Metabolic consequences of ENPP1 overexpression in adipose tissue

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EXCESSIVE CALORIC INTAKE AND FAT DEPOSITION is a common cause of adipose tissue dysfunction that leads to a clustering of systemic metabolic changes identifiable in the metabolic syndrome (22) and to the associated increased risk for both type 2 diabetes and cardiovascular disease (CVD).

Among the complex changes occurring in metabolic syndrome, ectopic fat deposition (particularly fatty liver) is increasingly recognized as causative of changes in lipid and glucose metabolism, which in turn leads to type 2 diabetes and CVD (6, 7). These changes are induced by positive caloric balance and obesity. However, it has become increasingly clear that several groups of people are “metabolically obese” even when body mass index (BMI) and waist circumference are not increased (23). The opposite is also true, and obese people may be found to have mild or no metabolic abnormalities associated with the metabolic syndrome and to maintain low risk for type 2 diabetes and CVD (18). Although fat distribution with predominant abdominal deposition can account for some of these clinical observations, an alternative explanation can be found in variability of overall functional capacity of adipose tissue as a “buffer” of excessive caloric intake. In other words, if storage capacity of adipose tissue is low, as it happens in its extreme manifestation in patients with lipodystrophy (13), fatty liver, and metabolic syndrome, and its consequences will be present at low body weight and with no or minimal increase in adipose tissue mass, including abdominal adipose tissue. If storage capacity is high, then obesity (or even morbid obesity) can be compatible with no fatty liver and no systemic metabolic abnormalities (18, 28). Recent research in various laboratories has focused on identifying mechanisms of reduced triglyceride storage capacity of adipose tissue. Among those, we have reported previously on the effects of ectonucleotide pyrophosphate phosphodiesterase (ENPP1) overexpression in adipocytes (17).

ENPP1 is a class II transmembrane glycoprotein that belongs to the ENPP family of enzymes known to hydrolyze 5'-phosphodiester bonds in nucleotides (11). ENPP1 also modulates insulin action by physical interaction with the α-subunit of the insulin receptor and inhibition of β-subunit activation (12, 13, 19). Interestingly, increased expression of ENPP1 in adipose tissue of insulin-resistant subjects has been reported previously (10). However, the specific effects of adipose tissue ENPP1 on systemic lipid and glucose metabolism are not yet known. Previous transgenic models have demonstrated systemic insulin resistance with ENPP1 overexpression in multiple tissues (except adipose), and the effects are attributed mainly to insulin resistance induced in skeletal muscle and liver (20). In this study, we turned our attention to the specific effects of ENPP1 overexpression in mature adipocytes.

On the basis of the above observations, we have hypothesized that ENPP1 overexpression in adipocytes is an important mechanism of adipose tissue dysfunction leading to adipocyte insulin resistance, reduced triglyceride storage capacity, fat deposition in ectopic tissues (including liver), and systemic manifestations of the metabolic syndrome. In support of our hypothesis, this study addresses the “in vivo” effects of ENPP1 overexpression in adipocytes using the adipose ENPP1 transgenic (AdiposeENPP1-TG) mice, a murine model obtained with targeted overexpression of human ENPP1 in adipocytes using the aP2 promoter in the transgenic construct.

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MATERIALS AND METHODS

Animals. Transgenic mice were produced by the University of Texas Southwestern (UTSW) Transgenic Core Laboratory using a C57Bl/6J background. The transgene was constructed using the human ENPP1 cDNA from Dr. Vincenzo Trischitta (Sapienza University of Rome, Italy), a 5.4-kb region of the mouse aP2 promoter/enhancer from Dr. Bruce Spiegelman (Dana Farber Cancer Institute), and the pSTEC-1 vector containing restriction sites for promoter and cDNA insertion, an intron, and SV40 polyA sequences from Dr. C. D. Sigmund (University of Iowa). A c-myc epitope tag was added to the amino terminus located in the intracellular domain of ENPP1. The sequence of the final transgene was confirmed in both directions. The transgene was transmitted in the expected Mendelian ratios, and litter sizes were normal, indicating that it does not impede normal mouse fertility or embryo development. All transgenic mice used in this study were male and obtained by crossing a male transgenic from a single colony with a C57Bl/6J female. Multiple generations of transgenic mice have been used for the various protocols of this study, and stable gene expression has been confirmed with RT-PCR and protein quantification. Mice were housed in the animal facility at UTSW, where the experiments were initiated, and subsequently at UT Medical Branch (UTMB) in Galveston, TX, where the experiments were completed. The Institutional Animal Care Boards of both UTSW and UTMB approved the study protocol.

Diet. After weaning, male mice were assigned to groups based on the presence or absence of the ENPP1 transgene. Different diets were started at 8 wk of age to conduct experiments with free access to food and also experiments with pair-feeding design. For the “ad libitum” diet experiments each group had free access to water and standard chow (4% fat by calories, Teklad 7001; Teklad, Madison, WI) or high-fat chow (60% fat by calories; Research Diets D12492, New Brunswick, NJ). For the pair-feeding experiments, a quantity of high-fat chow was chosen for the wild-type (WT) mice based on the intake recorded in the transgenic mice the day before.

Tissue collection. After mice were euthanized, blood, epididymal adipose tissue, skeletal muscle, and liver tissue were obtained and stored at −80°C. About 5 mg of tissues was fixed in formalin for histology.

mRNA quantification. Total RNA was isolated from frozen tissues using RNA STAT-60 (Tel-Test, Friendswood, TX). Genomic DNA was removed from the total RNA preparations using DNAse 1 (DNA Free; Ambion). RNA from each sample was diluted to 5 ng/μl, and 100 ng of RNA was reverse-transcribed in a 100-μl reaction using random hexamers (TaqMan Reverse Transcription kit; Applied Bio...
Adipose tissue was collected from 7 AdiposeENPP1-TG mice and 4 wild-type mice 30 min after intraperitoneal insulin injection at a dose of 0.5 U/kg body wt. All mice were studied after being exposed to 12 wk of a 60% fat diet with pair-feeding protocol. The Western blots show ENPP1 protein and Akt phosphorylation in each animal. The mean optical density and SD for phosphorylated Akt Ser473 (60 kDa) is shown in the bar graph for the 2 study groups.

Oil Red O staining. Liver tissues were fixed with 10% formalin in PBS for 15 min and stained for ≥1 h in freshly diluted Oil Red O solution (6 parts Oil Red O stock solution and 4 parts H2O; Oil Red O stock solution is 0.5% Oil Red O in isopropanol). The stain was then removed, and the cells were washed twice with water with or without counterstain (0.25% giemsa for 15 min) and then photographed.

Table 1. AdiposeENPP1-TG and wild-type littermate mice were given either regular chow diet or diet containing 60% fat without food quantity restrictions (“ad libitum”)

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>AdiposeENPP1-TG</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>26.6 ± 1.6</td>
<td>25.9 ± 0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Week 16</td>
<td>31.4 ± 2.8</td>
<td>27.2 ± 2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Week 24</td>
<td>37.3 ± 2.9</td>
<td>33.9 ± 2.5</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Food intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.08</td>
<td>0.8</td>
</tr>
<tr>
<td>Week 16</td>
<td>4.0 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Week 24</td>
<td>3.8 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

60% fat diet (“ad libitum”)

| **Weight**          |           |                 |         |
| Week 8              | 26.9 ± 0.7| 27.1 ± 1.4      | 0.7     |
| Week 16             | 41.4 ± 6.8| 36.5 ± 3.6      | 0.09    |
| Week 24             | 48.4 ± 5.8| 42.2 ± 5.2      | 0.02    |

Table 2. A group of AdiposeENPP1-TG (n = 5) and a group of wild-type littermate mice (n = 5) underwent metabolic cage studies during last week of 14-wk feeding with 60% fat diet using pair-feeding protocol

<table>
<thead>
<tr>
<th></th>
<th>AdiposeENPP1-TG</th>
<th>Wild Type</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at age 20 wk, g</td>
<td>32.3 ± 2.4</td>
<td>32.0 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total body fat at age 20 wk, %body weight</td>
<td>26.5 ± 3.3</td>
<td>26.2 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Epididymal fat weight, g</td>
<td>1.4 ± 0.3</td>
<td>1.6 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.09 ± 0.10</td>
<td>1.09 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>0.17 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>0.33 ± 0.03</td>
<td>0.35 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Food intake, g/body weight</td>
<td>0.18 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Oxygen consumption, ml·h⁻¹·kg⁻¹</td>
<td>1.363 ± 105</td>
<td>1.445 ± 306</td>
<td>NS</td>
</tr>
<tr>
<td>Respiratory exchange rate</td>
<td>0.95 ± 0.00</td>
<td>0.96 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Total locomotion (ambulatory and rearing)</td>
<td>5,364 ± 1,716</td>
<td>4,933 ± 1,343</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SD. NS, not significant. Body fat was measured using MRI after 5 h of fasting and soon before dissection for measurements of other organs at age 24 wk.
nology), and human ENPP1 antibody (Imgenex, San Diego, CA) were available commercially.

**Body composition and indirect calorimetry measurements.** Body composition was measured by magnetic resonance imaging (MRI) using an Echo MRI apparatus (Echo Medical Systems, Houston, TX). For indirect calorimetry measurements, animals were housed individually in metabolic chambers maintained at 20–22°C on a 12:12-h light-dark cycle with lights on at 0700. Metabolic measurements (oxygen consumption, respiratory exchange ratio, and locomotor activity) were obtained continuously using an open-circuit indirect calorimetry Comprehensive Laboratory Animal Monitoring System (Columbus Instruments, Columbus, OH). Mice were provided with the standard chow diet mentioned above and tap water ad libitum. Presented results contain data collected for a period of 8 days following 3 days of adaptation to the metabolic cages. For food intake measurements, mice were housed individually, and food was weighed each day at noon for 7 days. Food intake is expressed as the grams of food ingested per gram of body weight.

**Liver triglyceride measurements.** Triglycerides were extracted from frozen liver tissues (200 mg), as described by Folch et al. (8). Triaclyglycerol content was assayed using the GPO-Trinder Sigma kit (Sigma-Aldrich). Hepatic lipids were also quantitated using TLC separation followed by gas chromatographic quantitation. For this method, liver tissue was pulverized under liquid nitrogen, and ~20–50 mg tissue/reaction was weighed and used. The samples were vortexed with 3 ml of extraction solution [1:2 (vol/vol) methanol-chloroform containing 0.05 mg/ml butylated hydroxytoluene] and appropriate volumes of internal standards and left overnight to allow further extraction of the lipids. The next day, the samples were centrifuged at 3,500 rpm for 30 min at 4°C. The supernatant was collected and dried under gentle nitrogen flow. The lipids in the tubes were redissolved in 50 μl of chloroform-methanol (2:1 vol/vol) spotted on TLC plates and separated using the solvent mixture heptane-ethyl ether-acetic acid [70:30:1 (vol/vol)]. After the run the plates were sprayed with primuline solution, and the lipid spots were detected under the UV lamp with a wavelength of 365 nm. The spots corresponding to triacylglycerols and diacylglycerols were scrapped into separate tubes and dissolved in 50 μl of hexane and 1 ml of 14% BF₃·CH₂OH by vortex and then heated at 100°C for 4 min and then cooled down. Then, 1 ml of dH₂O and 2 ml of hexane were added into each tube, and the samples were shaken for 15 min and centrifuged for 15 min at 2,000 rpm; the upper phase was removed and dried under gentle nitrogen flow. The lipids were redissolved in 50–100 μl of heptanes and analyzed using GC-FID.

**Quantitation of adipocyte size.** Tissue sections from epididymal and mesenteric adipose tissue were stained with hematoxylin and eosin. Adobe Photoshop CS3 extended and Adobe Extendscript Technology, and human ENPP1 antibody (Imgenex, San Diego, CA) were available commercially. Adipose tissue sections from wild-type and AdiposeENPP1-TG mice were stained with hematoxylin and eosin. Adobe Photoshop CS3 extended and Adobe Extendscript Technology, and human ENPP1 antibody (Imgenex, San Diego, CA) were available commercially.

**RESULTS**

**Intraperitoneal glucose tolerance test.** Glucose was administered by intraperitoneal injection at a dose of 1 g/kg body wt following a 5-h fast. Blood samples were obtained via the tail vein at baseline and 15, 30, 60, 90, and 120 min after intraperitoneal glucose injection. Blood glucose levels were measured using an Ascensia glucometer (Bayer). Insulin was measured from plasma obtained at time 0, 15, 30, 60, 90, and 120 min during intraperitoneal glucose tolerance test (IPGTT). Ultra Sensitive Mouse Insulin ELISA Kit (cat. no. 90080; Crystal Chem) was used for insulin measurement.

**Insulin tolerance test.** After a 5-h fast, mice had 0.5 U/kg body wt regular insulin injected intraperitoneally. Blood glucose was measured at baseline and at 30-min intervals for 2 h using an Ascensia glucometer (Bayer).

**Biochemical quantitations.** Plasma glucose concentration was assayed using a glucose oxidase method. The plasma concentrations of free fatty acids were measured by enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany). Plasma triglycerides were measured using the GPO-Trinder Sigma kit (Sigma-Aldrich). Insulin was assayed using Ultra Sensitive Mouse Insulin ELISA Kit (cat. no. 90080; Crystal Chem). Leptin, adiponectin, resistin, and TNFα were measured using commercially available multiplex immunoassays (Millipore, Billerica, MA).

**Statistics.** Data were analyzed using SAS version 9.2 (SAS Institute, Cary, NC) and are presented as means ± SD. Because of skewness, plasma triglyceride and fatty acid were log-transformed prior to analysis. A two-tailed Student t-test was used for group comparison where appropriate. One-way ANOVA followed by least square contrast of the ANOVA model was used for multiple comparisons. Significance was defined as P < 0.05.
proportionate to the variability in Akt phosphorylation even within the AdiposeENPP1-TG group.

Cross-reactivity of the murine antibody with human ENPP1 can account for the differences in ENPP1 protein content between the two mice groups. To confirm that increased ENPP1 protein in adipose was attributable to the human transgene, we evaluated differences in human and murine ENPP1 gene expression in adipose tissue of the two mice groups. Figure 3 shows that RT-PCR results in epididymal adipose tissue, using primers designed for human and murine ENPP1. These primers had no cross-reactivity. Only AdiposeENPP1-TG mice were found to have human ENPP1 expression, whereas the endogenous ENPP1 expression was comparable with that of WT littermates.

AdiposeENPP1-TG and WT littermates had similar body weights at week 24 if exposed to regular chow diet (Table 1). However, when exposed to high fat diet (60% fat), the transgenic mice had lower weight than the WT \((P = 0.02)\), and food intake was also lower \((P < 0.0001)\). Therefore, a pair-feeding protocol was followed to compare the studied variables while minimizing differences in body weight between the AdiposeENPP1-TG and the WT littermates.

A group of pair-fed animals (60% fat diet) was used to perform calorimetric studies. These animals had similar body weights, total body fat, and organ weight for AdiposeENPP1-TG and WT littermates (Table 2). Furthermore, the food intake, oxygen consumption, respiratory exchange, and total locomotion were comparable. Despite similar adipose tissue mass \((8 \pm 3 \text{ and } 9 \pm 2 \text{ g for Adipose-TG and WT, respectively})\) we found that adipocyte size was significantly lower \((P = 0.038)\) in the AdiposeENPP1-TG mice (Fig. 4).

Figure 5 shows comparable ENPP1 protein content in the liver of AdiposeENPP1-TG and WT littermates. We did not find differences in the liver insulin receptor Tyr1361 phosphorylation between the two mice groups. However, Akt Ser473 phosphorylation was reduced by \(~30\%\) in the liver of AdiposeENPP1-TG \((P = 0.05)\).

The liver of AdiposeENPP1-TG transgenic mice was found to be significantly enriched with triglycerides (Fig. 6). This finding was further confirmed in independent experiments using gas chromatographic quantitation after TLC separation \((150 \pm 7 \text{ and } 100 \pm 6 \text{ mg triglyceride/g tissue for 10 AdiposeENPP1-TG mice and 8 WT littermate mice, respectively, } P = 0.02)\).
Figure 7 summarizes the results on plasma free fatty acids after 5 h of fasting and during the 30 min following intraperitoneal insulin administration at a dose of 0.5 U/kg body weight. Plasma glucose and insulin concentrations were similar in the two study groups on regular chow diet. However, the AdiposeENPP1-TG mice pair-fed with a 60% fat diet had higher plasma glucose (P < 0.05 at time points 30, 60, and 120 min) and insulin concentrations (P < 0.05 at time points 0 and 15 min) than the other three groups during IPGTT. Figure 8C shows that glucose suppression following insulin administration was decreased in the AdiposeENPP1-TG mice on a high-fat diet (P < 0.05 at time points 15–120 min from insulin administration). Possible mechanisms involved in the observed systemic effect of adipose tissue-specific ENPP1 overexpression are presented schematically in Fig. 9.

Of note, transgene expression was observed in white and brown adipose tissue and absent in other tissues relevant for systemic glucose and lipid metabolism (Fig. 10). Also, we failed to detect group differences for ENPP1 protein quantity, insulin signaling activation, and tissue fat content in the skeletal muscle (21 ± 11 and 20 ± 12 mg triglyceride/g tissue for AdiposeENPP1-TG and WT, respectively, P = 0.9; Fig. 11). Skeletal muscle diacylglycerol content was similar in the two study groups (data not shown).

Table 3 summarizes plasma concentrations of lipids and adipokines. AdiposeENPP1-TG mice had increased plasma concentrations of fatty acids and triglycerides and decreased plasma concentrations of leptin. A trend toward lower plasma adiponectin concentrations did not reach statistical significance. Plasma concentrations of inflammatory adipokines IL-6 and TNFα were similar in the two study groups. Adipose tissue gene expression of inflammatory cytokines was also comparable (data not shown). However, there was a nonsignificant trend toward higher adipose tissue expression of CD68, a marker of increased macrophage recruiting (Fig. 12).

**DISCUSSION**

The findings of this study support the view that adipocyte ENPP1 can modulate the overall caloric “buffering” function...
of adipose tissue in the presence of excessive fat intake. As depicted in the schematic representation of Fig. 9, our results support the hypothesis that when storage demand is increased by high-fat feeding, \textit{ENPP1} overexpression in adipocytes of \textit{AdiposeENPP1-TG} mice impairs the physiological increase in adipocyte size (triglyceride storage) we found in the WT siblings. This change in adipose tissue function likely reflects decreased triglyceride storage capacity in adipose tissue and appears to be a major cause of fatty liver and systemic changes in lipid and glucose metabolism commonly observed in the metabolic syndrome.

The possibility that \textit{ENPP1} would determine fatty liver and impact systemic glucose and lipid metabolism “in vivo” through an effect on adipocyte function had not been investigated previously. This gap in the literature is of importance in light of the observation that \textit{ENPP1} is often overexpressed in adipose tissue of insulin-resistant people (10). Of note, this previous study in humans included nonobese individuals, thus suggesting that \textit{ENPP1} overexpression is an early defect in the development of systemic insulin resistance independent of obesity.

Our mouse model provides information on the isolated effects of \textit{ENPP1}-induced adipose tissue dysfunction on systemic handling of glucose and lipid metabolism. Although no systemic consequences of \textit{ENPP1} overexpression were found when diet did not demand triglyceride storage in adipose tissue (regular chow fed mice), \textit{AdiposeENPP1-TG} mice on high-fat diets manifested fatty liver (Fig. 6).

Typically, fatty liver in obesity is associated with hyperinsulinemia and defective insulin signaling directed to hepatic glucose metabolism (4, 7, 21). However, insulin signaling directed to sterol regulatory element-binding protein-1c (SREBP-1c) activation has been described to be intact and upregulated by hyperinsulinemia, thus contributing to lipogenesis (3, 16, 26). This mechanism explains about 10% of “de novo” lipogenesis in obesity and insulin resistance (25) and up to 24% in human with fatty liver (5). Interestingly, our animal model was found to have diminished hepatic Akt phosphorylation even without changes in \textit{ENPP1} expression (Fig. 5C). In addition, no defective insulin receptor activation was measurable in the hepatocytes of these animals (Fig. 5B). Methodological problems related to insulin receptor phosphorylation quantification may
have precluded us from finding small differences. On the other hand, postreceptor dysregulation could also be postulated. The reduction of hepatic Akt-phosphorylation of AdiposeENPP1-TG mice could determine defective insulin-mediated suppression of hepatic glucose output and contribute to the dysglycemia observed “in vivo” using IPGTT and insulin tolerance test (Fig. 8). Since decreased Akt phosphorylation would predict decreased SREBP-1c activations (3), fatty acid synthesis might not be significant contributor to the fatty liver of AdiposeENPP1-TG mice. As depicted in the schematic of Fig. 9, increased fatty acid “spillover” from adipose tissue could be a reasonable consequence of defective triglyceride storage. This would provide an important substrate for the liver to synthesize triglyceride. The concomitant changes in glucose metabolism may contribute to provide the additional substrate to promote the triglyceride synthesis in the liver of this animal model. However, the possibility that a yet-unidentified signal from adipose tissue to the liver may be a contributory mechanism cannot be excluded.

The possibility of such an adipocyte-secreted molecule has been postulated to explain the fatty liver phenotype of another model of fatty liver induced by adipocyte insulin resistance, the adipose Glut4-deficient mice (1). Similarly to our model, the decreased insulin-mediated glucose utilization in adipocytes of Glut4-deficient mice induces hepatic steatosis, hepatic insulin resistance, and systemic insulin resistance (1, 15). However, a major difference between the two models is the presence of increased plasma fatty acid in the AdiposeENPP1-TG mice and its absence in the adipose Glut4-deficient mice. Likely as the consequence of upstream adipocyte insulin resistance induced by the ENPP1 overexpression, increased baseline fatty acid and incomplete suppression during the hyperinsulinemia induced by the IPGTT (Fig. 8) would support our mechanistic hypothesis depicted in Fig. 9. The concept that adipocyte fatty acid handling plays a major role in hepatic insulin sensitivity and triglyceride deposition is supported further by the findings...
in adipose hormone-sensitive lipase-deficient mice (27). In that model, decreased lipolysis was shown to be associated with low plasma fatty acid, improved insulin sensitivity, and reduced triglyceride content in the liver.

Table 3. Plasma concentrations of lipids and adipose tissue metabolites in AdiposeENPP1-TG and their wild-type littermates after 12 wk of a 60% fat diet with pair-feeding protocol.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AdiposeENPP1-TG</th>
<th>Wild Type</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mg/dl</td>
<td>116 ± 34</td>
<td>79 ± 11</td>
<td>0.03</td>
</tr>
<tr>
<td>Free fatty acid, ng/ml</td>
<td>110 ± 11</td>
<td>79 ± 8</td>
<td>0.02</td>
</tr>
<tr>
<td>Adiponectin, mg/ml</td>
<td>19 ± 5</td>
<td>23 ± 3</td>
<td>0.11</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>31 ± 30</td>
<td>116 ± 10</td>
<td>0.05</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>2.1 ± 0.9</td>
<td>2.5 ± 1.8</td>
<td>0.53</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>2.0 ± 1.3</td>
<td>2.3 ± 1.6</td>
<td>0.61</td>
</tr>
<tr>
<td>IL-6, ng/ml</td>
<td>12.9 ± 8.6</td>
<td>16.7 ± 17.9</td>
<td>0.40</td>
</tr>
<tr>
<td>TNFα, ng/ml</td>
<td>1.8 ± 1.9</td>
<td>2.7 ± 4.6</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD. PAI-1, plasminogen activator inhibitor-1. Student t-test for independent variables was used for group comparison.
Another important link between adipose tissue function, systemic insulin resistance, and hepatic insulin resistance/steatosis is production of inflammatory cytokines in adipose tissue. Adipose tissue inflammation has been reported to play a role in hepatic steatosis and insulin resistance in obesity. Mice lacking the Jnk1 gene in adipose tissue do not develop high-fat diet-induced inflammation of adipose tissue and do not develop systemic insulin resistance, hepatic insulin resistance, or fat deposition despite developing obesity (24). Similarly, mice with specific deficiency of Fas in adipose tissue have been shown to be protected from obesity-induced insulin resistance, liver insulin resistance, and steatosis (29). Fas is known to mediate inflammation in obesity, particularly in adipocytes, and contribute to adipose tissue inflammation and metabolic dysregulation. We found that the adipose tissue of AdiposeENPP1-TG mice has no significant increase in expression of genes involved in inflammation, such as IL-6 and TNFα. However, there was a trend toward increased expression of CD68, a marker of macrophage recruiting (Fig. 12). Because plasma concentrations of IL-6 and TNFα were not increased (Table 1), there is no evidence at this point for a role of adipose tissue inflammation in the pathogenesis of hepatic insulin resistance and steatosis of the AdiposeENPP1-TG mice. Interestingly, overexpression of adipose ENPP1 but not TNFα was found to be associated with whole body and adipose tissue insulin resistance in nondiabetic humans without diabetes (10).

The adipocyte insulin resistance induced by the ENPP1 overexpression in our model appears to be similar to the adipocyte-specific insulin receptor knockout model (adipose-IRKO or -FIRKO) regarding its effect on adipocyte size (2). Similarly to our model, the adipocytes of FIRKO mice remain small with higher caloric intake. However, FIRKO mice do not develop systemic insulin resistance. An explanation for these different responses to adipocyte insulin resistance can be found in the lack of excessive lipolysis in the FIRKO mice. FIRKO mice have been reported to have adipose tissue uncoupling protein 1 activation and increased energy expenditure during hyperphagia (2, 14). This metabolic response can account for a lack of increased plasma nonesterified fatty acids and determine the different systemic metabolic response to induced adipocyte insulin resistance. Understanding the mechanisms for the different effects of ENPP1-induced vs. insulin receptor knockout-induced insulin resistance in adipocytes may provide important insights into systemic regulation of glucose and lipid metabolism.

Of interest, we did not find increased triglyceride or diacylglycerol deposition, nor did we find any evidence of insulin-signaling defects in skeletal muscle (Fig. 11). These findings suggest that skeletal muscle utilization of glucose is intact and that the dysglycemia of AdiposeENPP1-TG mice is originating mainly from increased hepatic glucose production and decreased glucose uptake in adipose tissue. However, because gene expression studies, including hepatic phosphoenolpyruvate carboxykinase expression, have been thus far unrevealing (data not shown), further studies will be required to quantify hepatic glucose output in fasting conditions and during hyperinsulinemia to elucidate the mechanistic details for hyperglycemia in this model.

The similarities in metabolic changes observed between AdiposeENPP1-TG on a high-fat diet and patients with metabolic syndrome are supportive of a mechanistic role of adipose ENPP1 expression in increasing risk for type 2 diabetes by inducing adipose tissue insulin resistance and increasing fatty infiltration of the liver. As a consequence, more severe manifestations of the metabolic syndrome can be predicted even with mild increases in caloric intake and body fat mass, when ENPP1 is overexpressed in adipose tissue. Clearly, overexpression of ENPP1 in humans with insulin resistance is not exclusive of the adipose tissue, and the mechanisms involved in the ENPP1-related systemic metabolic changes are more complex than in the AdiposeENPP1-TG mouse. For example, decreased insulin receptor phosphorylation in concomitance with increased ENPP1 expression in skeletal muscle is not part of the AdiposeENPP1-TG mouse phenotype but can be found in nondiabetic, nondiabetic, insulin-resistant individuals (9). Regardless, the new animal model we report in this study can help to understand better the role of adipocyte insulin resistance vs. hypertrophy (not present in our model) and also the role of ENPP1 as a biomarker of increased susceptibility to type 2 diabetes and CVD independently of BMI or waist circumference. Our model could ultimately help identify novel intervention strategies to optimize adipocyte response to increased caloric demand and to reduce the risk for systemic metabolic complications of weight gain.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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