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High circulating levels of RBP4 and mRNA levels of aP2, PGC-1 α and UCP-2 predict improvement in insulin sensitivity following pioglitazone treatment of drug-naïve type 2 diabetic subjects

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Abstract

Hammarstedt A, Pihlajamäki J, Graham TE, Kainulainen S, Kahn BB, Laakso M, Smith U. (The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden; University of Kuopio and Kuopio University Hospital, Kuopio, Finland; Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA). High circulating levels of RBP4 and mRNA levels of aP2, PGC-1 α and UCP-2 predict improvement in insulin sensitivity following pioglitazone treatment of drug-naïve type 2 diabetic subjects.

Context—High levels of circulating retinol-binding protein 4 (RBP4) and baseline expression of adipogenic genes correlate with subsequent improvement in insulin sensitivity following Thiazolidinedione (TZD) treatment.

Objective—The aim was to identify baseline characteristics and early changes related to TZD treatment that could predict a good treatment response.

Design—Subjects were examined with oral glucose tolerance test, intravenous glucose tolerance test, hyperinsulinaemic euglycaemic clamp, body composition and standard blood sampling at baseline and after 4 and 12 weeks treatment. Subcutaneous adipose tissue biopsies were taken from the abdominal region at baseline, after 3 days and 4 weeks treatment to examine the gene expression profile.

Setting—Research laboratory in a University hospital.

Participants—Ten newly diagnosed and previously untreated type 2 diabetic subjects were treated with pioglitazone for 3 months.

Main outcome measures—Baseline characteristics and early changes related to TZD treatment that could predict the response after 3 months.

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Conflict of interest

Drs Graham and Kahn are listed as inventors on a patent application and Dr. Kahn has a research grant from Takeda Pharmaceutical Company.

Results—Pioglitazone improved insulin sensitivity after 4 weeks combined with lower glucose and insulin levels without any change in BMI. It was accompanied by lower circulating resistin and plasminogen activator inhibitor-1 levels rapidly increased levels of circulating total and high molecular weight adiponectin as well as adiponectin and adipocyte fatty acid-binding protein (aP2) mRNA expression in the adipose tissue. High levels of circulating RBP4 at baseline and adipose tissue expression of aP2, proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α) and uncoupling protein 2 (UCP-2) predicted a good treatment response measured as improvement in insulin-stimulated whole-body glucose uptake after 3 months.

Conclusions—Circulating levels of RBP4 as an index of insulin sensitivity and mRNA levels of adipogenic genes correlate with the subsequent improvement in insulin sensitivity following TZD treatment.

Keywords

adipose tissue; aP2; PGC-1 α ; RBP4; thiazolidinediones; UCP-2

Introduction

Thiazolidinediones (TZDs) are widely used for the treatment of type 2 diabetic patients, either as monotherapy or in combination with other agents. TZDs have consistently been shown to lower fasting and postprandial glucose concentrations, circulating free fatty acids (FFAs) and, in most clinical studies, also to decrease serum insulin levels [1–4]. Studies in nondiabetic subjects with impaired glucose tolerance and in nondiabetic first-degree relatives with insulin resistance [5] have shown that TZDs have similar beneficial effects in these prediabetic groups. In addition to the effects on insulin sensitivity and glucose homeostasis, TZDs have favourable effects on the inflammatory and atherogenic processes associated with type 2 diabetes [6].

However, not all patients respond with improved insulin sensitivity and lowering of glucose levels after TZD treatment. As many as 1/3 do not have a clinically relevant response to the treatment. Today no patient characteristics are known that can predict whether a patient is a responder or a nonresponder. It is, therefore, important to study baseline characteristics and early changes related to TZD treatment in order to find predictors of a good treatment response. This is particularly important since the clinical evaluation of the effect of TZD, measured as lowering of the HbA1c levels, is considered to require three or more months.

The objective of the present study was to search for baseline characteristics and/or early changes in gene expression in the adipose tissue of drug-naïve type 2 diabetic patients that could predict the response to TZD after 3 months treatment.

Subjects and methods

Subjects

Ten drug-naïve type 2 diabetic patients with mild diabetes (fasting plasma glucose ≤ 8.0 mmol L⁻¹), four men and six postmenopausal women were included, as discussed previously [7]. Exclusion criteria were evidence of peripheral vascular disease or heart disease, blood pressure $\geq 160/85$ mmHg, treatment with calcium channel blockers or nonsteroidal anti-inflammatory drugs (NSAID) on a regular basis. No prior anti-diabetic treatment was allowed. The thiazolidinedione, pioglitazone, was used as monotherapy at 30 mg for 2 days; the dose was then increased to 45 mg once daily at day 3 and throughout the remaining study period.

Informed consent was obtained from all subjects after the purpose and the potential risks of the study were explained to them. The protocol was approved by the Ethics Committees of the

University of Kuopio and the University of Göteborg and in accordance with the Declaration of Helsinki.

Body fat composition and fat distribution

Body composition was determined at baseline and after 12 weeks by bioelectric impedance (RJL Systems, Detroit, MI, USA) in the supine position after a 12 h fast. Abdominal fat distribution was evaluated by computed tomography (Somatom Plus S, Siemens, Germany). Scanning was performed at 120 kV and with a slice thickness of 10 mm. The fourth lumbar vertebra was mapped with a topogram and subcutaneous and intra-abdominal fat areas were calculated.

Oral glucose tolerance test

All participants underwent a standardized oral glucose tolerance test (OGTT) at baseline (75 g glucose) and were considered as diabetic according to the WHO definition [8].

Intravenous glucose tolerance test

The first-phase insulin secretion was estimated at baseline, after 4 weeks and after 12 weeks by calculating the area under the curve (AUC) for the insulin response during the first 10 min of the intravenous glucose tolerance test (IVGTT) (samples taken at 0, 2, 4, 6, 8 and 10 min after the glucose bolus).

Euglycaemic hyperinsulinaemic clamp

The degree of insulin resistance was evaluated at baseline, after 4 weeks and after 12 weeks with the euglycaemic clamp technique [9] after a 12 h fast as previously described [5]. After drawing baseline blood samples, a priming dose of insulin (Actrapid 100 U mL⁻¹; Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to raise the insulin concentration quickly to the desired level. It was then maintained by a continuous insulin infusion of 80 mU m⁻² body surface per minute. Blood glucose was clamped at 5.0 mmol L⁻¹ for the next 180 min by the infusion of 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals. The mean value for the last hour was used to calculate the rate of insulin-stimulated whole-body glucose uptake (WBGU) which was assumed equal to the glucose infusion rate.

Biochemical analysis

Plasma glucose levels in the fasting state, after an oral glucose load as well as blood glucose during the euglycaemic clamp, were measured by the glucose oxidase method (2300 Stat Plus; Yellow Springs Instrument Co Inc, Yellow Springs, OH, USA). For the determination of plasma insulin, blood was collected in EDTA-containing tubes and, after centrifugation, the plasma was stored at -20 °C until the analysis. Plasma insulin concentration was determined by a commercial double-antibody, solid-phase radio-immunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). Cholesterol and triglyceride levels in serum were assayed by automated enzymatic methods (Boehringer-Mannheim GmbH, Mannheim, Germany) and FFA by an enzymatic method (Wako Chemicals GmbH, Neuss, Germany).

Measurement of circulating factors

The level of circulating factors was measured at baseline, after 4 weeks and after 12 weeks. Circulating levels of adiponectin, resistin and serum amyloid A (SAA) were measured in plasma by ELISA (B-Bridge International, Sunnyvale, CA, USA; BioCat GmbH, Heidelberg, Germany; Biosource International Inc. Camarillo, CA, USA respectively). High sensitivity C-

reactive protein (CRP) was measured with an immunoenzymatic assay for quantitative determination of human CRP (CRP IEMA Test; Medix biochemica, Kauniainen, Finland). Circulating levels of cytokines i.e. tumour necrosis factor α (TNF α), interleukin-6 (IL-6) and IL-8 were measured by ELISA (R&D Systems, Inc. Minneapolis, MN, USA). Plasminogen activator inhibitor-1 (PAI-1) activity was measured by a standard enzymatic assay. High molecular weight (HMW) adiponectin was measured by Western blot under nonreducing conditions and quantified using a densitometer (Molecular Dynamics, Sunnyvale, CA, USA) and S_A was calculated by dividing HMW adiponectin by total adiponectin [10]. Circulating Retinol-binding protein 4 (RBP4) was measured by quantitative Western blot [11].

mRNA isolation and quantification

Subcutaneous adipose tissue biopsies were obtained from the peri-umbilical, abdominal region after an overnight fast at baseline, after 3 days and after 4 weeks treatment. Total cellular RNA was extracted with the guanidinium thiocyanate method as described [12] and TaqMan Real Time RT-PCR was used to quantify mRNA expression. The mRNA samples were treated with DNase and single-stranded random-hexamer-primed cDNA was synthesized. Gene-specific probes and primer pairs were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA) (available upon request). The quantification was performed using the standard protocol of ABI PRISM 7700 (Applied Biosystems). For each primer/probe set, a standard curve was generated. Each sample was run for 40 PCR cycles in duplicate in 25 μ L reactions with 5 μ L cDNA corresponding to 10 ng mRNA. The mean value was used to calculate mRNA levels. The quantity of a particular gene in each sample was normalized to that of ribosomal 18s.

Statistical analysis

Data are presented as mean $\pm$ SD. Data analyses were carried out with the STATVIEW version 5.0.1 for Macintosh (SAS Institute, Cary, NC). Wilcoxon's non-parametric signed rank test was used for paired observations and correlation coefficients (r_s) were obtained from Spearman's nonparametric rank correlations. Similar results were obtained with parametric tests. Multiple regression analysis was used to investigate independence of predictive parameters.

Results

Clinical characteristics and insulin sensitivity

Four men and six middle-age (57 ± 3 years) women were included in the study. Fasting plasma glucose and the 2 h glucose during the OGTT performed at baseline was 6.4 ± 0.3 and 12.3 ± 0.5 mmol L⁻¹, respectively, thus fulfilling the criteria of diabetes.

All but one subject were overweight and five were obese (BMI > 30 kg m⁻²). Body weight or BMI were not significantly changed by TZD treatment. No significant change in the intra-abdominal/subcutaneous fat ratio was seen after treatment (baseline, 0.29 ± 0.03 ; 12 weeks, 0.26 ± 0.03) but the deep subcutaneous adipose tissue increased significantly after treatment (baseline, 242 ± 29 cm²; 12 weeks, 268 ± 28 cm², $P = 0.02$) (Table 1).

Fasting glucose as well as the AUC_{glucose} during the IVGTT were significantly reduced already at 4 weeks and were not further changed during week 4–12 (Fig. 1a). Fasting insulin and AUC_{insulin} during the IVGTT were significantly reduced and, also for this, no further reduction was seen after week 4 (Fig. 1a). WBGU measured by the euglycaemic, hyperinsulinaemic clamp was improved by 26% ($P = 0.06$) after 4 weeks and further significantly improved by 33% ($P < 0.01$) after 12 weeks treatment (Table 1). Insulin levels during the clamps did not

differ significantly between the three occasions. However, the suppressive effect of insulin on circulating FFA was enhanced after 12 weeks treatment (Fig. 1b).

No significant effects were seen on blood lipids after 12 weeks TZD treatment (Table 1).

Circulating factors

Circulating adiponectin levels increased, as expected, robustly after 4 weeks treatment (258%, $P < 0.05$), and no further increase was seen after week 4–12 (Table 2). Neither the baseline value nor the increase in adiponectin correlated with the improvement in insulin sensitivity expressed as WBGU (data not shown). HMW adiponectin and S_A (HMW/total adiponectin) were measured at baseline and after 12 weeks and both increased significantly (Table 2). However, neither baseline values nor the increase in these two parameters correlated to the improvement in insulin sensitivity.

Several inflammatory markers were measured as well as the pro-coagulant factor PAI-1. No significant effect of TZD was seen on the circulating levels of TNF α , IL-6, CRP or SAA although a tendency for a decrease can be seen for most factors (Table 2). However, resistin levels were significantly reduced after 4 and 12 weeks, PAI-1 activity was also reduced by ~50% already after 4 weeks (Table 2). No correlation was seen between the reduction of either resistin or PAI-1 and WBGU (data not shown).

Measurements of the circulating levels of RBP4 showed that the baseline levels were inversely correlated to insulin sensitivity ($r_s = -0.67$, $P = 0.04$) as well as the treatment response to TZD ($r_s = 0.61$, $P = 0.07$) (Fig. 1c). In fact, Univariate regression analysis showed that circulating levels of RBP4 was the best predictor of improvement in WBGU ($r_p = 0.82$, $P = 0.0035$). Due to the small sample size we did not obtain a valid model for multiple regression analysis with more than two independent parameters. However, adjusting for the various parameters in repeated analyses with RBP4 as one of the independent parameters showed that RBP4 levels independent of baseline bodyweight ($P = 0.015$), HbA1c ($P = 0.013$), circulating levels of resistin ($P = 0.006$), PAI-1 ($P = 0.007$), adiponectin ($P = 0.009$) and mRNA levels of aP2 ($P = 0.007$) and proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α) ($P = 0.02$) predicted the improvement in the rates of WBGU. However, the P -value lost its statistical significance following adjustment for baseline insulin sensitivity. After 12 weeks of TZD treatment, RBP4 levels were reduced in eight out of ten subjects (Table 2). The two subjects who did not decrease their RBP4 levels showed a smaller increase in WBGU (5% and 12% vs. 33% for the whole group) after treatment. However, the over-all reduction did not significantly correlate with the improvement in insulin sensitivity ($r_s = -0.46$, $P = 0.17$) (Fig. 1c).

Adipose tissue gene expression

Gene expression was measured at baseline and after 3 days and 4 weeks to detect early changes in gene expression related to improvement in insulin sensitivity.

As TZDs are primarily thought to exert their effects through an enhanced differentiation of the (pre-)adipocytes, we measured several genes involved in both the early and late stages of adipogenesis. Although the expected trends related to adipogenesis were seen, only a few genes were significantly altered after treatment. Both adiponectin and aP2 were significantly increased; the effect on aP2 was rapid and it was significantly upregulated after 3 days and remained increased also after 4 weeks (Table 3).

Furthermore, we studied the effect of TZD treatment on genes involved in fatty acid transport and storage such as LXRalpha, CD36, lipoprotein lipase (LPL), lipin and perilipin. Most genes tended to increase after treatment but the changes were not statistically significant in this small

group of individuals. Similarly, genes related to mitochondrial function and biogenesis such as PGC-1 α , nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mtTFA) tended to increase. The mRNA levels of proteins involved in FFA transport and oxidation, such as UCP2, carnitine palmitoyltransferase 1 (CPT1), cytochrome c oxidase subunit 7a (COX7a), COX8a, ATP synthase subunit 5 (ATPb5) and cytochrome c (CYCS) also tended to increase.

As TZDs have anti-inflammatory effects, we measured the mRNA expression of several cytokines [TNF α , IL-6, IL-8, resistin, monocyte chemoattractant protein-1 (MCP-1)], CD68, EGF-like module containing, mucin like, hormone receptor-like 1 (EMR1), integrin, alpha M (ITGAM), SAA1, SAA2, CRP and PAI-1 and chemokines but no robust and significant changes could be detected after treatment (data not shown).

The baseline expression of only three genes that previously have been shown to be associated with measures of insulin sensitivity correlated with the subsequent improvement of insulin sensitivity; aP2 ($r_s = 0.59$, $P = 0.08$), PGC-1 α ($r_s = 0.76$, $P = 0.02$) and UCP-2 ($r_s = 0.81$, $P = 0.02$) (Fig. 1d).

Discussion

Thiazolidinediones are considered to have a fairly slow onset of action. In addition, ~20–30% of the patients do not show a clinically relevant benefit. Thus, it is important to identify markers that predict a good response to these drugs. The key question addressed in this study was if markers could be found at an early stage which could predict a favourable treatment response measured as improvement in insulin sensitivity (rates of whole-body glucose uptake).

Thiazolidinediones are selective and potent ligands for the nuclear transcription factor Peroxisome proliferator-activated receptor γ (PPAR γ). PPAR γ is, together with CCAAT/enhancer binding protein (C/EBP α), the key transcription factor for induction and maintenance of adipocyte differentiation and expression of adipocyte-specific genes like aP2 and adiponectin. The improvement of insulin sensitivity following TZD treatment is thought to be mainly due to PPAR γ -induced differentiation of the (pre-)adipocytes.

Although several adipocyte differentiation-related genes showed a changed expression, many changes were fairly small and did not reach statistical significance in this small group of individuals. Other studies of gene expression in the adipose tissue after as long as 16 weeks TZD treatment have also shown very moderate changes in gene expression in the adipose tissue [13]. However, aP2 was rapidly and robustly increased already after 3 days. The effect on adiponectin was less robust at this time point, but the adiponectin gene was significantly upregulated after 4 weeks. Thus, the effect of TZD on adipocyte differentiation, measured as aP2 and adiponectin induction, is clearly rapid and likely to precede the improvement in whole-body insulin sensitivity. The effect of pioglitazone on adiponectin mRNA expression was less pronounced than the effect on circulating adiponectin levels. Baseline levels of total or HMW adiponectin did not predict the improvement in WBGU. Furthermore, neither the change in total or HMW adiponectin nor in S_A correlated with the enhanced WBGU after treatment. These findings are not unexpected as the current opinion is that, in addition to effects on mitochondrial biogenesis and fatty acid oxidation in skeletal muscle [14]), adiponectin mainly has effects on hepatic insulin sensitivity and glucose production [10]), and this was not measured in the present study.

Proliferator-activated receptor-gamma coactivator 1 alpha is an important co-factor for PPAR γ . It is a versatile co-activator involved in a variety of processes related to energy homeostasis such as fatty acid oxidation, glucose uptake, gluconeogenesis, mitochondrial biogenesis and uncoupling of respiration [15]. PGC-1 α is expressed in human white adipose

tissue and reduced in states of insulin resistance and obesity [16,17], Ectopic expression of PGC-1 α in cultured human white preadipocytes induces the cells to acquire features of a typical brown adipocyte, i.e. increased fatty acid oxidation and energy dissipation through uncoupled respiration [18]. PGC-1 α has also been shown to be activated by TZDs in 3T3-L1 adipocytes [19]. Although we did not find a significant increase in PGC-1 α mRNA levels by TZD treatment in this study we did find that a high expression of PGC-1 α in the adipose tissue is predictive of a good treatment response. Interestingly, addition of TZD to differentiating white adipocytes leads to mitochondrial biogenesis as well as an increase in mitochondrial gene expression both in 3T3-L1 cells [20] and in human adipocytes *in vitro* [21] and *in vivo* [22]. Taken together, these data are in accordance with the concept that an enhanced brown adipocyte phenotype of the white fat cells is beneficial for the whole-body insulin sensitivity and that part of the mode of action of TZD is to induce such a phenotype in the existing or newly recruited adipocytes.

Circulating levels of RBP4 were recently found to be increased in states of insulin resistance and to be reduced by TZD treatment in mice [23,24]. Circulating levels of RBP4 were also shown to be inversely related to Glucose transporter 4 (GLUT4) expression in adipose tissue in man as well as associated with many components of the Metabolic Syndrome and to be reduced after exercise training in subjects that also improved their insulin sensitivity [11]. These findings provide a rationale for anti-diabetic therapies aimed at lowering circulating RBP4 levels. The effect of TZD on circulating levels of RBP4 in humans has previously been studied in HIV-positive diabetic patients where it was shown to reduce circulating levels slightly without any improvement in insulin sensitivity estimated as HOMA-index [25]. Furthermore, no effect of TZD on circulating RBP4 levels could be seen when used as add on therapy to previous anti-diabetes treatment [26]. However, this is the first time that the effect of TZD treatment on circulating RBP4 levels has been measured in drug-naïve type 2 diabetic subjects. Baseline circulating RBP4 levels correlated negatively with insulin sensitivity also in this small group of type 2 diabetic subjects and TZD treatment reduced the RBP4 levels in eight of 10 subjects. Interestingly, high circulating RBP4 levels at baseline predicted a good treatment response and, thus, this can be one possible measurement to identify responders to TZDs. As the elaborate clamp technique is not suitable for screening patients for responders or non-responders, measurement of circulating levels of RBP4 in a routine blood sample could be a very convenient way to predict response to TZDs. Although the reduction in circulating RBP4 levels was not significantly correlated with the improvement in WBGU, this may be related to effects of RBP4 on hepatic insulin sensitivity and glucose production [23], which was not measured in this study.

The present study is limited by the small number of subjects examined. However, in spite of this, the results show that TZD treatment leads to a rapid induction of some adipogenic genes like aP2 and adiponectin. The expression of aP2 as well as genes involved in mitochondrial biogenesis and function, like PGC-1 α and UCP-2, are related to the improvement in WBGU after 3 months treatment with pioglitazone. Furthermore, measurements of circulating RBP4 levels could represent a simple way to identify responders and this should now be verified in larger prospective studies.

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Fig. 1.

(a) Glucose (left) and insulin (right) levels during IVGTT. Baseline AUCglucose: 124 ± 12 , 4 weeks AUCglucose: $116 \pm 9^{\ddagger}$, 12 weeks AUCglucose: $113 \pm 12^{\S}$. Baseline AUCinsulin: 1427 ± 699 , 4 weeks AUCinsulin: $1145 \pm 419^{\ddagger}$, 12 weeks AUCinsulin: $1062 \pm 366^{\S}$. No differences were seen between 4 and 12 weeks for either glucose or insulin levels. Values are mean $\pm$ SD. ‡ significant difference baseline versus 4 weeks, § significant difference baseline versus 12 weeks. Circle, baseline; square, 4 weeks; triangle, 12 weeks. (b) Insulin (left) and FFA (right) levels during clamp steady-state. Values are mean $\pm$ SD. § Significant difference baseline-12 weeks. Circle, baseline; square, 4 weeks; triangle, 12 weeks. (c) Circulating levels of RBP4 were negatively correlated to insulin sensitivity at baseline (left). Furthermore, high baseline

levels of RBP4 predicted a good response to pioglitazone treatment measured as improvement in WBGU during the euglycaemic clamp (middle). The improvement in WBGU was not significantly correlated to the change in circulating RBP4 (right). (d) The gene expression of aP2, PGC-1 α and UCP2 in the adipose tissue at baseline is predictive of a good treatment response to pioglitazone treatment.

Table 1

Clinical characteristics

	Baseline (n = 10)	4 weeks (n = 7)	P-value*	12 weeks (n = 10)	P-value**
Age (years)	57 ± 8				
BP systolic (mmHg)	153 ± 25				
BP diastolic (mmHg)	93 ± 10				
Waist (cm)	98.1 ± 16.1				
Hip (cm)	105.6 ± 8.0				
WHR	0.93 ± 0.10				
Hight (cm)	164.9 ± 9.4				
Weight (kg)	83.6 ± 17.8	85.8 ± 19.1	n.s.	84.4 ± 18.5	n.s.
BMI (kg m ⁻²)	30.5 ± 4.4	32.0 ± 4.2	n.s.	30.8 ± 4.6	n.s.
IA fat (cm ²)	271 ± 141			265 ± 125	n.s.
SC fat (cm ²)	473 ± 200			515 ± 176	n.s.
SC fat superf (cm ²)	231 ± 125			246 ± 108	n.s.
SC fat deep (cm ²)	242 ± 91			268 ± 90	0.02
IA/SC fat	0.29 ± 0.10			0.26 ± 0.08	n.s.
Tot cholesterol (mmol L ⁻¹)	5.21 ± 0.77			5.10 ± 0.96	n.s.
LDL cholesterol (mmol L ⁻¹)	4.42 ± 0.40			3.19 ± 0.63	n.s.
HDL cholesterol (mmol L ⁻¹)	1.09 ± 0.22			1.17 ± 0.18	0.08
Tot TG (mmol L ⁻¹)	1.92 ± 1.39			1.66 ± 1.16	n.s.
VLDL TG (mmol L ⁻¹)	1.28 ± 1.29			1.19 ± 0.95	n.s.
f-glucose (mmol L ⁻¹)	5.9 ± 1.2	5.4 ± 0.4	0.03	5.2 ± 0.6	0.02
f-ins (mU L ⁻¹)	13.3 ± 10.2	11.5 ± 6.8	<0.05	8.9 ± 3.4	<0.05
WBGU (mg kg ⁻¹ min ⁻¹)	6.6 ± 2.3	7.2 ± 1.7	0.06	8.2 ± 2.1	0.005

Values are mean ± SD.

BP, blood pressure; WHR, waist/hip ratio; IA, intra abdominal; SC, subcutaneous; TG, triglycerides.

* P-value for significant change day 0–4 weeks.

** P-value for significant change day 0–12 weeks.

Table 2

Circulating levels of adipokines and inflammatory markers

	Baseline (<i>n</i> = 10)	4 weeks (<i>n</i> = 7)	<i>P</i> -value [*]	12 weeks (<i>n</i> = 10)	<i>P</i> -value ^{**}
Adiponectin ($\mu\text{g mL}^{-1}$)	6.82 $\pm$ 3.38	17.60 $\pm$ 8.31	0.02	17.48 $\pm$ 9.58	0.005
HMW adiponectin	1.61 $\pm$ 0.69	n.d.		2.95 $\pm$ 1.0	0.005
S _A adiponectin ^a	0.61 $\pm$ 0.05	n.d.		0.68 $\pm$ 0.03	0.005
RBP4 ($\mu\text{g mL}^{-1}$)	40.0 $\pm$ 13.9	n.d.		34.1 $\pm$ 10.7	0.11
Visfatin ($\text{pg } \mu\text{L}^{-1}$)	47.7 $\pm$ 25.2	50.1 $\pm$ 43.2	n.s.	56.9 $\pm$ 36.3	n.s.
IL-6 (pg mL^{-1})	0.75 $\pm$ 0.46	0.57 $\pm$ 0.38	n.s.	0.75 $\pm$ 0.49	n.s.
TNF α (pg mL^{-1})	0.43 $\pm$ 0.39	0.38 $\pm$ 0.34	n.s.	0.34 $\pm$ 0.20	n.s.
Resistin (ng mL^{-1})	1.91 $\pm$ 0.88	1.72 $\pm$ 0.71	0.02	1.54 $\pm$ 0.55	0.01
PAI-1 (kIE L^{-1})	33.0 $\pm$ 34.5	13.2 $\pm$ 9.2	0.02	15.2 $\pm$ 13.5	0.01
CRP (mg L^{-1})	1.17 $\pm$ 0.83	0.77 $\pm$ 0.30	n.s.	0.97 $\pm$ 0.31	n.s.
SAA ($\mu\text{g mL}^{-1}$)	26.4 $\pm$ 38.7	14.6 $\pm$ 5.5	n.s.	12.2 $\pm$ 7.7	n.s.

Values are mean $\pm$ SD.

HMW, high molecular weight; SAA, serum amyloid A; CRP, C-reactive protein; PAI-1, Plasminogen activator inhibitor-1; TNF α , tumour necrosis factor α ; IL-6, interleukin-6.

^aS_A adiponectin; HMW/total adiponectin.

^{*}*P*-value for significant change day 0–4 weeks.

^{**}*P*-value for significant change day 0–12 weeks.

Table 3

mRNA expression

	Baseline (<i>n</i> = 10)	3 days (<i>n</i> = 10)	<i>P</i> -value [*]	4 weeks (<i>n</i> = 7)	<i>P</i> -value ^{**}
αP2	0.48 ± 0.11	0.62 ± 0.17	0.02	0.64 ± 0.20	0.03
adiponectin	1.16 ± 0.28	1.32 ± 0.42	n.s.	1.57 ± 0.54	0.04

Values are mean ± SD.

^{*} *P*-value for significant change day 0–3 days,

^{**} *P*-value for significant change day 0–4 weeks.