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Adipose tissue expression of *adipose* (WDTC1) gene is associated with lower fat mass and enhanced insulin sensitivity in humans

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Abstract

The overexpression of the *adipose* gene (*adp*/WDTC1) in mice inhibits lipid accumulation and improves the metabolic profile.

Objective—We evaluated subcutaneous fat *adp* expression in humans and its relation to metabolic parameters.

Design, Setting and Methods—Abdominal subcutaneous fat *adp* expression, insulin sensitivity (clamp) and respiratory quotient (RQ; indirect calorimetry) were assessed in: 36 obese and 56 BMI-, race- and sex-matched type 2 diabetic volunteers (Look AHEAD Adipose Ancillary Study); 37 non-diabetic Pima Indians including obese (n=18) and non-obese (n=19) subjects and; 62 non-obese non-diabetic subjects at the Pennington Center in the ADAPT study.

Results—In the Look AHEAD Study, *adp* expression normalized for cyclophilin B was higher in males vs. females (1.27±0.06 vs. 1.11±0.04; p<0.01) but not after controlling for body fat. *Adp* expression was not influenced by the presence of diabetes but was related to body fat (r=−0.23; p=0.03), insulin sensitivity (r=0.23; p=0.03) and fasting/insulin-stimulated RQ (r=0.31 & 0.33; p<0.01). In Pima Indians, *adp* expression was also higher in males vs. females (1.00±0.05 vs.

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0.77±0.05; p=0.02) and higher in non-obese vs. obese (1.02±0.05 vs. 0.80±0.06; p=0.03). In the ADAPT study, there was no difference in *adp* expression between males and females.

Conclusion—Consistent with animal studies, our results suggest that, high *adp* expression in human adipose tissue is associated with lower adiposity and enhanced glucose utilization.

Keywords

obesity; insulin sensitivity; body fat

Introduction

The regulation of the size of the adipose tissue is critical for lipid storage thus preventing an oversupply of lipid to ectopic tissues such as skeletal muscle, liver and heart where it leads to insulin resistance (1, 2). Adipogenesis and cell lipid accumulation are important processes controlling adipose mass. Multiple genes play a role in adipose tissue lipid accumulation (3, 4). Suh et al. (5) observed that PPAR-γ2 action, a critical factor involved in adipogenesis, is suppressed by an anti-obesity gene named adipose (*adp*, WDTC1 or DCAF9). The gene was originally identified in *Drosophila*, in which a loss of activity increases fat storage (6, 7). Similarly, *adp* overexpression in 3T3-L1 adipose cells led to strong inhibition of lipid accumulation (5), while fat tissue-specific transgenic mice were leaner and had enhanced glucose tolerance. In contrast, heterozygous mice ($adp^{+/-}$) or mice overexpressing a dominant negative form of the gene were obese and glucose intolerant (5).

In humans, the potential role of *adp* (or its ortholog gene) on adiposity or energy metabolism is unknown. Lai et al. (8) reported that homozygote and heterozygote carriers of a major allele of *adp* were heavier than non-carrier subjects. To provide insight about the relevance of the *adp* gene in human energy balance, we studied the relationship between subcutaneous adipose tissue *adp* expression, body composition and energy metabolism in samples from 3 independent studies.

Research Design and Methods

This study included volunteers from studies conducted at Pennington Biomedical Research Center, Baton Rouge (Look AHEAD Adipose Ancillary and ADAPT studies), University of Pittsburgh (Look AHEAD), St. Luke's–Roosevelt Hospital Center, New York (Look AHEAD) and Obesity and Diabetes Clinical Research Section, NIDDK, Phoenix (Pima Indian study). All subjects provided written informed consent.

Subjects

The subjects' characteristics are shown in Table 1. Adipose tissue samples were available from 3 different studies:

1. *Look AHEAD* Adipose Ancillary *Study.* Type 2 diabetic (n=56) were recruited from the Look AHEAD study (9) and compared to non-diabetic weight-stable volunteers (n=36) matched for body mass index (BMI), sex and race.

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2. *ADAPT study.* Healthy weight-stable volunteers (45 males, 17 females) were recruited. Females and males had similar age and BMI but differed in percent body fat.

3. *Pima Indian study*. Obese (n=18) and non-obese (n=19), non-diabetic volunteers of similar age and from both sexes were recruited.

Experimental design

Look AHEAD Adipose Ancillary Study

Ninety two volunteers were admitted on the evening preceding the metabolic studies. Body fat mass was measured on a Hologic DXA (QDR 4500; Hologic, Waltham, MA). After a standardized dinner and overnight fast, ~500 mg of adipose tissue was obtained from the superficial abdominal subcutaneous adipose layer under local anesthesia through an aspiration biopsy needle. All samples were washed before snap freezing in liquid nitrogen. Another 50-mg sample was placed in osmium tetraoxide for fat cell size determination. Adipose tissue samples were shipped to the Pennington Center for fat cell size and gene expression analyses. One hour later, resting metabolic (RMR) was determined by indirect calorimetry for 30 minutes (10). After blood drawing, a hyperinsulinemic clamp (80 mU/m^2/min) was initiated and continued for 3 hours (10) with plasma glucose maintained at ~100 mg/dl. The mean rate of exogenous glucose infusion during steady-state was corrected for changes in glycemia and divided by the estimated metabolic body size (fat-free mass [FFM] + 17.7) to assess insulin sensitivity (11). For the last 30-minute period of the clamp, RMR was repeated.

The ADAPT study was designed to examine inter-individual differences in fat oxidation on an isoenergetic high-fat diet (12). After screening, 62 participants presented at the Pennington Center after refraining from vigorous physical activity for 48 h. After an overnight fast, a hyperinsulinemic clamp (80 mU/m^2/min) was initiated and continued for 3 hours with glucose maintained at ~90 mg/dl. Body composition was measured by DXA (QDR 4500; Hologic, Waltham, MA) and glucose disposal rate was adjusted for estimated metabolic body size. Adipose tissue biopsies were performed as described above.

In the Pima Indian study, 37 non-diabetic healthy Pima Indians on no medications were admitted to the clinical research unit at the NIDDK in Phoenix and placed on a weight maintaining diet. Body fat was assessed by DXA (Lunar, Madison, WI) and adipose tissue biopsies were performed at least 5 days after admission as described above. Samples were then shipped to Pennington Center for gene expression analysis.

Real-time quantitative reverse transcriptase-PCR

Adipose tissue was homogenized and total RNA extracted using a kit from Qiagen (Valencia, CA). Taqman Real PCR technique was performed after cDNA preparation by the one-step reverse transcriptase method. mRNA levels of *adp* and cyclophilin B genes were then quantified (Applied Biosystem, Foster City, CA). Each sample was run in duplicate and normalized for cyclophilin B transcript level. Cyclophilin B expression was not different between type 2 diabetic and non-diabetic subjects, race and gender. Primer and probe

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sequences for *adp* were: (F) 5′CGCATGATCCATAACCACAGAAAG3′, (R) 5′GGTGACCTGCTACGTAATACTGG3′ and (P) 5′CAGAGCCCTTCAGCGGGTGTGCAC3′.

Statistical analysis

Data are presented as means ± SE. Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC). Differences in *adp*/cycB and other variables were analyzed by covariance analysis. Because we observed an independent effect of the Study (Look AHEAD, ADAPT and Pima Indians), we analyzed the three studies separately. Included covariates included sex and diabetes status, and their interaction in the *Look AHEAD Adipose Ancillary Study*; sex in the *ADAPT study*; and sex, obesity and their interaction in the *Pima Indian study*. When the mixed model provided significant effects, post-hoc Tukey-Kramer test were used. Spearman correlation analysis assessed relationships between *adp* and metabolic variables. Statistical significance was 5%.

Results

Adipose expression and relationship with obesity and sex

In the *Look AHEAD Adipose Ancillary Study*, subcutaneous fat *adp* expression was not different in type 2 diabetic vs. non-diabetic subjects (p=0.18; Table 1), whereas, lower *adp* expression was observed in females vs. males (p<0.01; Table 1). However, the difference in *adp* expression between sexes disappeared after controlling for body fat mass. In the whole group, an inverse association between *adp* expression and percent body fat was found (r= −0.23; p=0.03; Figure 1A). *adp* expression was also inversely associated with fat cell size (r=−0.24; p=0.03), fasting plasma insulin (r=−0.34; p<0.01) and fasting plasma FFA (r= −0.30; p<0.01). In contrast, *adp* expression correlated positively with insulin-stimulated glucose disposal rate (r=0.23; p=0.03. Figure 1B), respiratory quotient (RQ) in fasting (r=0.31; p<0.01) and insulin-stimulated (r=0.33; p<0.01) conditions. Since the above relationships may be influenced by body fat and diabetes status, we analyzed the data in each group after adjusting for body fat. *Adp* expression remained correlated with insulin-stimulated glucose disposal rate (r=0.28; p=0.04) and RQ (fasting: r=0.46, p<0.01; insulin-stimulated: r=0.37, p<0.01) only in type 2 diabetic participants.

We confirmed some of these observations in the *Pima Indian Study*. For instance, subcutaneous fat *adp* expression was lower in females vs. males (0.77 ± 0.05 vs. 1.00 ± 0.05; p=0.02), and *adp* expression was lower in obese vs. non-obese individuals (0.80 ± 0.06 vs. 1.02 ± 0.05; p=0.03) (Table 1). However, we failed to confirm our findings in non-obese individuals in the *ADAPT Stu*dy. In this leaner group, *adp* expression did not differ between females and males (p=0.81) and was not related to fat cell size (r=−0.13; p=0.31), fasting insulin (r=0.02; p=0.91), fasting FFA (r=0.05; p=0.68) or percent body fat (r=−0.08; p=0.55).

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Discussion

In this study, we assessed the relationship of human abdominal subcutaneous fat *adp* expression with adiposity, insulin sensitivity and energy metabolism. Adipose tissue samples were available from three different studies: i) obese with and without type 2 diabetes (*Look AHEAD Adipose Ancillary Study*); ii) *Pima Indians* with and without obesity; and iii) healthy, non-obese individuals (*ADAPT Study*). In general, increased subcutaneous fat *adp* expression was associated with lower adiposity. In addition, in type 2 diabetic individuals increased *adp* expression was also related with higher insulin sensitivity and RQ. However, such relationship between *adp* expression and obesity (% body fat) or insulin sensitivity (fasting insulin) was not replicated in healthier and leaner subject (ADAPT study) probably due to narrower ranges of body fat and insulin sensitivity.

These results were consistent with previous reports in genetically modified 3T3-L1 fat cells, fruit flies and mice. In all these models, increased *adp* expression was related with decreased lipid accumulation and enhanced metabolic control (5). At a molecular level, the *adp*-related metabolic phenotype appears to be explained by the inhibitory action of *adp* on PPAR-γ2 activity (5). Such findings are partially in line with a previous obese PPAR-γ2-knock-out mouse model (2). PPAR-$\gamma 2^{-/-}$ mice are characterized by impaired adipogenic capacity (consistent with transgenic *adp* mice), but with increased ectopic lipotoxicity and impaired metabolic control (2) (different from transgenic *adp* mice) (5). Since transgenic *adp* mice are lean, one can speculate that a decreased lipogenic capacity may not be critical for energy homeostasis and does not lead to the metabolic disturbances observed in obese PPAR-$\gamma 2^{-/-}$ mice. Therefore, it would be insightful to compare metabolic control in obese animals with and without overexpression of *adp* gene.

In the present study, we did not measure adipose tissue PPAR-γ2 expression/activity and/or adipose tissue lipogenic capacity, so we cannot provide a mechanistic explanation for our findings. Due to the cross-sectional nature of our study, we cannot establish a causal relationship for our findings, i.e., one can also argue that adipose tissue *adp* expression may well be driven by fat mass or insulin sensitivity. In conclusion and similar to previous findings in insects and mice (5), our results suggest that adipose tissue expression of *adp* might play a role in human obesity and energy metabolism. Future studies should explore which nutritional/hormonal factors could regulate adipose tissue *adp* expression.

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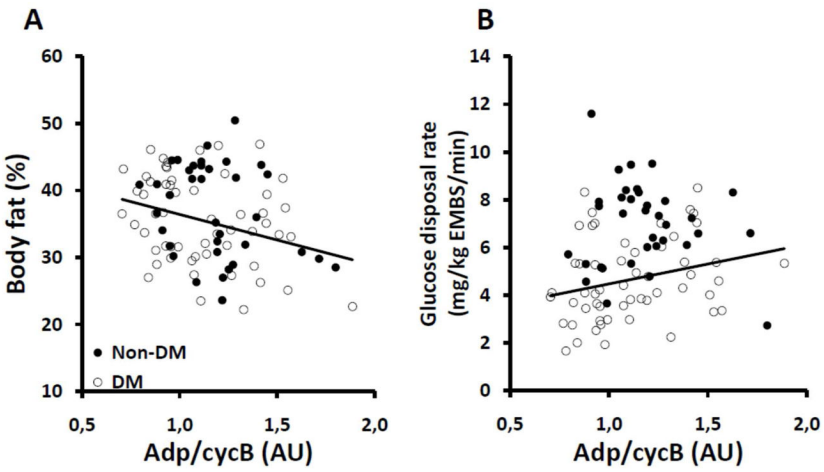

Figure 1.

Unadjusted Spearman correlation analysis in the Look AHEAD Adipose Ancillary Study group for non-diabetic and type 2 diabetic volunteers between human abdominal subcutaneous adipose tissue *adp* gene expression and body fat (A, r=−0.22; p=0.21 and r=−0.27; p=0.04, respectively), and insulin-stimulated glucose disposal rate (B, r=−0.01; p=0.94 and r=0.27; p=0.05, respectively).

● obese without type 2 diabetes (n = 36); ○ obese with type 2 diabetes (n = 56). Regression lines only indicated for significant associations.

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Table 1

Characteristics of the subjects

Look AHEAD Adipose Ancillary Study	**Non-diabetic subjects**		**Type 2 diabetic subjects**		**Sex (S)**	**Diabetes (D)**	**S × D**
	Male	**Female**	**Male**	**Female**			
African-American / White / Other	2 / 14 / 0	3 / 17 / 0	2 / 23 / 1	9 / 19 / 2			
Age (y)	54.8 ± 2.0	56.3 ± 1.8	61.6 ± 1.5	58.7 ± 1.3	0.66	<0.01	0.19
Body weight (kg)	106.0 ± 3.1[a]	87.2 ± 2.0[b]	101.2 ± 1.9[a]	92.0 ± 1.9[b]	<0.0001	0.99	0.03
Body mass index (kg/m^2)	33.2 ± 0.6[ab]	32.9 ± 0.6[a]	32.4 ± 0.5[a]	35.1 ± 0.6[b]	<0.05	0.25	0.02
Body fat (%)	30.2 ± 0.8	42.6 ± 0.7	29.8 ± 0.8	40.5 ± 0.7	<0.0001	0.11	0.26
Fasting glucose (mg/dl)	100 ± 2	100 ± 2	148 ± 6	134 ± 6	0.19	<0.0001	0.20
Fasting insulin (pmol/l)	50 ± 5	69 ± 10	68 ± 5	88 ± 8	0.01	0.02	0.92
GDR (mg/kg EMBS/min)	7. 0 ± 0.5	6.9 ± 0.4	4.6 ± 0.3	4.7 ± 0.4	0.88	<0.0001	0.77
Adipose/CycB	1.27 ± 0.06	1.11 ± 0.04	1.18 ± 0.05	1.06 ± 0.05	0.008	0.18	0.69
ADAPT	Male	Female					
African-American / White / Other	6 / 33 / 6	7 / 9 / 1					
Age (y)	22.7 ± 0.5	23.3 ± 0.9			0.53		
Body weight (kg)	83.6 ± 1.9	69.9 ± 2.8			0.0002		
Body mass index (kg/m^2)	26.8 ± 0.6	26.5 ± 1.2			0.82		
Body fat (%)	21.4 ± 0.9	33.6 ± 1.3			<0.0001		
Fasting glucose (mg/dl)	81 ± 1	76 ± 3			0.04		
Fasting insulin (pmol/l)	52 ± 4	49 ± 9			0.68		
GDR (mg/kg EMBS/min)	8.4 ± 0.5	9.4 ± 0.7			0.32		
Adipose/CycB	0.53 ± 0.01	0.52 ± 0.03			0.81		
PIMA INDIANS	Lean subjects		Obese subjects				
	Male	Female	Male	Female	Sex (S)	Obesity (O)	S×O
N	14	5	9	9			
Age (y)	26.8 ± 1.9	25.7 ± 2.8	29.5 ± 2.3	26.5 ± 2.3	0.48	0.39	0.69
Body weight (kg)	77.3 ± 1.7	62.4 ± 5.6	135.0 ± 5.2	116.9 ± 4.6	0.0004	<0.0001	0.70
Body mass index (kg/m^2)	26.3 ± 0.5	24.6 ± 2.2	45.3 ± 1.7	44.4 ± 1.0	0.31	<0.0001	0.74
Body fat (%)	26.4 ± 0.8	34.5 ± 4.1	38.2 ± 1.7	44.4 ± 1.0	0.0002	<0.0001	0.39
Fasting glucose (mg/dl)	90 ± 2	91 ± 2	92 ± 3	91 ± 2	0.96	0.64	0.81

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Look AHEAD Adipose Ancillary Study	Non-diabetic subjects		Type 2 diabetic subjects		Sex (S)	Diabetes (D)	S × D
	Male	Female	Male	Female			
Fasting insulin (pmol/l)	31 ±25	32 ± 4	53 ± 5	62 ± 7	0.31	<0.0001	0.54
Adipose/CycB	1.06 ± 0.04	0.89 ± 0.12	0.90 ± 0.11	0.70 ± 0.03	0.02	0.03	0.85

Unadjusted data are mean ± SE. Comparison of means by covariance analysis between groups with sex, diabetes and their interaction as main factors in the Look AHEAD Adipose Ancillary Study. Multiple comparisons between groups were adjusted by post-hoc Tukey-Kramer analysis. Statistical analysis with fat mass (kg) and fat-free mass (kg) as covariates. RQ, respiratory quotient; GDR, insulin-stimulated glucose disposal rate; EMBS, estimated metabolic body size (fat-free mass [kg] + 17.7).

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