British Journal of Nutrition (2008), **99**, 76–82
doi: 10.1017/S0007114507791857

Postprandial plasma adiponectin decreases after glucose and high fat meal and is independently associated with postprandial triacylglycerols but not with − 11388 promoter polymorphism

Diana Rubin[1,4]*, Ulf Helwig[1,4], Michael Nothnagel[2], Nina Lemke[1], Stefan Schreiber[3], Ulrich R Fölsch[4], Frank Döring[5] and Jürgen Schrezenmeir[1]

[1]*Institute of Physiology and Biochemistry of Nutrition, Federal Research Center of Nutrition and Food, Kiel, Germany*
[2]*Institute of Medical Informatics and Statistics, University Clinic Schleswig-Holstein, Campus Kiel, Germany*
[3]*Institute of Clinical Molecular Biology, Christian-Albrechts University, Kiel, Germany*
[4]*Department of General Internal Medicine, University Clinic Schleswig-Holstein, Campus Kiel, Germany*
[5]*Institute for Molecular Nutrition, Christian-Albrechts-University, Kiel, 24103 Kiel, Germany*

(Received 15 December 2006 – Revised 27 April 2007 – Accepted 4 June 2007)

Adiponectin is discussed to regulate energy balance and insulin sensitivity. Several studies indicated an association of fasting adiponectin with parameters of the metabolic syndrome. We investigated postprandial adiponectin release and its relation to traits of the metabolic syndrome. Serum adiponectin concentration after an oral glucose tolerance test and after ingestion of a standardised mixed, fat-containing meal in 110 male non-diabetic subjects was assessed. Fasting and postprandial adiponectin and the decrease of adiponectin were correlated with anthropometric and metabolic parameters. Subjects were genotyped for adiponectin − 11 388 G/A promoter single nucleotide polymorphism. Adiponectin slightly decreased after both test meals. A significant decrease was attained 5 and 6 h after the lipid load and 2 h after the glucose load. Particularly, the mixed meal postprandial adiponectin showed stronger correlations with most traits of the metabolic syndrome than fasting adiponectin: postprandial adiponectin with HDL (r 0·30) *v.* fasting adiponectin with HDL (r 0·23); with postprandial insulin (area under the curve): r −0·20 *v.* r −0·16; with fasting insulin: r 0·10 *v.* r 0·14; with BMI: r −0·23 *v.* r −0·20; with waist: r −0·18 *v.* −0·16; with systolic blood pressure: r −0·14 *v.* r −0·12; with diastolic blood pressure: r −0·18 *v.* r −0·15. In multivariate analysis, postprandial TAG were the only independent predictor of adiponectin. There was no significant association of adiponectin, NEFA and TAG with − 11 388 G/A adiponectin promoter polymorphism. Our findings favour the interpretation that postprandial adiponectin has the strongest and independent associations to postprandial TAG metabolism.

Adiponectin: Postprandial triacylglycerol: Insulin resistance: Glucose tolerance test: High-fat meal: Metabolic syndrome: Adiponectin promoter polymorphism

Diabetes type 2 is strongly associated with obesity but the mechanisms of how adipose tissue interacts with glucose metabolism are complex and still remain to be clarified. Higher BMI is associated with lower insulin sensitivity along with higher plasma levels of various adipokines, including leptin, resistin and TNF-α, which are promising candidates for linkage between insulin resistance and obesity[1]. Among the recently identified factors, the adipocyte-derived protein adiponectin is of particular interest because it may directly affect insulin-mediated glucose uptake[2]. Adiponectin is produced exclusively by white adipose tissue and is secreted into serum[3]. It is suggested to carry signals from adipose tissue to other organs in order to promote fat oxidation[4,5]and suppress hepatic glucose production[2,6]. Treatment of mice with purified adiponectin significantly decreased the elevated levels of plasma NEFA, caused either by a high fat/sucrose diet or by intravenous injection of lipids[5].

Several human studies revealed a strong positive correlation of fasting adiponectin and insulin sensitivity in normal subjects[7–9]. In obesity and type 2 diabetes, adiponectin levels are decreased, although it is produced by adipose tissue[10–13]. A circadian regulation of adiponectin was excluded before[13].

There are conflicting results on the effect of different meals on postprandial adiponectin levels in human subjects. Some studies found no significant difference between postprandial and fasting levels[14,15]. Others found a decrease in special subgroups of subjects or after special meals[16]. An increase of adiponectin was even reported for obese subjects[17]. The associations of adiponectin gene polymorphisms with parameters of the metabolic syndrome were also inconsistent.

Because of the strong association of postprandial TAG metabolism with early insulin resistance syndrome[17–21] our aim was to clarify the relationship between postprandial adiponectin and TAG, insulin and glucose in a population of

Abbreviations: AUC, area under the curve; HOMA, homeostasis model assessment; HOMA-IR, homeostasis model assessment of insulin resistance; oGTT, oral glucose tolerance test; oMTT, oral metabolic tolerance test.

* **Corresponding author:** Dr Diana Rubin, fax +49 431 6092472, email diana.rubin@bfel.de

British Journal of Nutrition

110 lean and obese subjects without diabetes. We further investigated the common promoter polymorphism − 11 388 G/A for associations with fasting and postprandial parameters after a liquid fat load and a plain glucose challenge test.

Materials and methods

Subjects

Data were analysed from 110 male subjects who participated in a study aimed to evaluate postprandial metabolic values after ingestion of a fat-containing meal. Subjects were selected by age from the general population *via* the registration office of the town of Kiel, Germany. Inclusion criteria were age 45–65 years and absence of self-reported diabetes. Exclusion criteria were intake of hormones or lipid-lowering medication, surgery of the intestinal tract in the last 3 months or other alterations of the gastrointestinal tract, malassimilation, active cancer, chronic renal or liver disease, anaemia and alcohol abuse. All subjects gave written informed consent before participating. The study was approved by the Medical Ethical Committee of the University Clinic Kiel. Length, weight, systolic and diastolic blood pressure, heart rate and waist:hip ratio were measured. Weight was measured with an electronic scale to the nearest 0·1 kg, and height was measured to the nearest cm (Seca, Hamburg, Germany). BMI was calculated as total body weight (kg)/height (m^2). For waist and hip measurements, participants were asked to roll down their undergarments. Waist circumference was measured with a constant tension tape to the nearest 0·1 cm midway between the lower rib margin and the upper iliac spine, which in most occasions was identical with the level of the umbilicus. Hip was measured at the height of trochanter femoris. Those measurements were done in an upright position with subjects breathing normally. Blood pressure was measured in a supine position after 5 min of rest by sphingomanometry (Boso, Jungingen, Germany) with the cuff at the same level as the heart during measurement. Subjects were classified as being hypertensive when treated with antihypertensive medications or their systolic/diastolic blood pressure was ≥130/85 mmHg. For classification of the metabolic syndrome Adult Treatment Panel III[22] criteria were used. The diagnosis of metabolic syndrome was established when three or more of the following risk factors were present: waist circumference >102 cm; TAG ≥ 150 mg/dl; HDL-cholesterol <40 mg/dl; blood pressure ≥130/85 mmHg; fasting glucose >110 mg/dl.

Oral glucose tolerance test

Dietary advice was given to ensure a carbohydrate intake of >150 g/d over the previous 3 d before the test. Following a 12 h overnight fast, an intravenous catheter was inserted into a forearm vein for blood sampling and a basal blood sample was obtained. Glucose tolerance was assessed by 75 g oral glucose. Venous blood samples for glucose, insulin and adiponectin determination were taken before and 30, 60, 120, 180 and 240 min after the glucose load.

Oral metabolic tolerance test

A minimum of 3 d after the oral glucose tolerance test (oGTT) participants visited our department after a 12 hour fast. An intravenous catheter was inserted into a forearm vein for blood sampling and a fasting blood sample was obtained. The subjects drank a standardised high-fat mixed meal (500 ml) containing the following ingredients: 30 g protein (11·9 kJ%); 75 g carbohydrate (29·6 kJ%) (93 % sucrose, 7 % lactose); 58 g fat (51·6 kJ%) (65 % SFA, 35 % unsaturated fatty acids); 10 g alcohol (6·9 kJ%); 600 mg cholesterol; 30·000 IU retinylpalmitate (Nutrichem, Roth, Germany). The total energy content was 4255 kJ (1017 kcal). The test meal was drunk within 10 min after the fasting blood withdrawal. Blood withdrawal was repeated 0·5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h and 9 h after ingestion of oral metabolic tolerance test (oMTT). Subjects were allowed to walk or sit, as they wished, but not to eat or exercise during the test. Drinking water was permitted *ad libitum*.

Laboratory analyses

TAG, NEFA, glucose, cholesterol and HDL-cholesterol were determined using enzymatic methods with Kone lab 20i analyzer (Kone, Espoo, Finland). Plasma insulin was measured with a standard RIA (Adaltis, Freiburg, Germany). Plasma adiponectin was measured with an ELISA development kit according to the manufacturers' recommendation (R&D Systems, Minneapolis, MN, USA). All samples were measured in duplicate. The interassay and intra-assay CV were <10 %. For the indirect determination of insulin sensitivity, the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows[22]: HOMA − IR = fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5. Genotyping of − 11 388 promoter polymorphism was performed by TaqMan allelic discrimination assay, using the ABI Prism 7000 sequence detection system (Applied Biosystems, Darmstadt, Germany). The probes were 5′-TTGCAAGAACCGGCTC-3′ (6-carboxyfluorescein fluorescence) and 5′-CTTGCAAGAACC-AGCTC-3′ (Vaccine-type-specific probe fluorescence). The primers were 5′-GGCATCCTAAGCCCTTGCT-3′ (forward) and 5′-TTGGCAGGCTCATGTTTTGTTTT-3′ (reverse). Using the TaqMan machine, PCR conditions were denaturation at 95°C, 15 min (1 cycle) followed by 45 cycles at 95°C for 10 s and 60°C for 1 min.

Statistical analyses

All data are expressed as means and standard deviations. The 0–9 h area under the curve (AUC) was calculated by the trapezoidal method. Parameters were tested for normal distribution by the Shapiro–Wilk test. Comparisons between the groups were performed using the Mann–Whitney *U* test. The signed rank test was used for dependent variables. Spearman's correlation coefficient was used to determine correlation between various parameters. The Friedman test as nonparametric test for dependent variables was used for statistical evaluation of postprandial changes. We used Bonferroni-Holm correction to assess the points where significant changes occurred, thus accounting for the compounding risk of multiple statistical comparisons. The independent influence of the studied variables on adiponectin levels was tested by multiple linear regression analysis, after variables were log-transformed to achieve a normal distribution. Comparisons between the clinical characteristics of the two

British Journal of Nutrition

Table 1. General characteristics and fasting metabolic parameters of the study group (*n* 110)* and subjects with (*n* 27) or without (*n* 83) the metabolic syndrome (MS) according to Adult Treatment Panel III criteria

(Values are means and standard deviations)

	All		No MS		MS	
Parameters	Mean	SD	Mean	SD	Mean	SD
Age (years)	57·2	6·2	57·4	6·4	56·4	5·7
BMI (kg/m^2)	28·2	5·3	26·5	3·9	32·8	6·2
Waist circumference (cm)	102·4	14·6	98·3	11·3	115·3	15·6
Systolic blood pressure (mmHg)	128·7	21·4	123·3	17·9	127·2	19·2
Diastolic blood pressure (mmHg)	81·3	12·9	78·0	10·2	92·0	14·3
Glucose (mg/dl)	108·3	18·4	103·9	12·7	125·3	26·7
TAG (mg/dl)	160·4	128	124·7	63·6	282·5	196·1
NEFA (mmol/l)	0·46	0·21	0·44	0·20	0·50	0·21
Cholesterol (mg/dl)	221·5	40·7	219·8	36·9	232·0	48·6
HDL-cholesterol (mg/dl)	50·9	13·2	54·2	12·2	39·3	11·5
LDL-cholesterol (mg/dl)	134·5	31·3	134·3	30·5	138·5	32·7
Insulin (mU/l)	16·2	12·5	14·0	10·0	28·4	27·0
HOMA-IR	4·6	4·6	3·8	4·3	7·0	4·6
Fasting adiponectin (pg/ml)	4071	2081	4222	2127	3637	1943

* For details of subjects and procedures, see Materials and methods.
HOMA-IR, homeostasis model assessment of insulin resistance.

genotype groups were made by Mann–Whitney *U* test. Bonferroni-Holm correction was applied for multiple genotype-phenotype testing. A *P* value of $<0{\cdot}05$ was considered statistically significant. Δ-Adiponectin was calculated by subtracting the minimum level of postprandial adiponectin from the fasting value. The increase of NEFA was defined by the difference of NEFA maximum and NEFA nadir, the decrease of NEFA was defined by the difference of fasting NEFA and the NEFA minimum.

Results

Subjects characteristics

On average, subjects were overweight with abdominal obesity at the Adult Treatment Panel III limit[23], insulin resistant referring to HOMA-normal limits ($<2{\cdot}6$) defined by Monzillo & Hamdy[24] and borderline hypertensive (Adult Treatment Panel III). Characteristics of the subjects are shown in Table 1. According to WHO criteria, there were nine formerly unknown type 2 diabetics among the 110 subjects. Fourteen additional subjects had either impaired fasting glucose or impaired glucose tolerance.

Fasting and postprandial adiponectin concentrations

Adiponectin levels decreased after oGTT and after oMTT (Fig. 1). After the glucose load, levels significantly decreased from 3964 (SD 177·7) to 3744 (SD 160·7) pg/ml after 2 h by 5·9 %. After the lipid-containing meal, the decrease attained significance at 5 h and 6 h postprandial from 3964 (SD 201·3) to 3638 (SD 178·1) ($P<0{\cdot}01$, 8·2 %) and to 3623 (SD 175·1) ($P<0{\cdot}0001$, 8·6 %). After Bonferroni corrections, the decrease was still significant (Fig. 1).

Fasting and postprandial (AUC) plasma adiponectin levels after oMTT and oGTT showed associations with traits of the metabolic syndrome and were negatively correlated with BMI (Tables 2 and 3). No significant correlation was found between the fasting or postprandial plasma levels of adiponectin and age found by Hotta *et al.*[10]. The correlation with metabolic parameters after oMTT revealed a significant negative correlation of fasting and postprandial plasma adiponectin with BMI, fasting TAG, postprandial TAG and a positive correlation with HDL-cholesterol. Postprandial insulin was associated with fasting and postprandial adiponectin, whereas fasting insulin was not (Table 3). After oGTT, fasting and postprandial adiponectin levels showed a correlation with the same parameters. After OGTT, there was also a negative correlation with HOMA-IR and postprandial HOMA-IR (AUC) (Table 2).

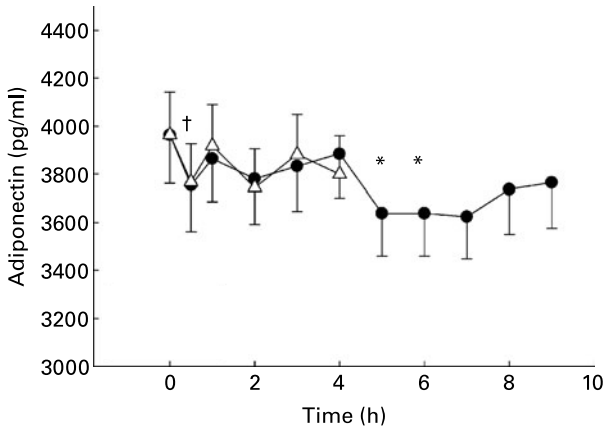

Fig. 1. Postprandial changes of adiponectin plasma levels after glucose load (oral glucose tolerance test, oGTT; Δ) and fat-containing mixed meal (oral metabolic tolerance test, oMTT; •) in 110 subjects aged 45–65 years recruited by the town registry of Kiel and excluding known diabetes. Mean values and standard deviations. Mean values were significantly different (*$P<0{\cdot}0065$ after oMTT; †$P<0{\cdot}017$ after oGTT compared with fasting levels as assessed by Wilcoxon test after correction according to Bonferroni Holm. For details of subjects and procedures, see Materials and methods.

Table 2. Correlation coefficients (r) of plasma adiponectin with clinical and metabolic parameters in the fasting state and after oral glucose tolerance test (oGTT)*

OGTT Variables	Fasting adiponectin		Postprandial adiponectin (AUC)		Δ adiponectin	
	r	*P*	*r*	*P*	*r*	*P*
Age	0·07	NS	0·14	NS	0·01	NS
BMI	−0·27	0·004	−0·26	0·005	0·16	0·07
Waist	−0·21	0·03	−0·19	0·05	0·18	NS
Systolic blood pressure	−0·14	NS	−0·14	NS	0·07	NS
Diastolic blood pressure	−0·12	NS	−0·14	NS	0·07	NS
Total cholesterol	−0·02	NS	0·01	NS	0·08	NS
HDL-cholesterol	0·38	<0·001	0·41	<0·001	−0·12	NS
LDL-cholesterol	−0·06	NS	−0·05	NS	0·05	NS
Fasting TAG	−0·35	<0·001	−0·34	<0·001	0·22	0·02
Postprandial TAG (AUC)	−0·37	<0·001	−0·36	<0·001	0·20	0·03
Fasting glucose	−0·19	0·04	−0·18	NS	0·21	0·03
Postprandial glucose (AUC)	−0·19	0·05	−0·19	0·05	0·17	NS
Fasting insulin	−0·30	0·002	−0·27	0·005	0·25	0·009
Postprandial insulin (AUC)	−0·26	0·007	−0·24	0·01	0·19	0·05
HOMA-IR	−0·31	0·001	−0·29	0·002	0·26	0·005
HOMA-IR (AUC)	−0·22	0·02	−0·26	0·007	0·13	NS
Fasting NEFA	−0·13	NS	−0·17	NS	−0·009	NS
NEFA increase	0·08	NS	0·08	NS	-0·01	NS
NEFA decrease	−0·07	NS	−0·04	NS	0·02	NS

* For details of subjects and procedures, see Materials and methods.
AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance; NEFA increase, NEFA maximum − NEFA nadir; NEFA decrease, fasting NEFA − NEFA minimum.

The postprandial decrease of adiponectin (Δ-adiponectin) after oGTT was correlated with the decrease of adiponectin after oMTT (r 0·24, $P=0·01$). After oGTT and oMTT Δ-adiponectin was correlated with fasting adiponectin (r −0·54, $P<0·0001$; r −0·64, $P<0·001$ respectively). There was a positive correlation of Δ-adiponectin after oGTT and fasting TAG, postprandial TAG, fasting glucose, fasting and postprandial insulin (AUC) and fasting homeostasis model assessment (HOMA) (Table 2). After oMTT, Δ-adiponectin correlated with fasting and postprandial TAG, postprandial insulin, postprandial NEFA increase and postprandial HOMA (Table 3).

The findings from the afore-mentioned bivariate correlation analyses were further explored using multivariate linear

Table 3. Correlation coefficients (r) of plasma adiponectin with clinical and metabolic parameters in the fasting state and after oral metabolic tolerance test (oMTT)

OMTT Variables	Fasting adiponectin		Postprandial adiponectin (AUC)		Δ adiponectin	
	r	*P*	*r*	*P*	*r*	*P*
Age	−0·03	NS	−0·08	NS	0·12	NS
BMI	−0·20	0·03	−0·23	0·01	0·14	NS
Waist	−0·16	NS	−0·18	NS	0·13	NS
Systolic blood pressure	−0·12	NS	−0·14	NS	0·08	NS
Diastolic blood pressure	−0·15	NS	−0·18	NS	0·08	NS
Total cholesterol	−0·18	NS	−0·12	NS	0·08	NS
HDL-cholesterol	0·23	0·02	0·30	<0·001	−0·06	NS
LDL-cholesterol	−0·19	0·049	−0·14	NS	0·05	NS
Fasting TAG	−0·22	0·018	−0·21	0·027	0·23	0·02
Postprandial TAG (AUC)	−0·26	0·006	−0·26	0·007	0·21	0·03
Fasting glucose	−0·06	NS	−0·07	NS	0·03	NS
Postprandial glucose (AUC)	−0·01	NS	−0·04	NS	0·17	NS
Fasting insulin	−0·10	NS	−0·14	NS	0·04	NS
Postprandial insulin (AUC)	−0·16	NS	−0·20	0·04	0·19	0·047
HOMA-IR	−0·16	NS	−0·14	NS	0·12	NS
HOMA-IR (AUC)	−0·08	NS	−0·08	NS	0·22	0·02
Fasting NEFA	−0·13	NS	−0·13	NS	0·18	NS
NEFA increase	0·003	NS	0·06	NS	0·20	0·04
NEFA decrease	−0·06	NS	0·003	NS	0·18	NS

* For details of subjects and procedures, see Materials and methods.
AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance; NEFA increase, NEFA maximum − NEFA nadir; NEFA decrease, fasting NEFA − NEFA minimum.

British Journal of Nutrition

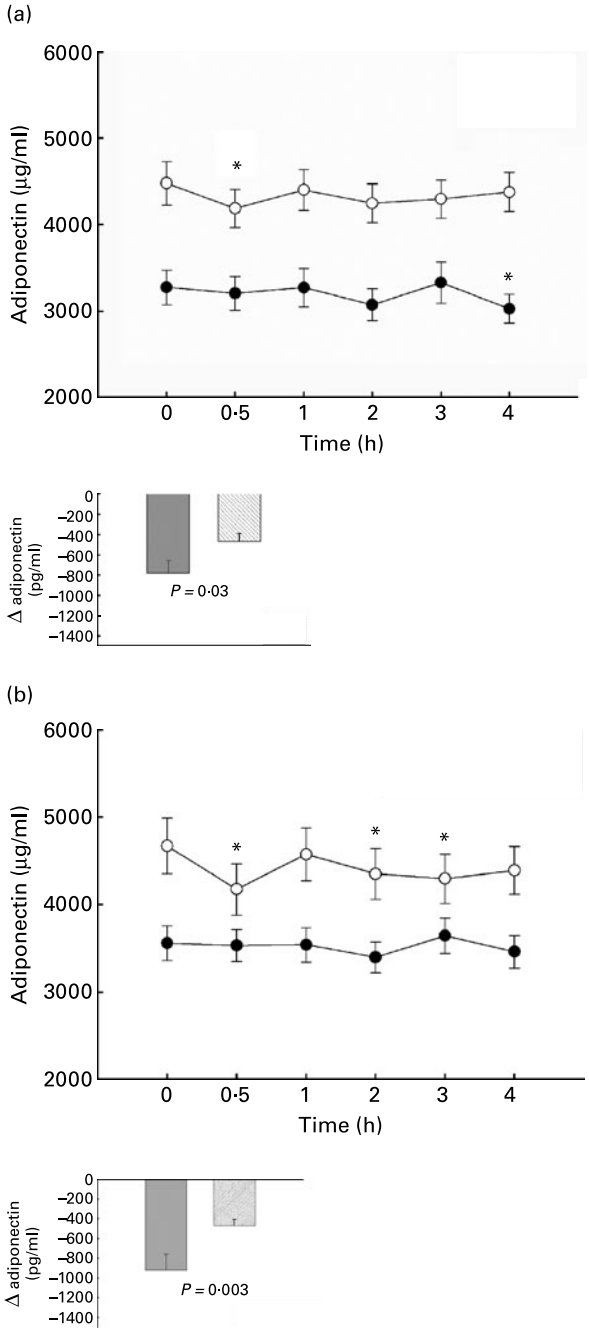

Fig. 2. Postprandial plasma adiponectin after oral glucose tolerance test in normal (○) and TAG high responders (●) (a) and in subjects with normal (○) or abnormal (●) homeostasis model assessment of insulin resistance (b). Mean values with their standard errors. *Significant decrease compared with fasting value with Bonferroni-Holm correction. For details of subjects and procedures, see Materials and methods.

analysis. We included log adiponectin as dependent variable and fasting and postprandial log glucose, log insulin, log NEFA and log TAG as independent variables. In this model, postprandial TAG (AUC) were the only independently associated variable. In another model, which included postprandial log adiponectin (AUC) as dependent variable and all the afore-mentioned factors as independent risk factors, we got the same results. Another model included log Δ-adiponectin as dependent variable and the afore-mentioned as independent. In this model, postprandial insulin, postprandial NEFA and fasting TAG remained independent predictors of Δ-adiponectin (data not shown).

Subgroup analysis

To study the effect of TAG response and insulin resistance on postprandial adiponectin course, we divided the participants into a high TAG responder group (postprandial TAG >280 mg/dl, *n* 47) and a normal responder group (postprandial TAG <280 mg/dl, *n* 63). The insulin-resistant group was divided according to the HOMA-IR index in an insulin-resistant group (HOMA $>2{\cdot}6$, *n* 70) and a group without insulin resistance (HOMA-index $<2{\cdot}6$, *n* 40)[23].

The postprandial plasma adiponectin after oGTT decreased significantly in both TAG groups, but the decrease occurred later and seemed to be more prolonged in the high TAG response group (4·0 h *v.* 0·5 h postprandial), whereas the normal responders return to fasting level after 4 h. Insulin-resistant subjects showed no significant decrease, normal subjects had an early response at 0·5 h postprandial. The decrease of adiponectin was significantly lower in high than in normal responders and in insulin resistant than in normal subjects (Fig. 2).

After oMTT, the decrease of adiponectin did not attain statistical significance in either group. There was no significant difference in adiponectin decrease between TAG normal and high responders and subjects with or without insulin resistance.

Association of adiponectin variant −11 388 with adiponectin levels and parameters of the metabolic syndrome

We applied a nonparametric test to all parameters for methodological consistency because most of the tested parameters did not follow a normal distribution. There was a slight, but non-significant association between adiponectin −11 388 promoter polymorphism and adiponectin concentrations (Table 4). The single nucleotide polymorphism showed significant association with the increase of NEFA after oMTT, but after correction for multiple testing this association was not significant. There was no correlation with other parameters of the metabolic syndrome.

Discussion

The results of the present study show that plasma adiponectin decreases after a plain glucose and after a single mixed high-fat meal. These decreases were small in the complete cohort, but more pronounced in normal subjects than in subjects with traits of the metabolic syndrome and accompanying low fasting adiponectin concentrations (Fig. 2(a),(b)). These results suggest that indeed nutrient intake is followed by a decrease of adiponectin levels. However, this decrease was less pronounced in subjects with low adiponectin levels, low insulin sensitivity and high TAG. A circadian regulation of adiponectin was excluded by the authors in a study in which subjects remained awake for 38 h and received small identical snacks every 2 h. In contrast to other adipokines, such as leptin, adiponectin showed no significant circadian rhythm[13].

Table 4. Fasting and postprandial adiponectin, Δ-adiponectin (oGTT), Δ-adiponectin (oMTT) and metabolic parameters according to adiponectin −11 388 G/A promoter polymorphism‡

(Values are means and standard deviations)

	GG (*n* 86)		GA + AA (*n* 24)		
	Mean	SD	Mean	SD	*P**
Fasting adiponectin (pg/ml)	4009	2123	4498	2028	NS
Postprandial adiponectin (oMTT) (pg/ml)	33 720	15 955	38 158	15 210	NS
Δ-adiponectin (oGTT) (pg/ml)	594·5	719·0	755	944	NS
Δ-adiponectin (oMTT) (pg/ml)	1210	1434	1381	1464	NS
BMI (kg/m^2)	28·2	5·7	27·4	3·7	NS
Waist (cm)	102·8	15·6	101·4	9·1	NS
Fasting glucose (mg/dl)	108·4	16·2	112·9	28·6	NS
Postprandial glucose (AUC) (mg/dl)	543·9	97·5	538·4	117·6	NS
Fasting insulin (mU/l)	17·2	17·6	20·0	16·2	NS
Postprandial insulin (AUC) (mU/l)	250·0	233·9	231·6	190·3	NS
Fasting TAG (mg/dl)	165·1	126·5	152·9	132·2	NS
Postprandial TAG (AUC) (mg/dl)	1877	1268	1759	1063	NS
Fasting NEFA (mmol/l)	0·46	0·21	0·45	0·19	NS
Postprandial NEFA (increase)(mmol/l)	0·32	0·23	0·24	0·20	0·039†
Fasting HOMA-IR	4·55	4·92	4·59	3·15	NS
Postprandial HOMA-IR^4 (AUC)	74·8	87·1	77·7	63·0	NS

* Obtained by Mann–Whitney *U* test.
† NS after Bonferroni Holm correction.
‡ For details of subjects and procedures, see Materials and methods.
oGTT, oral glucose tolerance test; oMTT, oral metabolic tolerance test; AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance.

In previous studies conflicting results were described for postprandial adiponectin levels with either no significant alteration or an increase of adiponectin in a small study[14,15,17,25]. In addition to the different meal composition in those studies, the measurement of postprandial adiponectin was not performed constantly over a longer postprandial period as in the present study. Those studies might have missed the postprandial change because adiponectin showed up and down changes. Another study also used a standard liquid meal and showed a mild decrease 4 h postprandial in Prader Willi syndrome patients[26].

There is only one former study with a reasonable statistical power (*n* 60). This study investigated the response of normal and diabetic subjects to three different meals[16]. After the high-fat meal, diabetic and control subjects showed a significant decrease at 4 h postprandial. However, the authors did not see a significant influence of a high-carbohydrate, high-fibre meal on adiponectin. In their study, only mixed solid meals were given; thus, the effect of single nutrients remained unresolved. In the present study, we showed for the first time that not only mixed lipid-containing meals decreased adiponectin levels but also single glucose ingestion was followed by a significant decrease in adiponectin.

Although adiponectin was higher in normal than insulin-resistant subjects (Fig. 2(b)), in a multivariate analysis model, only postprandial TAG were independent predictors of fasting and postprandial adiponectin levels. This underlines the importance of postprandial TAG for fasting and postprandial adiponectin levels. The strong and independent correlation of adiponectin with TAG is supported by other studies, where the adiponectin increase after weight loss was correlated with improvement in plasma lipids independently of insulin sensitivity changes[27].

Fasting and postprandial adiponectin after oMTT showed higher correlations with postprandial insulin and TAG than with their fasting values (Table 3). The importance of the postprandial metabolism is supported by an even stronger association of postprandial adiponectin with HDL, insulin, waist, BMI and blood pressure compared with fasting adiponectin. Interestingly, the postprandial decrease of adiponectin (Δ) itself was associated with postprandial TAG and postprandial insulin (Table 3). The common polymorphism −11 388 in the adiponectin promoter was investigated as a genetic factor influencing adiponectin plasma levels, which was shown in some studies. These studies were epidemiological studies with large numbers of subjects[28,29]. In our cohort, while we found strong associations of adiponectin plasma concentrations with several other parameters of the metabolic syndrome, we could not find significant associations of the adiponectin promoter variant with these phenotypes. The postprandial increase of NEFA after oMTT was lower in subjects with the rare allele of −11 388 promoter polymorphism ($P=0{\cdot}039$) but after accounting for multiple testing this effect was not significant. We conclude that the −11 388 promoter polymorphism has no main effect on adiponectin and parameters of the metabolic syndrome.

Our findings favour the interpretation that adiponectin has the strongest associations to postprandial lipid metabolism and is not causally related to insulin sensitivity. One could suggest that a high-fat or high-glycaemic-index diet contributes to a decrease of adiponectin levels on a long-term basis.

Acknowledgements

We thank A. Thoss, M. Hartelt, M. Gerull and S. Kaschner for excellent technical assistance. This work was financially supported by the BMBF-Project 'Fat and metabolism – gene variation, gene regulation and gene function' (MN 0313437A). D. Rubin and U. Helwig contributed equally to this study.

References

1. Kahn BB & Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* **106**, 473–481.
2. Berg AH, Combs TP, Du X, Brownlee M & Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* **7**, 947–953.
3. Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* **270**, 26746–26749.
4. Hu E, Liang P & Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* **271**, 10697–10703.
5. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE & Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* **98**, 2005–2010.
6. Combs TP, Berg AH, Obici S, Scherer PE & Rosetti L (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* **108**, 1875–1881.
7. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K & Saruta T (2002) Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci (Lond)* **103**, 137–144.
8. Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staiger H, Maerker E, Haring H & Stumvoll M (2003) Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes* **52**, 239–243.
9. Pellme F, Smith U, Funahashi T, Matsuzawa Y, Brekke H, Wiklund O, Taskinen MR & Jansson PA (2003) Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. *Diabetes* **52**, 1182–1186.
10. Hotta K, Funahashi T, Arita Y, *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* **20**, 1595–1599.
11. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE & Tataranni PA (2001) Hypoadiponectinemia in obesity and type 2 diabetes, close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**, 1930–1935.
12. Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M & Olefsky JM (2002) The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* **51**, 2968–2974.
13. Shea SA, Hilton MF, Orlova C, Ayers RT & Mantzoros CS (2005) Independent circadian and sleep/wake regulation of adipokines and glucose in humans. *J Clin Endocrinol Metab* **90**, 2537–2544.
14. Peake PW, Kriteros AD, Denyer GS, Campbell LV & Charlesworth JA (2003) The postprandial response of adiponectin to a high-fat meal in normal and insulin-resistant subjects. *Int J Obes Relat Metab Disord* **27**, 657–662.
15. Imbeault P, Pomerleau M, Harper ME & Doucet E (2004) Unchanged fasting and postprandial adiponectin levels following a 4-day caloric restriction in young healthy men. *Clin Endocrinol* **60**, 429–433.
16. Esposito K, Nappo F, Giugliano F, Di Palo C, Ciotola M, Barbieri M, Paolisso G & Giugliano D (2003) Meal modulation of circulating interleukin 18 and adiponectin concentrations in healthy subjects and in patients with type 2 diabetes mellitus. *Am J Clin Nutr* **78**, 1135–1140.
17. English PJ, Coughlin SR, Hayden K, Malik IA & Wilding JP (2003) Plasma adiponectin increases postprandially in obese, but not in lean, subjects. *Obes Res* **11**, 839–844.
18. Lewis GF, O'Meara NM, Soltys PA, Blackman JD, Iverius PH, Druetzler AF, Getz GS & Polonsky KS (1990) Postprandial lipoprotein metabolism in normal and obese subjects, comparison after the vitamin A fat-loading test. *J Clin Endocrinol Metab* **71**, 1041–1050.
19. Chen YD, Swami S, Skowronski R, Coulston A & Reaven GM (1993) Differences in postprandial lipemia between patients with normal glucose tolerance and noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* **76**, 172–177.
20. Schrezenmeir J, Keppler I, Fenselau S, Weber P, Biesalski HK, Probst R, Laue C, Zuchhold HD, Prellwitz W & Beyer J (1993) The phenomenon of a high triglyceride response to an oral lipid load in healthy subjects and its link to the metabolic syndrome. *Ann N Y Acad Sci* **683**, 302–314.
21. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, Mauriege P & Despres JP (1998) Postprandial triglyceride response in visceral obesity in men. *Diabetes* **47**, 953–960.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC (1985) Homeostasis model assessment, insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419.
23. Adult Treatment Panel III (2001) Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. *JAMA* **16**, 2486–2496.
24. Monzillo LU & Hamdy O (2003) Evaluation of insulin sensitivity in clinical practice and in research settings. *Nutr Rev* **61**, 397–412.
25. Heliovaara MK, Strandberg TE, Karonen SL & Ebeling P (2006) Association of serum adiponectin concentration to lipid and glucose metabolism in healthy humans. *Horm Metab Res* **38**, 336–340.
26. Caixas A, Gimenez-Palop O, Gimenez-Perez G, Potau N, Berlanga E, Gonzalez-Glemente JM, Arroyo J, Laferrere B & Mauricio D (2006) Postprandial adiponectin levels are unlikely to contribute to the pathogenesis of obesity in Prader-Willi syndrome. *Horm Res* **65**, 39–45.
27. Baratta R, Amato S, Degano C, Farina MG, Patane G, Vigneri R & Frittitta L (2004) Adiponectin relationship with lipid metabolism is independent of body fat mass, evidence from both cross-sectional and intervention studies. *J Clin Endocrinol Metab* **89**, 2665–2671.
28. Fumeron F, Aubert R, Siddiq A, *et al.* (2004) Epidemiologic data on the Insulin Resistance Syndrome (DESIR) Study Group. Adiponectin gene polymorphisms and adiponectin levels are independently associated with the development of hyperglycemia during a 3-year period, the epidemiologic data on the insulin resistance syndrome prospective study. *Diabetes* **53**, 1150–1157.
29. Vasseur F, Helbecque N, Dina C, *et al.* (2002) Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* **11**, 2607–2614.