Adipose Tissue Expression of Adipose (WDTC1) Gene Is Associated with Lower Fat Mass and Enhanced Insulin Sensitivity in Humans

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Objective: The overexpression of the adipose gene (adp/WDTC1) in mice inhibits lipid accumulation and improves the metabolic profile. Subcutaneous fat adp expression in humans and its relation to metabolic parameters was evaluated.

Design 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 nondiabetic Pima Indians including obese (n = 18) and nonobese (n = 19) subjects and; 62 nonobese nondiabetic 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 versus 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 and 0.33; P < 0.01). In Pima Indians, adp expression was also higher in males versus females (1.00 ± 0.05 vs. 0.77 ± 0.05; P = 0.02) and higher in nonobese versus 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.

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,
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 three 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.

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 nonobese (n = 19), nondiabetic 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, USA). After a standardized dinner and overnight fast, approximately 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 specimens. Adipose tissue biopsies were performed at least 5 days after admission as described above. Body fat was assessed by DXA (Lunar, Madison, WI, USA) 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, USA). Taqman Real PCR technique was performed after cDNA preparation by the one-step reverse transcription method. mRNA levels of adip and cyclophilin B genes were then quantified (Applied Biosystem, Foster City, CA, USA). Each sample was run in duplicate and normalized for cyclophilin B transcript levels. Cyclophilin B expression was not different between type 2 diabetic and non-diabetic subjects, race and gender. Primer and probe sequences for adip were: (F) 5’CCGATGATCCATAACCACAGAAAG3’, (R) 5’GGTGACCTGTACGAATCTGG3’, and (P) 5’CAGAGCCCTTCAGGTTGTCAC3’.

Statistical analysis

Data are presented as means ± SE. Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA). Differences in adip/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. 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 adip 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 adip expression was not different in type 2 diabetic vs. non-diabetic subjects (P = 0.18; Table 1), whereas, lower adip expression was observed in females vs. males (P < 0.01; Table 1). However, the difference in adip expression between sexes disappeared after controlling for body fat mass. In the whole group, an inverse association was observed in females vs. males (P = 0.03; Figure 1A). adip 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, adip expression correlated positively with insulin-stimulated glucose disposal rate (r = 0.23; P = 0.03. Figure 1B), RQ in fasting (r = 0.31; P < 0.01) and insulin-stimulated (r = 0.33; P < 0.01) conditions. Because the above relationships may be influenced by body fat and diabetes status, we analyzed the data in each group after adjusting for body fat. Adip 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 adip expression was lower in females versus males (0.77 ± 0.05 vs. 1.00 ± 0.05; P = 0.02), and adip
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: (1) obese with and without type 2 diabetes (Look AHEAD Adipose Ancillary Study); (2) Pima Indians with and without obesity; and (3) healthy, nonobese 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.

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: (1) obese with and without type 2 diabetes (Look AHEAD Adipose Ancillary Study); (2) Pima Indians with and without obesity; and (3) healthy, nonobese 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.
related metabolic phenotype appears to be explained by the inhibitory action of
on PPAR-2 activity (5). Such findings are
partially in line with a previous obese PPAR-2-knock-out mouse
model (2). PPAR-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). Because transgenic  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-2/- mice. Therefore, it would be insightful to compare metabolic control in obese animals with and without overexpression of  gene.

In this study, we did not measure adipose tissue PPAR-2 expression/ activity and/or adipose tissue lipogenic capacity, so we cannot provide a mechanistic explanation for our findings. Because of the cross-sectional nature of our study, we cannot establish a causal relationship for our findings, that is, one can also argue that adipose tissue  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/ activity and/or adipose tissue lipogenic capacity, so we cannot provide a mechanistic explanation for our findings. Because of the cross-sectional nature of our study, we cannot establish a causal relationship for our findings, that is, one can also argue that adipose tissue  expression may well be driven by fat mass or insulin sensitivity.

Acknowledgments
We acknowledge the other members of the Look AHEAD adipose research group, not included in the writing group, who contributed generously to this research project. We are grateful to the participants of the primary Look AHEAD trial for their enthusiastic willingness to participate in this ancillary study. We are also grateful to the participants from Look AHEAD Ancillary, ADAPT, and Pima Indian Studies and to the nursing and nutritional staffs of each Research Center involved.

Wake Forest University School of Medicine (the Coordinating Center for Look AHEAD): Mark A. Espeland, PhD; Judy Bahnsen, BA; Lynne Wagenknecht, DrPH; David Reboussin, PhD; W. Jack Rejeski, PhD; Wei Lang, PhD; Alain Bertoni, MD, MPH; Mara Vitolins, DrPH; Gary Miller, PhD; Paul Ribisl, PhD; Kathy Dotson, BA; Amelia Hodges, BS; Patricia Hogan, MS; Kathy Lane, BS; Carrie Combs, BS; Christian Speas, BS; Delia S. West, PhD; William Herman, MD, MPH.

Pennington Biomedical Research Center: Donna H. Ryan, MD; Donald Williamson, PhD; Frank L. Greenway, MD; Allison Strate, RN; Elizabeth Tucker; Kristi Rau; Brandi Armand, LPN; Mandy Shipp, RD; Kim Landry; Jennifer Perault.

St. Luke’s Roosevelt Hospital Center: Xavier Pi-Sunyer, MD; Jennifer Patricio, MS; Jennifer Mayer, MS; Stanley Heshka, PhD; Carmen Pal, MD; Mary Anne Holowaty, MS, CN; Diane Hirsch, RNC, MS, CDE.

University of Pittsburgh: Jacqueline Wesche-Thobaben, RN, BSN, CDE; Lewis Kuller, MD, DrPH; Andrea Kriska, PhD; Daniel Edmundowicz, MD; Mary L. Klem, PhD, MLIS; Janet Bonk, RN, MPH; Jennifer Rush, MPH; Rebecca Danchenko, BS; Barb Elmeczyk, MA; Karen Vujevich, RN-BC, MSN, CRNP; Janet Krulia, RN, BSN, CDE; Donna Wolf, MS; Juliet Mancino, MS, RD, CDE, LDN; Pat Harper, MS, RD, LDN; Anne Mathews, MS, RD, LDN.

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