

PAPER

The postprandial response of adiponectin to a high-fat meal in normal and insulin-resistant subjects

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OBJECTIVE: Adiponectin is an adipose-specific protein with short-term effects *in vivo* on glucose and fatty acid levels. We studied the plasma concentration and the proteolytic activation status of adiponectin following the consumption of a high-fat, low-carbohydrate meal.

DESIGN: Analysis of adiponectin concentration and polypeptide structure after consumption of a fat meal.

SUBJECTS: Normal subjects ($n = 24$) and first-degree relatives of patients with type II diabetes ($n = 20$).

MEASUREMENTS: All subjects had a normal fasting plasma glucose and glucose tolerance. Blood was collected for the determination of plasma insulin, adiponectin, triglyceride, and free fatty acids. Body composition was assessed with dual-energy X-ray absorptiometry and whole-body insulin sensitivity with a euglycaemic, hyperinsulinaemic clamp. Postprandial response over 6 h was determined for plasma adiponectin, glucose, insulin, triglyceride, and free fatty acids. Adiponectin was measured by commercial RIA and its polypeptide structure examined by Western blotting.

RESULTS: The relatives were more insulin resistant and had increased adiposity compared with control subjects. There was no significant difference in postprandial response in fatty acids, triglyceride, or insulin between the groups. Postprandial levels of adiponectin measured by radioimmunoassay were not significantly different from fasting levels, and no breakdown products of adiponectin were detectable in postprandial samples by Western blotting.

CONCLUSIONS: Levels of circulating adiponectin do not alter in response to a fat meal, despite evidence in mice that acute changes in adiponectin significantly affect postprandial fatty acid flux. Moreover, a fat meal challenge did not lead to significant activation of adiponectin by proteolytic conversion.

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Introduction

There is increasing evidence that adipose tissue is an important endocrine organ producing a range of polypeptides which act locally and systemically.¹ These include adiponectin, an adipose-specific protein which circulates at high concentration in the plasma.² Circulating levels of adiponectin correlate negatively with cardiovascular disease in patients with type II diabetes,³ consistent with the hypothesis that this protein acts as a protective factor for the cardiovascular system.⁴ This effect may be mediated by its anti-inflammatory activity; adiponectin inhibits NF- κ B

signalling, and modulates the expression of adhesion molecules by endothelial cells.⁵

A strong correlation between plasma adiponectin levels and whole body insulin sensitivity has been demonstrated. In both Pima Indians and Caucasians, decreased levels of adiponectin were related to insulin resistance and hyperinsulinaemia.⁶ Adiponectin is believed to act via an insulin sensitising action to suppress glucose production.⁷ Insulin sensitising drugs such as thiazolidinediones significantly increase plasma adiponectin in both humans and rodents.⁸

Adiponectin has also been reported to have a role in fat metabolism. While the native molecule appeared inactive, acute treatment of mice with a 16 kDa fragment of adiponectin significantly decreased the elevated levels of plasma-free fatty acids caused either by administration of a high-fat test meal or by Intralipid. This was accompanied by an acute increase in fatty acid oxidation by muscle. Furthermore, daily administration of this fragment to mice

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fed a high-fat/sucrose diet caused significant and long-term weight reduction, without affecting food intake.⁹ In humans, levels of adiponectin are inversely related to adiposity,³ despite it being the product of adipose tissue.

Patients with type II diabetes commonly show insulin-resistance, low levels of high-density lipoprotein cholesterol (HDL), and increased circulating fatty acids. McGarry has proposed that elevated levels of fatty acids contribute to the development of glucose intolerance by their influence on the supply of excess lipid to muscle and to the pancreatic beta-cell.¹⁰ It is not clear whether oral lipid handling is also abnormal.

Given evidence of short-term metabolic effects of adiponectin and its fragments *in vivo* and *in vitro*, we investigated the relation between levels of adiponectin and plasma lipid after consumption of a high-fat, low-carbohydrate meal. We also examined the plasma for evidence of the postprandial activation of adiponectin by the generation of proteolytic fragments. The subjects investigated comprised a clinically healthy control group with no family history of diabetes, and normoglycaemic first-degree members of families with a high incidence of type II diabetes (≥ 2 per family). This latter group had a significantly greater body mass index (BMI) and %body fat, and lower insulin sensitivity.

Patients and methods

Subjects

Healthy, sedentary, nonsmoking subjects (29 females/15 males), with no history of cardiovascular disease, dyslipidaemia, or other major health problems, were included. In the control group there were 14 female and 10 male subjects, and in the relatives group, 15 female and five male subjects. The St Vincent's Hospital Human Research Ethics Committee approved the study and all subjects gave written informed consent. The subjects' self-assessed weight was stable (< 2 kg change) for the previous 3 months.

All women were premenopausal, and five control women and three among the relatives were taking oral contraceptives at the time of the study. No control on the time of the menstrual cycle was imposed in this study, but all were not pregnant, and were not lactating.

Protocol

Procedures were undertaken following a 10 h fast on three separate occasions. On the first visit, all subjects had a normal fasting plasma glucose (< 6.1 mmol/l) and were shown to have normal glucose tolerance following a 75 g oral glucose tolerance test. After 7–10 days, blood was collected for the determination of plasma insulin, adiponectin, total cholesterol, triglyceride, HDL-cholesterol, and free fatty acids. During the same morning, body composition was assessed with dual-energy X-ray absorptiometry (DEXA) and a 120 min euglycaemic, hyperinsulinaemic clamp performed to determine whole-body insulin sensitivity.

Approximately 1 week later, a fat meal was given and the postprandial response measured over the subsequent 6 h.

Anthropometry and body composition

At 8:00 h on the first visit, weight and height were measured in light clothing with footwear removed. Weight was measured to the nearest 0.1 kg using a digital electronic scale. Height was measured to the nearest 0.1 cm using a stadiometer. BMI was calculated as weight divided by height squared (kg/m^2). Whole body DEXA (Lunar DPX-Lunar Radiation Corporation, Madison, WI, USA software version 1.35 y) was used to analyse the body composition according to a three compartmental model, comprising fat mass, lean tissue, and bone mineral content. Measurement of central body fat has been described elsewhere,¹¹ and includes both visceral and subcutaneous abdominal fat.

Oral glucose tolerance test (OGTT)

Following an overnight fast and after dietary advice was given to ensure a carbohydrate intake of > 150 g/day over the previous 3 days, glucose tolerance was assessed by a 75 g OGTT. Blood samples for glucose and insulin were taken immediately before and 30, 60, 90, and 120 min after the glucose load.

Euglycaemic, hyperinsulinaemic clamp

Subjects underwent a 120 min euglycaemic hyperinsulinaemic clamp (insulin dose $50 \text{ mU}/\text{m}^2/\text{min}$; Actrapid HM, Novo Industries, Denmark), after a 10 h overnight fast as previously described.^{11,12} A variable-rate glucose infusion (25% dextrose) was used to maintain blood glucose levels close to 5 mmol/l. The steady-state glucose infusion rate (GIR; measured here over the final 30 min of the clamp) provided an index of whole-body insulin sensitivity.

Oral fat meal test

Subjects ate a meal containing ~ 80 g of dietary fat with an energy content of 4250 kJ. The meal contained 19 g carbohydrate, 40 g saturated fat, and 47 g protein. Subjects remained supine for 1 h prior to and 6 h following the meal. The meal was consumed within 20 min, accompanied by 600 ml water. No other food or beverage was consumed through the testing period.

The meal consisted of: cheese (44 g), beef (100 g), fried egg (118 g), coha (coconut oil; 8 g), cracker biscuit (12.5 g), cream (fat $> 48\%$) (25 g), strawberries (130 g), and macadamia nuts (20 g); total 458 g.

Biochemical analysis

Plasma glucose was measured by the glucose oxidase method (Yellow Springs Instruments Model 23AM Glucose Analyser,

Yellow Springs, OH, USA). Plasma insulin was measured by radioimmunoassay (Linco RIA Charles, MO, USA). Serum total cholesterol, HDL-cholesterol, triglyceride, and free fatty acid concentrations were determined spectrophotometrically using enzymatic colourimetric kits (CHOD-PAP kit, C.f.a.s. HDL-C kit, GPO-PAP kit, Roche Diagnostics, Basel, Switzerland and NEFA kit, Wako Inc., Japan). Inter- and intra-assay CV were <10% for these assays in our laboratory.

Analysis of adiponectin

Adiponectin levels in basal and postprandial sera were measured by commercial RIA (Linco, St Charles, USA). Adiponectin in serum samples was also examined by separation on 11% reducing SDS-PAGE gels, and blotting onto nitrocellulose. A volume of 70 µl of a 1/20 dilution of serum in reducing sample buffer were loaded in each lane. Blots were probed with a monoclonal antibody to adiponectin (BD Biosciences, Sydney, Australia), which was detected with an affinity purified, alkaline phosphatase-conjugated antibody to mouse Ig (Sigma, Sydney, Australia). Development was by nitroblue tetrazolium/ bromochloroindolyl phosphate.

Trypsin digest

To confirm the ability of Western blotting to demonstrate fragments of adiponectin in serum, samples were diluted with two volumes of PBS containing TPCK-treated trypsin (Sigma) at a final concentration of 1.7, 0.3, 0.07, 0.01, and 0% w/v. Samples were incubated for 90 min at 37°C before analysis as above.

Statistical analysis

Comparisons between groups at baseline or postprandially were performed using one-way ANOVA with Dunnett post-tests, and paired and unpaired *t*-tests. Relations between continuous variables were assessed by simple and multiple

regression, or two-tailed Spearman's correlation analyses, as appropriate. These analyses were performed using GraphPad Prism. Results are presented as mean ± s.e.m.

Results

Subject characteristics

Subjects formed part of a larger group chosen to have an age range of 25–45 y and BMI <35 kg/m². Characteristics of subjects in this study are shown in Table 1. Male subjects had significantly reduced percentage body fat and fasting adiponectin levels compared with female subjects (Table 1), and fasting levels of NEFA were higher in women: 0.54 ± 0.04 vs 0.28 ± 0.03 mmol/l in males (*P* < 0.0001). First-degree relatives of patients with type II diabetes had a higher % body fat, and lower insulin sensitivity. However, mean fasting levels of insulin and adiponectin were not significantly different in the two groups. Moreover, the basal level of glucose did not significantly differ in relatives of patients with diabetes compared with control subjects: 5.05 ± 0.09 vs 4.96 ± 0.10 mmol/l.

Postprandial response

Postprandial levels of insulin, NEFA, and triglyceride changed significantly after the fat meal, levels of insulin rising around two-fold 1 h postprandially (Figure 1). Postprandial responses in NEFA, triglyceride, and insulin were similar in the relatives of patients with diabetes and control subjects. Plasma glucose was little changed in both groups from 2 h postprandially (Figure 1).

Analysis of adiponectin

Levels of adiponectin observed postprandially are shown in Figure 2. Both groups showed no significant alteration in postprandial adiponectin over the 6 h period when analysed by paired-comparison *t*-test. There was also no significant change over 6 h when postprandial adiponectin levels were

Table 1 Subject characteristics

	All Subjects N = 44	Controls N = 24	Relatives N = 20	Females N = 29	Males N = 15
Age (y)	31.57 ± 1.05	32.06 ± 1.47	30.98 ± 1.53	31.20 ± 1.42	32.30 ± 1.48
Weight (kg)	73.43 ± 2.64	72.10 ± 3.44	75.03 ± 4.16	67.11 ± 2.64	85.66 ± 4.45*
Height (cm)	171.0 ± 1.6	173.4 ± 2.3	168.2 ± 1.9	165.9 ± 1.4	180.9 ± 1.9*
BMI (kg/m ²)	24.93 ± 0.70	23.77 ± 0.78	26.32 ± 1.17	24.39 ± 0.93	25.96 ± 0.99
Total body fat (kg)	23.3 ± 1.7	20.1 ± 1.9	27.1 ± 2.8***	24.8 ± 2.2	20.3 ± 2.7
% Body fat	31.0 ± 1.6	27.6 ± 2.0	35.0 ± 2.3***	35.3 ± 1.7	22.5 ± 2.2*
Fat-free mass (kg)	50.1 ± 1.9	52.1 ± 2.7	47.8 ± 2.5	42.3 ± 0.7	65.3 ± 2.3*
Adiponectin (µg/ml)	18.49 ± 1.32	19.02 ± 1.66	17.86 ± 2.14	22.12 ± 1.46	11.47 ± 1.44*
Insulin (mU/ml)	10.45 ± 0.98	9.163 ± 1.07	11.99 ± 1.69	10.02 ± 0.96	11.28 ± 2.23
Insulin sensitivity (µmol/min/kg)	41.24 ± 2.74	46.97 ± 4.03	34.36 ± 3.04***	35.92 ± 2.60	51.52 ± 5.49**

All data shown as mean ± s.e.m. Significance was determined by unpaired *t*-test.

****P* < 0.05 for differences between subgroups.

***P* ≤ 0.01 for differences between subgroups.

**P* < 0.001 for differences between subgroups.

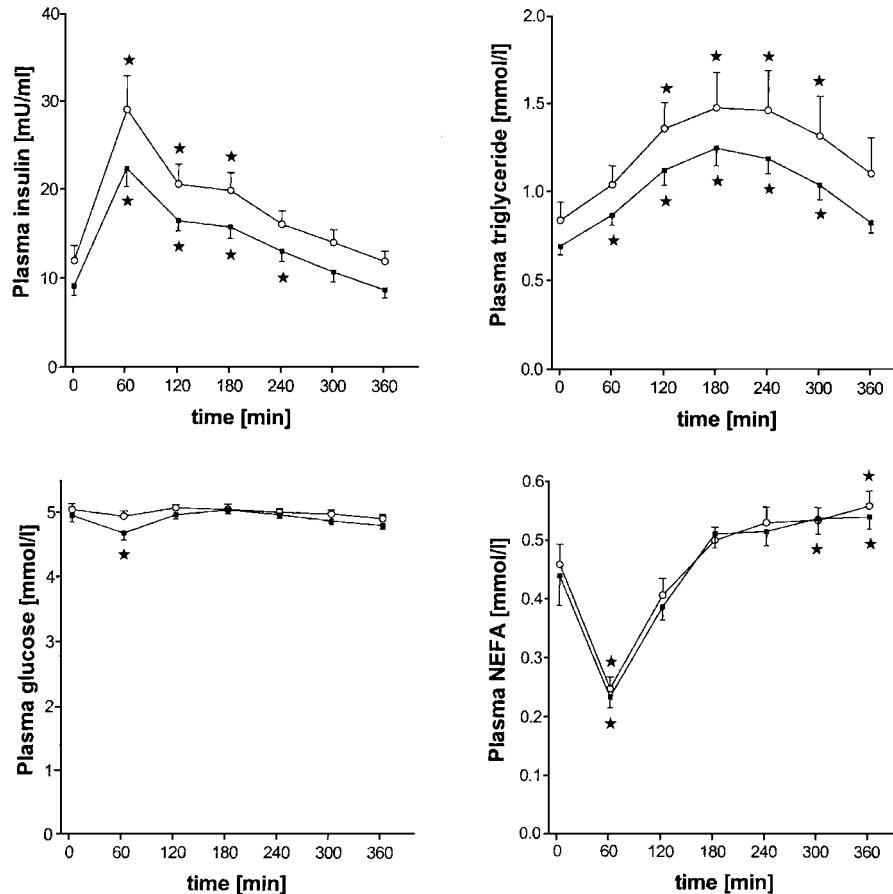


Figure 1 Levels of plasma insulin, NEFA, glucose, and triglyceride for control subjects (■) and relatives (○) of patients with diabetes following ingestion of a fat meal. Fasting levels are shown at $t=0$ min. Levels of analytes significantly different from fasting levels are highlighted (*). Data are shown as mean \pm s.e.m.

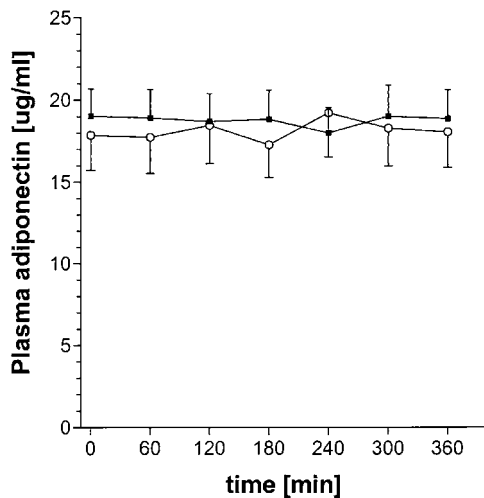


Figure 2 Levels of plasma adiponectin for control subjects (■) and relatives (○) of patients with diabetes following ingestion of a fat meal. Fasting levels are shown at $t=0$ min. Data are shown as mean \pm s.e.m.

compared between male and female subjects. The results obtained by RIA were confirmed semiquantitatively by a Western blot of plasma samples (Figure 3). Blots were performed on all subjects in this study and showed no evidence of the postprandial generation of breakdown products of adiponectin consistent with the 16 kDa species previously reported.⁹ To confirm the ability of this technique to demonstrate such fragments, adiponectin in plasma was digested with increasing quantities of TPCK-treated trypsin.⁹ Numerous species were detected following digestion, including one consistent with the 16 kDa band metabolically active in mice (Figure 3).

Discussion

Adiponectin is present in the circulation in significant ($\mu\text{g/ml}$) quantities, but its metabolic role and manner of regulation remain uncertain. Both obesity and type II diabetes are associated with low plasma adiponectin concentrations, and the degree of insulin resistance and

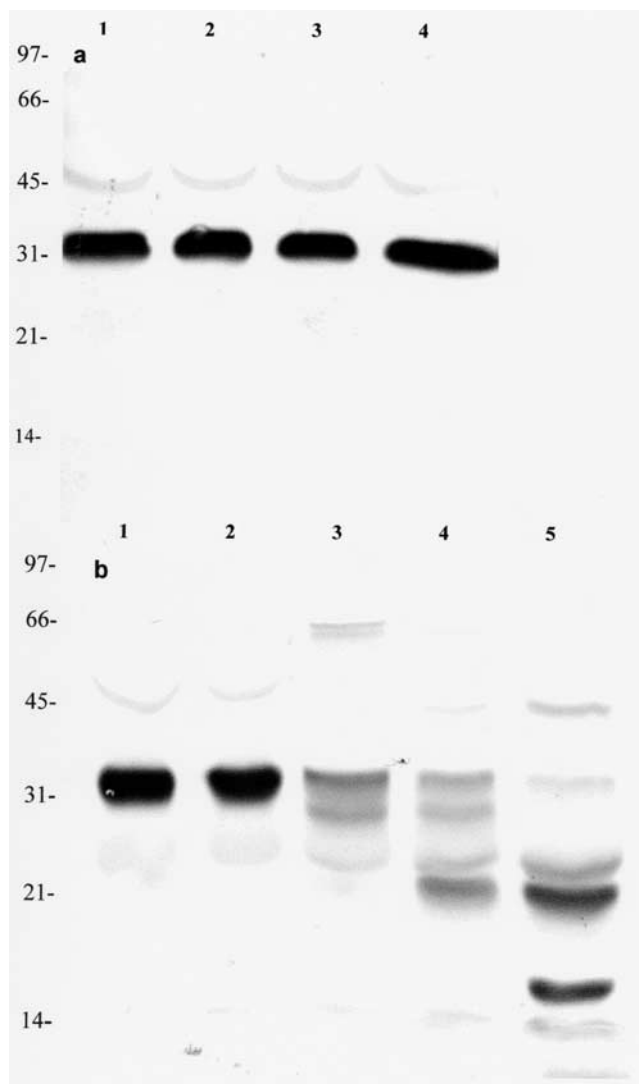


Figure 3 (a) A Western blot of a reducing SDS-PAGE gel showing plasma adiponectin in samples from a representative control subject fasting (lane 1), and at 2 (lane 2), 4 (lane 3), and 6 h (lane 4) postprandially. Molecular weight markers (kDa) are shown at left. (b) A Western blot of a reducing SDS-PAGE gel showing plasma adiponectin in the same fasting plasma sample incubated for 90 min with final trypsin concentrations of 0 (lane 1), 0.01 (lane 2), 0.07 (lane 3), 0.3 (lane 4) and 1.7% w/v (lane 5). Molecular weight markers (kDa) are shown at left.

hyperinsulinaemia are more important predictors of adiponectin levels than the degree of adiposity and glucose intolerance.⁶ Adiponectin deficiency may play a role in the development of insulin resistance since levels of adiponectin decrease early in the development of insulin resistance in the live rhesus model.¹³ Insulin resistance is an important risk factor for the development of atherosclerosis, which was suggested to be a 'postprandial phenomenon' over 20y ago.¹⁴

Our study subjects were carefully characterised in terms of body composition, insulin sensitivity, and glucose tolerance.

This facilitated the interpretation of postprandial data, where it is possible that the adiponectin/lipid relation is heterogeneous, and may be related to the degree of insulin sensitivity. In addition, the choice of normoglycaemic subjects avoided any secondary effects of elevated glucose on lipid profiles. The very low-carbohydrate content of the fat meal was designed to minimise insulin response, since fasting levels of adiponectin are correlated with levels of insulin.³ In a mixed test meal, where significant levels of carbohydrate were present, insulin levels rose five- to six-fold, and there were significant differences between the insulin response of normal subjects and that of relatives of patients with type II diabetes.¹⁵ Our meal data showed around a two-fold increase in insulin 60 min postprandially, and levels of NEFA and triglyceride also changed significantly in this period, with no significant differences between patient groups. However, the control subjects and relatives of patients with type II diabetes differed in BMI and % body fat, while the relatives had significantly lower insulin sensitivity, a finding in agreement with previously reported data.¹⁵

The mechanism whereby adiponectin exerts its effects on the body is not well understood. A receptor for adiponectin has yet to be described, and it is unclear whether the protein must be metabolised to a smaller, pharmacologically active form in order to function. A 16 kDa proteolytic fragment of adiponectin, gAcrp30, acutely reduced the postprandial increase in fatty acids normally observed in mice, whereas the full-length molecule was reported to be inactive.⁹ However, our study of human subjects suggests that there is no significant production of fragments of adiponectin in the postprandial state.

Our data show that in subjects fully characterised for %fat and insulin sensitivity, there are no marked changes in plasma adiponectin over 6h in response to a high-fat challenge. A previous study of subjects, given three undefined meals, detected no evidence of significant postprandial alteration in adiponectin levels over 22 h.³ Data from these two studies suggest that, despite evidence in mice that acute changes in adiponectin levels affect postprandial fatty acid flux,⁹ levels of circulating adiponectin do not respond to fatty acid levels in human subjects.

In summary, these data show that despite evidence that adiponectin can acutely control fatty acid and glucose metabolism, circulating levels of adiponectin in both insulin-sensitive and insulin-resistant subjects do not acutely respond to a high-fat load, either in terms of circulating levels, or by proteolytic conversion to a different molecular form. Instead, it is possible the activity of adiponectin is regulated by changes in the relative abundance of oligomeric isoforms of adiponectin.¹⁶

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