

## RESEARCH

# Changes in adipose tissue lipolysis gene expression and insulin sensitivity after weight loss

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## Abstract

**Objective:** Insulin resistance is a major pathophysiological link between obesity and its metabolic complications. Weight loss (WL) is an effective tool to prevent obesity-related diseases; however, the mechanisms of an improvement in insulin sensitivity (IS) after weight-reducing interventions are not completely understood. The aim of the present study was to analyze the relationships between IS and adipose tissue (AT) expression of the genes involved in the regulation of lipolysis in obese subjects after WL.

**Methods:** Fifty-two obese subjects underwent weight-reducing dietary intervention program. The control group comprised 20 normal-weight subjects, examined at baseline only. Hyperinsulinemic-euglycemic clamp and s.c. AT biopsy with subsequent gene expression analysis were performed before and after the program.

**Results:** AT expression of genes encoding lipases (*PNPLA2*, *LIPE* and *MGLL*) and lipid-droplet proteins enhancing (*ABHD5*) and inhibiting lipolysis (*PLIN1* and *CIDEA*) were decreased in obese individuals in comparison with normal-weight individuals. The group of 38 obese participants completed dietary intervention program and clamp studies, which resulted in a significant WL and an improvement in mean IS. However, in nine subjects from this group IS did not improve in response to WL. AT expression of *PNPLA2*, *LIPE* and *PLIN1* increased only in the group without IS improvement.

**Conclusions:** Excessive lipolysis may prevent an improvement in IS during WL. The change in AT *PNPLA2* and *LIPE* expression was a negative predictor of the change in IS after WL.

## Key Words

- ▶ weight loss
- ▶ insulin sensitivity
- ▶ adipose tissue
- ▶ hormone-sensitive lipase
- ▶ adipose triglyceride lipase

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## Introduction

Obesity predisposes to the development of type 2 diabetes, dyslipidemia, hypertension, atherosclerosis, several types of cancer and other disorders. Insulin resistance is a major pathophysiological link between obesity and its complications (1). Adipose tissue (AT) function and the balance between lipid storage and mobilization play an important role in regulating insulin action. Inability of AT to store fat leads to free fatty acids (FFA) overflow to other tissues and to insulin resistance (2).

Lipolysis is a process of hydrolysis of triacylglycerols (TG) into fatty acids and glycerol, which results in a release of FFA from AT into circulation. In the first step, the hydrolysis of TG into diacylglycerol and fatty acid is catalyzed by the enzyme adipose triglyceride lipase (ATGL), encoded by *PNPLA2*. Diacylglycerol is further hydrolyzed to monoacylglycerol and fatty acid with hormone-sensitive lipase (HSL), encoded by *LIPE*, and monoacylglycerol is ultimately hydrolyzed to glycerol

and fatty acid with monoacylglycerol lipase (MGLL) (2). Lipolysis is stimulated by catecholamines, which act through  $\beta_{1,3}$  adrenergic receptors (encoded by *ADRB1-3*), in humans mostly through  $\beta_{1,2}$  receptors and natriuretic peptides, whereas it is inhibited mainly by insulin (2, 3). An important mediator of hormonal regulation of lipolysis is phosphodiesterase 3B (PDE3B), which may inhibit lipolysis in response to insulin signal transduction and may counteract the lipolytic effect of an increased adrenergic signaling (2, 3).

In adipocytes, TGs are packed in lipid droplets, and proteins associated with lipid droplet surface play an important role in the regulation of lipolysis (3). Among these proteins, perilipin (PLIN), Cell Death-Inducing DFFA (DNA Fragmentation Factor- $\alpha$ )-Like Effector A (CIDEA) and caveolin 1 (CAV1) inhibit lipolysis through stabilization of lipid droplet and protection against TG hydrolysis, whereas G0/G1 switch gene 2 (*G0S2*) inhibits ATGL activity (4, 5, 6, 7, 8). In contrast, comparative gene identification 58 (CGI-58, also known as alpha/beta-hydrolase domain-containing protein 5, ABHD5) is ATGL coactivator and thus enhances lipolysis (4). ABHD5 bound to PLIN is unable to activate ATGL. Upon hormonal stimulation, PLIN is phosphorylated, and ABHD5 is dissociated from PLIN and may interact with ATGL promoting its activation (4).

The inter-relationships between AT lipolysis and insulin sensitivity are complex and still not fully understood and the existing data are contradictory. Genetic adipose tissue-specific ATGL deletion (9), HSL haploinsufficiency (10) or pharmacological ATGL or HSL inhibition (10, 11) improved glucose tolerance and insulin sensitivity in mice, without increasing body weight. MGLL deficiency attenuated high-fat diet-induced insulin resistance in mice (12). *G0S2* overexpression (8) and ABHD5 knockdown in mice (13) also protected against diet-induced insulin resistance and glucose intolerance. In contrast, mice with *G0S2* knockout display increased AT lipase activity and lipolysis, and they are lean, resistant to high-fat diet-induced weight gain and are glucose tolerant and insulin sensitive (14).

In human adipocytes, HSL gene silencing led to improved insulin-stimulated glucose uptake (10). On the other hand, genetic or pharmacological ATGL or HSL knockdown in human multipotent adipose-derived stem cells disrupted mitochondrial respiration, impaired insulin signaling and decreased insulin-stimulated glucose uptake (15).

In humans, white AT lipolytic rate is positively associated with insulin resistance (10). However, a defect

in lipolysis (16) and decreased AT HSL and ATGL protein expression and/or activity (16, 17, 18), as well as mRNA expression (19, 20, 21), were reported in obesity and insulin resistance, although the opposite findings were also described (22). Null mutation in HSL gene leads to impaired AT lipolysis, dyslipidemia, hepatic steatosis, insulin resistance and diabetes (23). Decreased AT expression of *MGLL*, *PLIN1*, *CIDEA* and *G0S2* in different conditions associated with insulin resistance was observed (5, 24, 25, 26). The differences in human studies may come from different characteristics of the study groups and different clinical situations. Thus, there is need for further studies on the association between AT lipases and insulin sensitivity in different conditions/interventions in humans.

Weight loss is an effective tool to prevent obesity-associated insulin resistance and related comorbidities; however, the precise mechanism of an improvement in insulin sensitivity remains unclear. In our previous study we demonstrated that diet-induced moderate (11.3%) weight loss resulted in a significant increase in insulin sensitivity by 27%, without any effect on AT inflammation (27). During weight loss, AT mobilizes fat stores to provide FFA as a source of energy to other organs. Lipolysis plays a crucial role in this process. Data on the effect of weight loss on AT lipase and lipid droplet protein expression are conflicting. Furthermore, it remains unclear to what extent they are related to the concurrent change in insulin sensitivity.

We hypothesized that the changes in AT expression of genes associated with lipolysis may be related to the concurrent improvement in insulin sensitivity during weight loss. To test this hypothesis, we measured AT lipolysis gene expression in the samples obtained before and after weight loss in obese subjects and analyzed them in relation to the concurrent change in insulin sensitivity.

## Subjects and methods

### Study groups

We examined 52 subjects with marked overweight or obesity (BMI >28 kg/m<sup>2</sup>, 27 males and 25 females, age 32.0±7.9 years). The cut-off point of 28 kg/m<sup>2</sup> instead of 25 kg/m<sup>2</sup> was chosen to enhance the probability of achieving greater body weight reduction. Twenty normal-weight subjects (8 males and 12 females, age 23.5±1.8 years) served as a control group. The detailed description of the study groups has been described previously (27). All participants were nonsmokers, had no serious diseases, morbid obesity (thus, the BMI range in the overweight/obese group was 28–40 kg/m<sup>2</sup>), impaired fasting glucose,

impaired glucose tolerance or diabetes and were not taking any drugs. Body weight of the participants had remained stable ( $\pm 1$  kg) for at least 3 months prior to the study. Participants underwent clinical examination, anthropometric measurements and appropriate laboratory tests (27, 28). All the studies were performed after overnight fast. All human studies have been approved by the Local Ethics Committee of the Medical University of Białystok. All persons gave their written informed consent prior to their inclusion in the study.

### Study protocol

The detailed study protocol has been described previously (27). Overweight/obese subjects underwent a 12-week dietary intervention program, which consisted of individually planned low-calorie diet (20 kcal per kg of proper body weight). The ideal body weight was calculated for the Broca formula. Before the program, none of the participants were on a special diet (like vegetarian, vegan, gluten-free, etc.). The dietary habits were assessed with the questionnaire. The sources of energy in the diet were carbohydrate 55–60%, fat 25% and protein 15–20%. Participants received a detailed instruction about low-calorie diet. Each participant received the detailed menu for every day of the 2-week period. The body weight changes were assessed every 2 weeks. The compliance was also assessed every 2 weeks by qualified dieticians – each participant reported non-adherence to the prescribed diet in a diary. Furthermore, at each visit, participants presented the detailed meal diary from the selected 2 days in every 2-week period. Physical activity was assessed by a questionnaire and all subjects were sedentary. Subjects were advised to maintain their daily physical activity, without specific advice to increase it. Physical activity was assessed with a questionnaire every 2 weeks and was unchanged during the program. All analyses were performed before and after dietary intervention.

Normal-weight subjects were examined only at baseline. Forty out of 52 subjects completed the program. Twelve individuals did not complete the program due to the non-adherence to the prescribed diets. Insulin sensitivity was assessed in 38 subjects before and after the program, because two individuals were not willing to undergo the clamp study at the end of the program.

### Measurement of insulin sensitivity

Insulin sensitivity was measured with the 2h hyperinsulinemic-euglycemic clamp (27, 28). On the

morning of the study, two venous catheters were inserted into antecubital veins, one for the infusion of insulin and glucose and the other in the contralateral hand for blood sampling, with that hand heated to  $\sim 60^{\circ}\text{C}$ . Insulin (Actrapid HM; Novo Nordisk) was given as a primed-continuous i.v. infusion for 120 min at  $40 \text{ mU} \times \text{m}^{-2} \times \text{min}^{-1}$ , resulting in constant hyperinsulinemia of  $\sim 80 \text{ } \mu\text{IU}/\text{mL}$ . Arterialized blood glucose was obtained every 5 min, and 20% dextrose (1.11 mol/L) infusion was adjusted to maintain plasma glucose levels at 5.0 mmol/L. The rate of whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate during the last 40 min of the clamp, corrected for the glucose space, and normalized for fat-free mass (ffm).

### Subcutaneous AT biopsy

Subcutaneous AT biopsy from the umbilical region was performed using biopsy needle under local anesthesia with 1% lidocaine, after small skin incision of less than 1 cm (27, 28). Tissue were collected to 1 mL of RNA stabilization reagent (Allprotect tissue reagent; QIAGEN GmbH) and were kept at  $-80^{\circ}\text{C}$  until analyses.

### Isolation of RNA from adipose tissue and determination of gene expression

Total RNA was isolated from AT as described (27, 28). AT mRNA expression was analyzed with quantitative Real-Time PCR. The samples were quantified with the Light Cycler 480 II Real-Time PCR Instrument (Roche Diagnostics, GmbH) using Roche LightCycler480 Probes Master (Roche Diagnostics GmbH). Data were analyzed with LC480 Software 1.5.0 SP3. *LIPE* and *PNPLA2* mRNA expression was measured using primers in combination with predesigned mono-color hydrolysis probes from the Universal Probe Library Roche (UPL) (Roche Diagnostics GmbH): *LIPE* forward 5'-cttcaccgtggctcttcg, reverse 5'-ggtaggctgcatgatgc, UPL probe #66; *PNPLA2* forward 5'-ctccaccaatccagag, reverse 5'-ccctgcttcacatctctc, UPL probe #89. For other genes, the validated PrimePCR Probe Assays were purchased from Bio-Rad: *MGLL* (qHsaCEP0058399), *PLIN* (qHsaCEP0053667), *ABHD5* (qHsaCEP0057650), *CIDEA* (qHsaCEP0024748), *CAV1* (qHsaCIP00333230), *GOS2* (qHsaCEP0051262) and *PGK1* (qHsaCEP0050174), *ADRB1* (qHsaCEP0032252), *ADRB2* (qHsaCEP0032222), *ADRB3* (qHsaCEP0055256), *PDE3B* (qHsaCEP0052820). All samples were run in triplicate and average values were calculated. All results were normalized to the levels of *PGK1*, since its expression

was the most stable among a few housekeeping genes tests. Relative quantification was calculated using the  $\Delta\Delta C_t$  formula cycle threshold.

### Statistical analysis

The statistics were performed with the STATISTICA 12.5 (Statsoft, Krakow, Poland). All data are presented as mean  $\pm$  s.d. The variables which did not have normal distribution were log-transformed before analyses. For the purpose of data presentation, absolute values are shown in the Results. Differences between the groups were analyzed with the unpaired Student's *t*-test. Differences in the estimated parameters before and after weight loss program in the entire obese group were assessed with the paired Student's *t*-test. Differences before and after weight loss program in the subgroups of subjects with and without the concurrent improvement in insulin sensitivity were analyzed with repeated-measures ANOVA with time vs group interaction. For all the observed differences, Benjamini–Hochberg correction for multiple comparisons was applied. To assess the effect of the initial insulin sensitivity and the decrease in body weight during the intervention between subjects, in whom insulin sensitivity increased and did not increase, we used analysis of covariance. To assess the differences among the initial BMI quartiles, we used one-way ANOVA with *post hoc* Tukey test. Differences in categorical variables were assessed with the chi-square test. Relationships between variables were studied with the Pearson product moment correlation analysis and with multiple regression analysis. The level of significance was accepted at *P* value lower than 0.05.

## Results

### Baseline (before dietary intervention) differences between normal-weight and obese subjects

Obese subjects had lower baseline insulin sensitivity in comparison with normal-weight subjects ( $6.59 \pm 2.97$  vs  $9.77 \pm 3.36$  mg/kg ffm/min,  $P < 0.0001$ ). Differences in the baseline AT expression of the studied genes are shown in Table 1. Obese subjects had lower AT *LIPE*, *MGLL*, *ABHD5*, *CIDEA*, *PDE3B* ( $P < 0.001$ ), *PNPLA2* ( $P = 0.002$ ), *PLIN* expression ( $P = 0.01$ ) and *ADRB1-3* expression ( $P < 0.005$ ) (Table 1). All the differences remained significant after adjustment for age. AT *GOS2* and *CAV1* expression did not differ between the groups (Table 1).

**Table 1** Baseline (before dietary intervention) AT gene expression (A.U.) in normal-weight ( $n = 20$ ) and obese subjects ( $n = 52$ ).

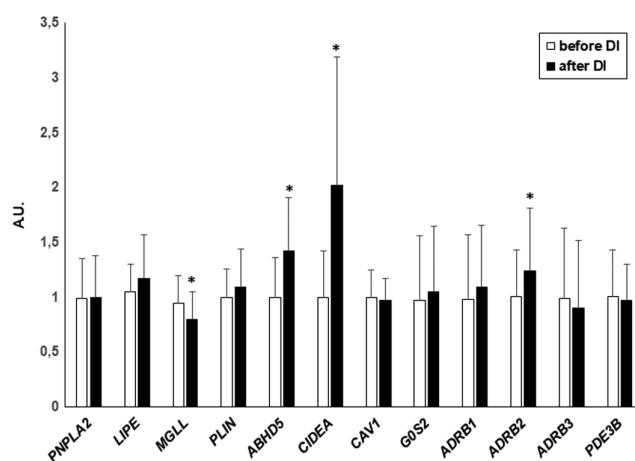
	Normal-weight	Obese
<i>PNPLA2</i>	1.37 $\pm$ 0.41	1.00 $\pm$ 0.36 <sup>a</sup>
<i>LIPE</i>	1.60 $\pm$ 0.44	1.05 $\pm$ 0.25 <sup>a</sup>
<i>MGLL</i>	1.29 $\pm$ 0.44	0.96 $\pm$ 0.25 <sup>a</sup>
<i>PLIN</i>	1.19 $\pm$ 0.24	1.00 $\pm$ 0.25 <sup>a</sup>
<i>ABHD5</i>	2.69 $\pm$ 1.65	1.00 $\pm$ 0.36 <sup>a</sup>
<i>CIDEA</i>	3.05 $\pm$ 1.42	1.00 $\pm$ 0.42 <sup>a</sup>
<i>CAV1</i>	0.99 $\pm$ 0.18	1.00 $\pm$ 0.25
<i>GOS2</i>	1.37 $\pm$ 1.04	1.00 $\pm$ 0.62
<i>ADRB1</i>	1.74 $\pm$ 1.04	1.00 $\pm$ 0.59 <sup>a</sup>
<i>ADRB2</i>	1.52 $\pm$ 0.83	1.00 $\pm$ 0.42 <sup>a</sup>
<i>ADRB3</i>	1.72 $\pm$ 0.89	1.00 $\pm$ 0.63 <sup>a</sup>
<i>PDE3B</i>	1.58 $\pm$ 0.40	1.00 $\pm$ 0.41 <sup>a</sup>

<sup>a</sup> $P < 0.05$  vs the normal-weight group. A.U., arbitrary units.

### The effect of diet-induced weight loss on AT gene expression in the entire overweight/obese group

All 40 subjects who completed the program had a reduction in body weight. The range of body weight reduction was from 5.5 kg to 21.8 kg.

Weight loss resulted in an increase in AT expression of *ABHD5*, *CIDEA* ( $P < 0.0001$ ) and *ADRB2* ( $P = 0.017$ ) and a decrease in the expression of *MGLL* ( $P = 0.01$ ) (Fig. 1). The expression of *LIPE* and *PLIN* increased after weight loss; however, it lost its significance after correction for multiple comparisons. The expression of other genes studied did not change in response to weight loss in the entire study group (Fig. 1).



**Figure 1** The effect of diet-induced weight loss on AT gene expression in the entire obese group ( $n = 38$ ). Data are shown as mean  $\pm$  s.d.  $*P < 0.05$  vs before dietary intervention. A.U., arbitrary units.

Next, we divided the obese group according to the initial BMI quartiles (lower quartile – 29.9, median – 32.7, upper quartile – 35.2 kg/m<sup>2</sup>). Weight loss and the change in insulin sensitivity did not differ among the BMI quartiles. However, we observed the relationship between the initial BMI and the change in BMI after weight loss ( $r=-0.35$ ,  $P=0.032$ ), that is, the higher the initial BMI, the greater decrease in BMI in response to weight loss. An increase in AT *CIDEA* expression was higher in the lowest vs the highest initial BMI quartile ( $P=0.039$ ). The change in AT expression of other analysed genes did not differ among the BMI quartiles.

### The effect of diet-induced weight loss on anthropometric and metabolic parameters in subjects in whom insulin sensitivity increased vs subjects in whom insulin sensitivity did not increase

Although mean insulin sensitivity increased during the dietary intervention, in 9 out of 38 subjects it not only did not improve, but even worsened in response to weight loss. The frequency of no improvement in insulin sensitivity was similar in males and females (chi-square  $P=0.57$ ).

Individuals without an improvement in insulin sensitivity had higher initial M value ( $P=0.001$ ); however, they did not differ in any other of the initial anthropometric and metabolic parameters from subjects, in whom insulin sensitivity increased after the dietary intervention (Table 2). Furthermore, body weight, waist circumference, body fat, serum insulin and total

cholesterol reduction during the dietary intervention was similar in both groups ( $P<0.05$ ) (Table 2). Serum triglycerides (TG) decreased in the group with an increase in insulin sensitivity ( $P=0.0007$ ) (Table 2). Fasting serum FFA increased in the group, in which insulin sensitivity did not improve ( $P=0.01$ ) (Table 2). There was a significant correlation between initial insulin sensitivity and the change in M value after weight loss ( $r=-0.44$ ,  $P=0.006$ ).

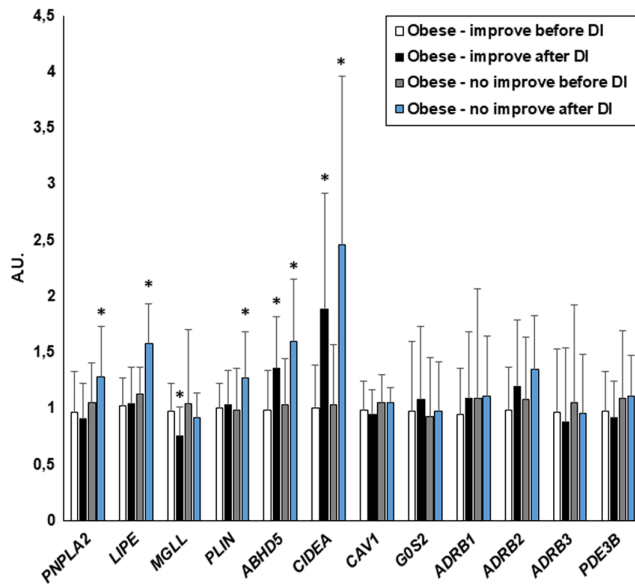
### The effect of diet-induced weight loss on AT gene expression in subjects in whom insulin sensitivity increased vs subjects in whom insulin sensitivity did not increase

AT expression *PNPLA2* ( $P=0.01$ ), *LIPE* ( $P<0.001$ ) and *PLIN* ( $P=0.007$ ) increased after weight loss only in subjects without an improvement in insulin sensitivity and did not change in subjects with an improvement in insulin sensitivity (Fig. 2). The expression of *MGLL* decreased significantly only in subjects with an improvement in insulin sensitivity ( $P=0.002$ ) (Fig. 2). The difference in response ( $\Delta$ ) between the groups without improvement and with improvement in insulin sensitivity in *PNPLA2* ( $0.23\pm 0.19$  vs  $-0.06\pm 0.26$  A.U.,  $P=0.016$ ) and *LIPE* ( $0.43\pm 0.25$  vs  $0.02\pm 0.33$  A.U.,  $P=0.013$ ) was significant, whereas the difference in *MGLL* and *PLIN* response lost its significance after correction for multiple comparisons ( $P=0.052$  and  $P=0.059$  after correction, respectively). Furthermore, the difference in *PNPLA2* and *LIPE* response to weight loss remained significant after adjustment for the initial insulin sensitivity and for the concurrent decrease in BMI and the changes in FFA ( $P<0.05$ ).

**Table 2** The effect of diet-induced weight loss on anthropometric and metabolic parameters in obese subjects, in whom insulin sensitivity increased (Obese - improve,  $n=29$ ) or did not increase (Obese - no improve,  $n=9$ ).

	Obese - improve		Obese - no improve	
	Before	After	Before	After
Body weight (kg)	99.1 ± 15.7	87.7 ± 13.9 <sup>a</sup>	100 ± 11.9	88.9 ± 12.0 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	33.3 ± 3.1	29.5 ± 2.8 <sup>a</sup>	31.3 ± 2.6	27.9 ± 3.1 <sup>a</sup>
Waist (cm)	107 ± 10.1	98.3 ± 8.9 <sup>a</sup>	105 ± 7.4	96.2 ± 7.9 <sup>a</sup>
Fat mass (kg)	38.9 ± 8.4	29.5 ± 7.5 <sup>a</sup>	36.9 ± 6.5	27.5 ± 7.9 <sup>a</sup>
Fasting plasma glucose (mg/dL)	88.2 ± 4.5	86.1 ± 6.5	87.3 ± 8.2	86.3 ± 8.8
Fasting serum insulin (μU/mL)	15.7 ± 5.9	10.9 ± 3.2 <sup>a</sup>	13.5 ± 5.0	10.3 ± 1.9 <sup>a</sup>
M (mg/kg ffm/min)	5.63 ± 2.49	8.55 ± 3.42 <sup>a</sup>	9.16 ± 3.00 <sup>b</sup>	7.19 ± 2.21 <sup>a</sup>
Fasting serum FFA (mmol/L)	0.71 ± 0.21	0.76 ± 0.35	0.58 ± 0.08	0.89 ± 0.26 <sup>a</sup>
Cholesterol (mg/dL)	192 ± 25.7	171 ± 31.0 <sup>a</sup>	176 ± 27.5	159 ± 29.8 <sup>a</sup>
TG (mg/dL)	114 ± 58.6	80.2 ± 33.1 <sup>a</sup>	94.0 ± 39.7	72.6 ± 21.7
HDL-cholesterol (mg/dL)	52.5 ± 10.5	49.9 ± 10.5	51.1 ± 8.3	48.6 ± 5.5
LDL-cholesterol (mg/dL)	123 ± 25.4	110 ± 29.4	112 ± 36.4	103 ± 31.1

<sup>a</sup> $P<0.05$  vs before dietary intervention; <sup>b</sup> $P<0.05$  vs improve in the respective time-point.



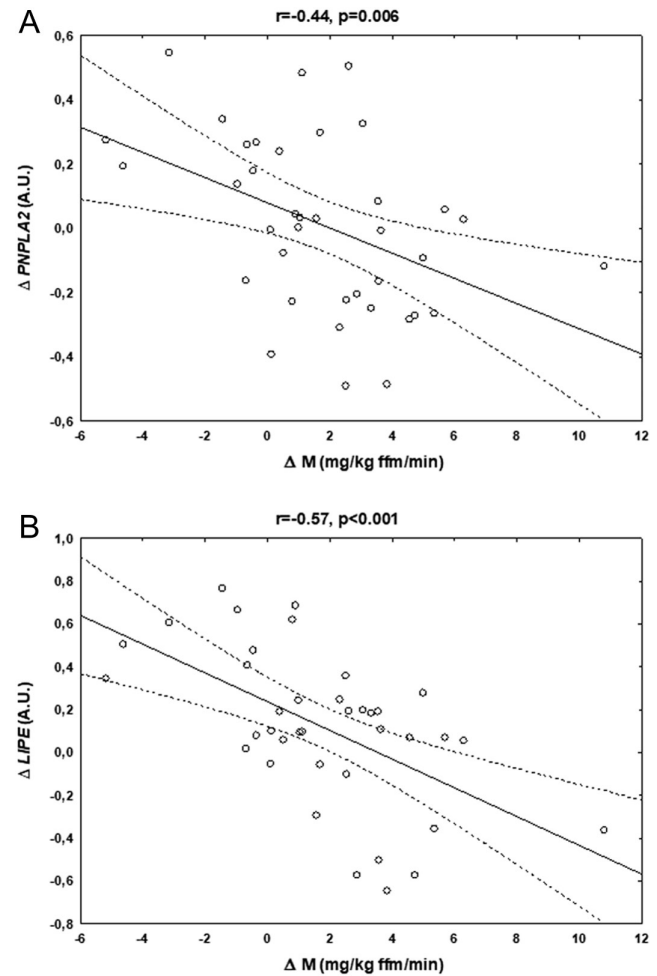
**Figure 2**  
The effect of diet-induced weight loss on AT gene expression in obese subjects, in whom insulin sensitivity increased (Obese - improve,  $n = 29$ ) or did not increase (Obese - no improve,  $n = 9$ ). Data are shown as mean  $\pm$  s.d. \* $P < 0.05$  vs before dietary intervention. A.U., arbitrary units.

AT expression of *ABHD5* and *CIDEA* increased similarly in both groups after the intervention ( $P < 0.005$ ), whereas the expression of *GOS2*, *CAV1*, *ADRB1*, *ADRB3* and *PDE3B* did not change in any of the groups (Fig. 2). AT *ADRB2* expression did not change in the groups with and without increase in insulin sensitivity analyzed separately.

### The correlation between the analyzed genes and insulin sensitivity

At baseline, before dietary intervention, AT *PNPLA2* ( $r = 0.30$ ,  $P = 0.019$ ), *LIPE* ( $r = 0.45$ ,  $P < 0.001$ ), *PLIN* ( $r = 0.30$ ,  $P = 0.021$ ), *ABHD5* ( $r = 0.26$ ,  $P = 0.042$ ) and *CIDEA* ( $r = 0.35$ ,  $P = 0.006$ ) expression was positively related to insulin sensitivity in the entire study group. Only correlation of *LIPE* with insulin sensitivity remained significant after adjustment for BMI ( $\beta = 0.29$ ,  $P = 0.04$ ). Baseline *LIPE* ( $r = 0.42$ ,  $P = 0.007$ ) expression was also positively related to insulin sensitivity within the group of obese subjects.

The changes in the AT *PNPLA2* ( $r = -0.44$ ,  $P = 0.006$ ; Fig. 3A) and *LIPE* ( $r = -0.57$ ,  $P < 0.001$ ; Fig. 3B) after weight loss were inversely related to the concurrent changes in insulin sensitivity (i.e. the higher increase in the respective gene expression, the lower increase in insulin sensitivity) in the entire overweight/obese group. Both correlations remained significant after adjustment for initial insulin sensitivity and the concurrent change in BMI (*PNPLA2*,  $\beta = -0.33$ ,  $P = 0.046$ ; *LIPE*,  $\beta = -0.45$ ,  $P = 0.007$ ), as well as



**Figure 3**  
Correlations between the changes in insulin sensitivity ( $\Delta M$ ) and the concurrent changes in *PNPLA2* (A) and *LIPE* (B) expression after diet-induced weight loss in obese subjects ( $n = 38$ ). A.U., arbitrary units.

for the concurrent changes in FFA ( $P < 0.05$ ). In contrast, the relationship between initial insulin sensitivity and the change in this parameter during the intervention remained significant after adjustment for the change in *PNPLA2* ( $\beta = -0.34$ ,  $P = 0.036$ ), but not in *LIPE* expression. All the associations remained significant after exclusion of one subject with the highest increase in insulin sensitivity (data not shown).

Post-intervention insulin sensitivity was related to post-intervention AT *CIDEA* expression ( $r = 0.38$ ,  $P = 0.019$ ); however, this association disappeared after adjustment for BMI in the respective time-point.

### Discussion

The most important finding of the present study is that obese subjects, in whom insulin sensitivity does not

increase in response to weight loss, demonstrate higher initial insulin sensitivity (Table 2) and an increase in AT HSL and ATGL expression after dietary intervention (Fig. 2). Only the change in gene expression encoding HSL and ATGL independently predicted the changes in insulin sensitivity during the weight loss program in multiple regression analysis.

The decrease in body weight and body fat was very similar in the groups of subjects with and without an improvement in insulin sensitivity. Subjects without an improvement in insulin sensitivity had higher initial M value and exhibited a decrease in their insulin sensitivity after the dietary intervention program. Subjects with lower initial insulin sensitivity achieved greater metabolic benefit from lifestyle intervention (29). Findings similar to ours regarding worsening (29, 30), or the lack of improvement in insulin sensitivity (31) after weight loss in subjects with high initial insulin sensitivity, were observed by other researchers. In the cited studies, AT gene expression was not reported. In our study, initial insulin sensitivity value was not an independent predictor of the subsequent change in insulin sensitivity, therefore, changes in AT gene expression seem to be of particular importance.

At baseline, before the intervention, obese subjects had lower AT expression of the genes associated with lipolysis. Our data are in agreement with other studies, which also reported lower AT lipase expression in obesity (16, 17, 18, 19, 20, 21). Furthermore, decreased AT expression of *PLIN* (25), *ABHD5* (32, 33) and *CIDEA* (6, 33) in obesity was also reported. Decrease in AT *GOS2* (33, 34) and an increase in *CAVI* (35) were previously reported; however, we were unable to confirm these findings in our study. Our study subjects were young and had uncomplicated obesity, which may potentially explain the difference in the results. Interestingly, AT expression of the factors increasing (*PNPLA2*, *LIPE*, *MGLL*, *ABHD5*) and decreasing lipolysis (*PLIN*, *CIDEA*) were simultaneously decreased in obese subjects. This finding may suggest that the balance between pro- and anti-lipolytic factors is maintained in obesity; however, it is set at the altered level.

Before the dietary intervention, expression of *PNPLA2*, *LIPE*, *PLIN*, *ABHD5* and *CIDEA* was positively related to insulin sensitivity; however, most of these correlations (except *LIPE*) lost their significance after adjustment for BMI. In other studies, AT HSL and ATGL were associated with different measures of insulin sensitivity, independent of adiposity (19, 21). Similar findings were reported for AT *PLIN* and *CIDEA* (5, 36). Although we did observe positive correlations between the estimated genes

and clamp-derived insulin sensitivity, most of these associations were dependent on BMI. However, it should be noted that our study group were young and that obese subjects did not have overt metabolic disturbances and had relatively mild degree of insulin resistance (decrease by approx. 30% in comparison to normal-weight subjects), which may potentially influence the results. Anyway, our results do not indicate the genes associated with lipolysis as primary factors in obesity-related insulin resistance, with the exception of HSL.

We did not observe a significant increase in AT *PNPLA2* and *LIPE* expression in the entire study group. Data on the effect of weight loss on AT lipases are conflicting. Jocken *et al.* reported a decrease in AT HSL and ATGL mRNA and protein expression after a 10-week hypocaloric diet, whereas insulin resistance, measured as HOMA-IR, did not significantly change (19). In another study, both HSL and ATGL s.c. AT expression decreased after a 2-month low-calorie diet, which was preceded by a 1 month very low-calorie diet (37). No correlations between the change in lipase expression and anthropometric and metabolic parameters were found (37). In contrast, there are also data showing a normalization of an initially low AT HSL with an unchanged ATGL expression after weight loss (38) and an increase in ATGL after a 10-month weight maintenance period, but not during the preceding weight loss program (39). An increase in AT ATGL protein expression after 5 weeks of a very low-calorie diet followed by a 3-week weight maintenance diet (40) and an increase in AT HSL and ATGL mRNA expression after bariatric surgery-induced weight loss (41) were reported. In the latter study, post-intervention lipase expression displayed some relationships with beneficial metabolic profile; however, no BMI or other adjustments were reported (41). All the aforementioned studies differ regarding subjects' characteristics, type and duration of weight-reducing intervention, the degree of weight loss and the method of AT sampling, which likely may contribute to the observed differences in the results.

In our study, the change in AT *PNPLA2* and *LIPE* expression was associated with the concurrent change in insulin sensitivity independently of other estimated variables. It is postulated that a decreased lipase expression and an impaired AT lipolysis may contribute to the development and maintenance of obesity (16, 19). However, this explanation is rather unlikely, as HSL-deficient animals are resistant to diet-induced obesity (42), and humans with HSL null mutation display an impaired lipolysis and metabolic disturbances; however, their BMI is usually within the range of overweight and not obese (24).

Alternatively, it was suggested that lipase downregulation in obesity may be a compensatory mechanism to prevent an excessive FFA outflow and to restrain obesity-related insulin resistance (16, 19). Girousse *et al.* demonstrated that a decrease in AT lipolysis correlated with the concurrent improvement in insulin sensitivity 2 years after bariatric surgery. Lipase expression was not reported in the clinical part of this work, whereas initially lipolysis correlated positively with insulin resistance across a wide range of BMI (10). Another issue is the predisposition to weight regain, which has been linked to the defect in lipolysis in recent animal study (43). Our data show that AT lipase expression response to weight loss should be analyzed together with the concurrent changes in insulin sensitivity as the differences in individual response exist. Our results also suggest that the relation of AT lipase expression with insulin action may be different in the baseline conditions and in the state of a negative energy balance.

It is possible that worsening in insulin sensitivity is associated mainly with an FFA excess and with lipotoxicity during weight loss, as suggested by an increase in the genes encoding ATGL and HSL and lack of decrease in MGLL. The changes in *PNPLA2* and *LIPE* expression were related to the change in insulin sensitivity independent of change in FFA; however, fasting FFA measurement does not reflect possible lipotoxicity effect during the 3-month intervention. It was also demonstrated that lipolysis during weight loss may influence inflammatory response in AT in mice (44). However, AT inflammation was not decreased by weight loss in our previous report (27) and it was unchanged or even worsened when insulin sensitivity improved after ATGL inhibition in experimental studies (9, 10). Therefore, we propose that sustained lipotoxic effect may be responsible for the lack of improvement in insulin sensitivity during dietary intervention. It should be noted that one cannot establish the cause-effect relationship between different changes occurring simultaneously during weight loss on the basis of our results, and an increase in lipase expression may simply reflect an impaired inhibitory effect of insulin due to the lack of improvement in insulin sensitivity. However, despite this lack of improvement, insulin sensitivity remained still relatively good in this group, which argues against such an interpretation of our results.

An increase in AT *PLIN*, the gene encoding perilipin, a protein that stabilizes lipid droplet and decreases lipolysis, in the group with a worsening of insulin sensitivity may reflect the inter-relationships of this factor with lipases and/or may represent a compensatory mechanism aimed

to balance an increase in lipase expression. In contrast to *PNPLA2* and *LIPE*, the change in *PLIN* was not related to the change in insulin sensitivity.

Weight loss is usually associated with the beneficial metabolic changes. Thus, it may seem paradoxical that some of the changes observed in the present study during weight loss prevented an increase in insulin sensitivity. One may hypothesize that the most beneficial for metabolic health would be to mobilize fat stores efficiently in response to the weight-reducing intervention without subsequent maintaining prolonged lipolytic response. Our data support the notion that the regulation of lipolysis may uncouple adiposity from insulin sensitivity (8) especially in the conditions of weight loss. Our results also suggest that inhibition of AT lipolysis as a treatment option for insulin resistance (2) may have a particular benefit during weight-reducing interventions.

In the entire study group, we observed an increase in AT *ABHD5* and *CIDEA* and a decrease in *MGLL* expression. An increase in AT *CIDEA* after diet- or bariatric surgery-induced weight loss was observed previously (6, 36, 45, 46), and some of the studies report also an increase in *ABHD5* and *PLIN* (41, 47) and the latter one was not observed in our study when the entire group was analyzed. AT *ABHD5* and *CIDEA* expression increased similarly in the group with and without an improvement in insulin sensitivity and was not related to the concurrent change in M value. In the study of Montastier *et al.* (48), changes in adipose tissue *CIDEA* paralleled variations in insulin sensitivity independently of changes in BMI during diet induced weight loss and weight maintenance program. Our data suggest that low AT *CIDEA* is related rather to obesity *per se* and secondarily to insulin resistance.

It should be noted that our dietary intervention consisted of calorie restriction and balanced diet, without any specific modification in diet composition. Physical activity maintained stable throughout the program. Furthermore, we observed a decrease in fasting serum insulin in both groups and triglycerides in the group with an improvement in insulin sensitivity. In the group without an improvement in insulin sensitivity, serum FFA increased after weight loss. These changes are typical for diet-induced weight loss. Thus, it is reasonable to assume that the metabolic alterations reported in the present study, including AT lipase expression, are due to the calorie restriction and associated weight loss. It should also be noted that that an increase in AT lipase expression was not related to the changes in adrenergic receptors and *PDE3B* expression and the mechanism of an increase in AT *PNPLA2* and *LIPE* expression specifically in the group



without improvement in insulin sensitivity should be studied further.

We did not measure AT protein expression, which may be considered as a limitation of the present study. Such measurement was impossible due to the limited tissue availability. An excellent correlation between AT lipase mRNA and protein expression was demonstrated, both at baseline and after weight loss (19). It was also impossible to analyze adipocyte size; however, in the study of Verhoef *et al.* (39) the changes in the adipocyte size were not paralleled by the changes in ATGL during weight loss and maintenance. The group without an improvement in insulin sensitivity comprised of only nine subjects. However, the proportion of subjects with and without an improvement in insulin sensitivity is similar to other aforementioned studies (29, 30, 31). Furthermore, the change in *PNPLA2* and *LIPE* expression was inversely associated with the concurrent change in insulin sensitivity in the entire study group.

In conclusion, our data suggest that excessive lipolysis may prevent an improvement in insulin sensitivity after weight loss. The change in AT *PNPLA2* and *LIPE* expression was a negative predictor of the change in insulin sensitivity after weight loss.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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