose greater than 126 mg/dL and/or 2-hour glucose over 200 mg/dL on 75-g oral glucose load, were excluded. Other exclusion criteria included body mass index (BMI) less than 20 kg/m^2, hemoglobin less than 9 g/dL, and use of oral or parental glucocorticoids, anabolic steroids, growth hormone, or antidiabetic agents within the 3 months prior to study initiation.

Written and informed consent was obtained from each subject before testing, in accordance with the Committee on Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology and the Subcommittee on Human Studies at the Massachusetts General Hospital.

Outpatient Screening Visit

After a 12-hour overnight fast, subjects reported to the General Clinical Research Center (GCRC) for a screening visit, at which time eligibility was determined based on fasting blood glucose level and 2-hour glucose level on a 75-g oral glucose tolerance test. Height, weight, BMI, and WHR were determined.

Inpatient Visits

Within 2 weeks of the screening visit, each subject was admitted to the GCRC at the Massachusetts General Hospital for 2 inpatient visits, separated by 1 week. During each visit, subjects underwent an overnight fast and were randomized to receive either acipimox (Olbetam, Pharmacia, Milan, Italy), 500 mg at 2 AM and again at 6 AM at the first visit and placebo at the second, or vice versa (Fig 1). Fasting blood glucose, insulin, adiponectin, and FFA levels were determined at 7:45 AM at each visit, 1 hour and 45 minutes after the second dose of acipimox or placebo.

Laboratory Methods

Serum adiponectin concentration was measured using a radioimmunoassay (RIA, Linco Research, St. Charles, MO). The inter- and intra-assay coefficients of variation (CVs) ranged from 6.4% to 8.4% and 3.6% to 6.9%, respectively. Insulin concentrations were determined in serum by radioimmunoassay (Diagnostic Product Corp, Los Angeles, CA). The interassay CV ranged from 4.9% to 9.4%. The

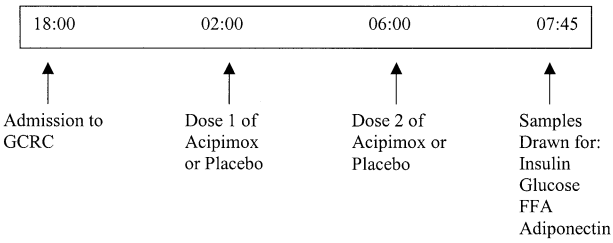

Fig 1. Schematic diagram of the study design.

intra-assay CV ranged from 3.5% to 8.9%. Nonesterified fatty acid concentrations were measured using an in vitro enzymatic colorimetric assay kit (Wako Chemicals USA, Richmond, VA). The intra-assay CV for fatty acids ranged from 1.1% to 2.7%. The published normal range for fatty acids is 0.1 to 0.6 mEq/L (mmol/L). Glucose and triglyceride concentrations were measured by standard techniques.[17] Homeostasis model assessment (HOMA) was calculated as an estimate of insulin resistance as described by Matthews et al.[18]

Statistical Analysis

Changes in adiponectin, FFA, HOMA, and fasting insulin concentration before and after treatment with acipimox were compared using paired *t* tests. The change in adiponectin was compared to the change in FFA and HOMA using a nonparametric multivariate Hoeffding's D test. All statistical analyses were made using SAS JMP Statistical Database Software (version 4; SAS Institute, Cary, NC). Statistical significance was defined as a 2-tailed α value of $P \leq .05$. Results are mean ± SEM unless otherwise indicated.

RESULTS

Subject Characteristics

The subjects' ages ranged from age 32 to 46 years with a mean of 39 years. Characteristics of the study subjects are shown in Table 1. Mean BMI was 28.0 ± 1.0 kg/m^2 and WHR was 0.91 ± 0.10. The fasting insulin, glucose, and triglyceride concentrations were normal.

Change in Response to Acipimox

FFA concentrations were significantly lower after acipimox than placebo administration (0.08 ± 0.02 mEq/L *v* 0.35 ± 0.53 mEq/L, $P < .05$). Adiponectin concentrations were also significantly lower after acipimox than placebo administration (7.4 ± 1.2 μg/mL *v* 10.3 ± 1.7 μg/mL, $P < .05$) (Table 2 and Fig 2). The change in FFA between acipimox and placebo was positively correlated with the change in adiponectin. Larger reductions in FFA in response to acipimox were associated with greater reductions in adiponectin ($r = 0.66$, $P < .05$). There was no significant change in fasting glucose, insulin, or HOMA between acipimox and placebo administration (Table 2).

DISCUSSION

Our study shows that acute administration of acipimox lowers FFA and simultaneously decreases adiponectin concentration in healthy male subjects. This finding contrasts with the observation that higher triglycerides and FFA are associated with lower adiponectin levels.[2,10,11] While an inverse relationship between adiponectin and FFA may exist in chronic equilibrium, the acute regulation of adiponectin by FFA remains largely unknown. Peake et al demonstrated that an increase in FFA produced by a high-fat meal did not change adiponectin postprandially over a 6-hour period.[19] The investigators used a high-fat, low-carbohydrate diet in an attempt to maintain steady insulin levels, since fasting insulin levels are correlated with adiponectin. Despite the content of the meal, insulin increased by approximately 200% while FFA increased by approximately only 25%. The absolute change of FFA may not have been adequate to produce a detectable change in adiponectin. Furthermore, hormonal changes in the setting of a meal may have acted as potential confounders to obscure the relationship between FFA and adiponectin.

Staiger et al investigated the acute effects of FFA on adiponectin and, in contrast to our study, demonstrated no effects of acute reduction in FFA by acipimox on adiponectin.[20] Staiger et al used a smaller total dose of acipimox (500 mg compared to 1000 mg in our study) but achieved a similar suppression of FFA levels.[20] The explanation for the contrasting results between our study and that of Staiger et al is not clear. However, different results may relate to the clinical characteristics of the study subjects (mixed male and female in the study by Staiger et al *v* all male in our study, lean subjects in the study of Staiger et al *v* healthy but moderately overweight subjects in our study), dosing issues, or other factors, including timing of acipimox administration and adiponectin determination (midmorning *v* early morning), or study design (open label administration *v* randomized, placebo-controlled crossover design). The significant correlation between change in FFA and change in acipimox seen in our study argues for a biological effect of FFA on adiponectin regulation, but further studies will be necessary to answer this important question.

Table 1. Demographics of Study Population

	Mean	Range
Age (yr)	39 ± 2	32-46
Race		
Caucasion (n)	7	
Black (n)	2	
Asian (n)	1	
BMI (kg/m^2)	28.0 ± 1.0	22-32
WHR	0.91 ± 0.10	0.84-0.98
HOMA	1.94 ± 0.26	0.93-3.66
Fasting insulin (mIU/L)	8.0 ± 1.0	4.2-13.6
Fasting glucose (mg/dL)	98 ± 3	87-111
Fasting triglycerides (mg/dL)	124 ± 30	27-324
Fasting FFA (mEq/L)	0.35 ± 0.05	0.07-0.61

Table 2. Hormone Concentrations in Response to Acipimox and Placebo

	Placebo	Acipimox	*P* Value*
Adiponectin (μg/mL)	10.3 ± 1.7	7.4 ± 1.2	<.05
FFA (mEq/L)	0.35 ± 0.53	0.08 ± 0.02	<.05
Fasting insulin (mIU/L)	7.4 ± 1.5	7.3 ± 1.7	NS
Fasting glucose (mg/dL)	92 ± 2	87 ± 3	NS
HOMA	1.7 ± 0.3	1.6 ± 0.4	NS

*Paired *t* test. NS, not significant.

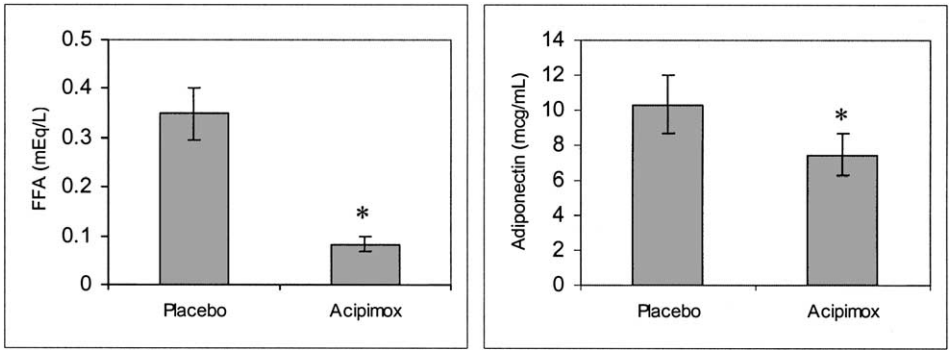

Fig 2. FFA and adiponectin concentration in response to acipimox and placebo.

Staiger et al also studied the acute effects of lipid infusion on adiponectin. Intralipid administration resulted in a 5.5-fold increase in FFA and a statistically significant increase in adiponectin 360 minutes after initiation of the lipid infusion.[20] Increased adiponectin after lipid infusion suggests potential acute regulation of adiponectin by FFA or related metabolic variables.

Animal studies suggest that adiponectin increases oxidation of FFA in skeletal muscle[7,9] and stimulates muscle fatty acid transporter,[1,8] resulting in accelerated FFA clearance from the blood. Mice fed a high-fat diet or intravenous intralipid with concomitant recombinant adiponectin showed blunted increases in FFA and triglyceride concentration compared to mice treated with a high-fat diet or intralipid alone.[9] In human studies, plasma adiponectin correlates inversely with intramyocellular lipid concentration.[21] Prior data, therefore, suggest that adiponectin may regulate FFA and lipid metabolism through effects on fat oxidation in the muscle. We now demonstrate that FFA may, in turn, acutely regulate adiponectin. This negative regulation of adiponectin by FFA may be useful to limit FFA clearance from the circulation, eg, a reduction in FFA results in reduced adiponectin and reduced clearance of FFA from the circulation. Conversely, our model would predict that an acute elevation in FFA may stimulate adiponectin to increase FFA clearance from the circulation as shown recently by Staiger et al,[20] although we did not directly investigate this question.

Adiponectin's effects on FFA may at least partially mediate its effects on insulin sensitivity. Weiss et al showed that the relationship between adiponectin and glucose disposal rate was lost when investigators controlled for triglycerides, suggesting that adiponectin's effects on glucose disposal may be mediated through changes in triglycerides.[11] Among obese subjects, higher endogenous FFA have been shown to correlate with hyperinsulinemia.[22,23] FFA impairs glucose uptake in peripheral muscle leading to decreased muscle glycogen synthesis and decreased glucose oxidation.[24,25] Studies have also suggested that FFA impair insulin's suppression of hepatic glucose output.[26,27] Administration of acipimox in obese and diabetic subjects results in lowered plasma insulin and glucose, and improved insulin-stimulated glucose uptake.[28,29]

Due to the effects of FFA on insulin sensitivity, it is possible that the decrease in adiponectin seen in the current study resulted from changes in insulin sensitivity, rather than directly from FFA reduction. While in vitro data on the effects of insulin on adiponectin are controversial, Mohlig et al demonstrated an acute reduction of adiponectin in the setting of hyperinsulinemic euglycemic clamps in 5 healthy control subjects.[16] In our healthy population, however, acipimox did not significantly change fasting insulin levels or insulin sensitivity measurable by HOMA. Moreover, acipimox would be expected to lower insulin levels thus raising adiponectin, the opposite of the observed effect. This suggests that the change in adiponectin resulted from either the lowering of FFA, as supported by the significant correlation between change in adiponectin and change in FFA, or from a direct effect of acipimox itself.

This study was limited to men because of the potentially different physiology of adiponectin in men and women. Subjects were in the fasting state, and changes during fasting may not duplicate the effects of FFA or triglyceride fluxes postprandially. Further studies are needed to investigate the relationship between FFA and adiponectin in women and in the postprandial state.

In summary, our data suggest that adiponectin concentrations decrease in association with acute lowering of FFA. Further studies are needed to clarify the acute regulation of adiponectin, the potential role of FFA in adiponectin regulation, and the molecular mechanisms whereby FFA might regulate adiponectin.

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