Time course of high-fat diet-induced reductions in adipose tissue mitochondrial proteins: potential mechanisms and the relationship to glucose intolerance

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Sutherland LN, Capozzi LC, Turchinsky NJ, Bell RC, Wright DC. Time course of high-fat diet-induced reductions in adipose tissue mitochondrial proteins: potential mechanisms and the relationship to glucose intolerance. Am J Physiol Endocrinol Metab 295: E1076–E1083, 2008. First published September 9, 2008; doi:10.1152/ajpendo.90408.2008.—Increasing evidence suggests that reduced adipose tissue mitochondrial content is associated with the pathogenesis of type 2 diabetes. These investigations have utilized severely insulin-resistant rodent models. Thus, it is difficult to ascertain the potential mechanisms that initiate these changes and whether reductions in adipose mitochondria are an initiating event in the development of impaired glucose homeostasis. Thus, we sought to determine the time course of high-fat diet-induced reductions of mitochondrial content in epididymal adipose tissue in relation to changes in purported mediators of mitochondrial biogenesis and the development of impaired glucose homeostasis. Male Wistar rats were fed a high-fat diet (~59% of kcais from fat) for 2, 4, or 6 wk. Six weeks of high-fat feeding resulted in reductions in CORE I, COX IV, cytochrome c, HSP60, relative mtDNA copy number, and PGC-1α expression. These changes were not associated with decreases in eNOS and AMPK or increases in markers of oxidative stress. Interestingly, ex vivo treatment of adipose tissue cultures with palmitate led to decreases in PGC-1α expression and COX IV and CORE I protein content as observed in vivo. Thus, the high-fat diet-induced reductions in adipose tissue mitochondrial proteins may be mediated by increases in plasma fatty acids. Importantly, reductions in adipose tissue mitochondrial content occurred after the development of impaired glucose homeostasis. Thus, reductions in adipose tissue mitochondrial proteins are most likely not a causal event in the development of impaired glucose homeostasis.

peroxisome proliferator-activated receptor-γ coactivator-1; insulin resistance; obesity; rat; type 2 diabetes

Adipose tissue has classically been viewed as an inert storage depot for excess calories. However, it is now widely recognized that adipose is an active endocrine organ and that derangements in adipose tissue function and morphology are closely associated with impaired glucose homeostasis (24). For instance, mounting evidence has suggested that reductions in adipose tissue mitochondrial content are involved in the pathogenesis of type 2 diabetes. In support of this hypothesis it has been shown that the protein content of mitochondrial respiratory chain enzymes such as, ubiquinone:cytochrome c oxidoreductase core subunit (CORE I), cytochrome c oxidase subunit IV (COX IV), and cytochrome c are reduced in epididymal adipose tissue and adipocytes of genetically modified, insulin-resistant rodents (4, 13, 29, 30). A similar pattern of reduced mitochondrial content has been reported in subcutaneous adipose tissue samples obtained from individuals with type 2 diabetes (1). Interestingly, the insulin sensitizing effects of thiazolidiones (TZDs) are closely matched by robust increases in adipose tissue mitochondrial biogenesis (1, 4, 30).

Reductions in adipose tissue mitochondrial content in insulin-resistant states could likely be the result of decreases in the expression of peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α), a transcriptional coactivator that has been shown to control the expression of mitochondrial genes in white adipocytes (27). For instance, PGC-1α expression is reduced in abdominal adipose tissue from insulin-resistant rodents (29) and in subcutaneous adipose tissue samples from insulin-resistant subjects (12) and individuals with type 2 diabetes (1).

It seems likely that decreases in PGC-1α expression could be a result of reductions in signaling pathways that induce PGC-1α expression and reciprocal increases in the activation of processes that downregulate this transcriptional coactivator. For instance, endothelial nitric oxide synthase (eNOS) (18, 29) and 5′-AMP-activated protein kinase (AMPK) (20, 22) have been linked to the induction of PGC-1α expression in white adipose tissue and the content and/or activity of these proteins are decreased in adipose tissue from severely insulin-resistant rodents (21, 29). On the other hand, it has recently been argued that hyperglycemia, elevations in free fatty acids, and/or the induction of oxidative stress can all lead to decreases in the expression of PGC-1α and reductions in mitochondrial content (2, 7).

The majority of studies that have explored the regulation of adipose tissue mitochondrial content have utilized genetic rodent models of severe insulin resistance. Although these investigations are useful in establishing associations between physiological end points, they are limited by the severe and prolonged insulin resistance present in these animals. Consequently, these studies lend little insight into the cellular events that trigger reductions in adipose tissue mitochondrial content and the relationship of these changes to the development of systemic insulin resistance. Establishing the temporal relationships between these variables will not only allow us to more accurately define the potential regulators of adipose tissue mitochondrial biogenesis in a physiological setting, but more importantly will help to determine whether reductions in adipose tissue mitochondrial content are an early, and perhaps causal, event in the development of impaired glucose ho-
meostasis. Within this context, the purpose of the present investigation was to determine the time course of high-fat diet-induced reductions in adipose tissue mitochondrial content in relation to changes in reputed mediators of mitochondrial biogenesis and the development of impaired glucose homeostasis. Given the close association between adipose tissue mitochondrial content and systemic insulin sensitivity, we hypothesized that reductions in adipose tissue mitochondria would occur prior to, or concurrent with, the onset of impaired glucose tolerance.

**MATERIALS AND METHODS**

**Materials.** Reagents, molecular weight marker, and nitrocellulose membranes for SDS-PAGE were purchased from Bio-Rad (Mississauga, ON, Canada). ECL Plus was a product of Amersham Pharamacia Biotech (Arlington Heights, IL). Antibodies against COX IV and CORE I were purchased from Molecular Probes (Eugene, OR). Phosphospecific (Thr172) AMPKα antibodies were from Cell Signaling (Danvers, MA). An anti-eNOS antibody was purchased from BD Transduction Laboratories (Mississauga, ON, Canada). Antibodies against heat shock protein-60 (HSP60) and cytochrome c were from Stressgen (Victoria, BC, Canada) and Mitosciences (Eugene, OR), respectively. An antibody against β-actin was a product of Sigma (St. Louis, MO). Horseradish peroxidase-conjugated donkey anti-rabbit and goat anti-mouse IgG secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Glucose standards were obtained from NERL Diagnostics (East Providence, RI) and glucose reagents from Diagnostic Chemicals Limited (PEI, Canada). Rat insulin enzyme-linked immunosorbent assay kits (ELISA) kits and adiponectin ELISA kits were purchased from Linco (St. Charles, MO). A nonesterified fatty acid (NEFA) assay kit was a product of Wako Diagnostics (Richmond, VA). RNeasy and DNeasy extraction kits were purchased from Qiagen (Mississauga, ON, Canada). Antibodies for these genes were from Applied Biosystems. Assay kits for thiocyanate were from Integrated DNA Technologies (Coraville, IA), while probes were from Cayman Chemicals (Ann Arbor, MI). All other chemicals were purchased from Sigma.

**Treatment of rats.** All protocols followed Canadian Council on Animal Care guidelines and were approved by the Animal Use and Welfare Committee at the University of Alberta. Male Wistar rats (Charles River, Wilmington, MA) weighing ~200 g were housed two per cage with a 12:12-h light-dark cycle and were provided with water and standard rat chow ad libitum. Rats were acclimated to the animal housing facility for 1 wk prior to the start of the diet manipulation.

Rats continued to receive either standard rat chow or were fed a high-fat diet ad libitum for 2, 4, or 6 wk. The standard chow diet contained 28.9% protein, 13.5% fat, and 58.0% carbohydrates, expressed as percentage of total energy. The high-fat diet contained 21% protein (casein), 59% fat (21.0% corn oil, 37.9% lard), and 20% carbohydrate, expressed as percentage of total energy. The diets were similarly enriched with protein, vitamins, fiber, and minerals.

**Intraperitoneal glucose tolerance tests.** After a 12-h overnight fast, animals received an intraperitoneal injection of glucose (2 g/kg body wt) between 8 AM and 10 AM. Blood was collected into heparinized tubes (Fisher Scientific, Edmonton, AB, Canada) from the tail veins at 0 min (prior to injection) and 15, 30, 45, 60, 90, and 120 min after injection. Whole blood was placed on ice and centrifuged for separation of plasma, and plasma was stored at −20°C until further analysis. Changes in glucose and insulin over time were plotted, and the area under the curve (AUC) was calculated for each. The product of the glucose and insulin AUCs was calculated, as this can be used as a marker of systemic insulin action (6, 26).

**Determination of plasma glucose, insulin, leptin, adiponectin and NEFAs.** Plasma glucose was analyzed using the glucose oxidase method. Samples were analyzed in triplicate on a microplate reader. The average coefficient of variation (CV) for this assay in our laboratory is <5%. Plasma insulin, leptin, and adiponectin were measured using ELISA assay kits specific for rat. Samples were run in triplicate, and the average CVs of these assays are <5%. NEFAs were measured in duplicate on a 96-well plate using a commercially available kit.

**Adipose tissue collection.** Animals were anesthetized with pentabarbital sodium (5 mg/100 g body wt). Epididymal adipose tissue was dissected free of the testes and immediately weighed. A portion was kept for adipose tissue organ culture, and the remainder was clamp frozen in tongs cooled to the temperature of liquid nitrogen and then stored at −80°C until further analysis.

**Adipose tissue organ culture.** Adipose tissue organ culture is a well-characterized technique that has previously been used to study alterations in adipose tissue metabolism and gene expression (9, 10, 14, 28). Under sterile conditions, ~500 mg of epididymal fat from each animal was placed into culture dishes containing 15 ml of M-199 supplemented with 1% antibiotic-antimycotic, 50 μU/ml insulin, and 2.5 nM dexamethasone. The tissue was minced into 1- to 2-mg pieces and placed in an incubator maintained at 37°C with a gas phase of 5% CO2. After 24 h, palmitate complexed to 7.5% BSA, or an equivalent volume of vehicle, was added to a final concentration of 500 μM. To study the effects of prolonged hyperinsulinemia on PGC-1α expression, M-199 was supplemented with 2 μM insulin or vehicle. After a 24-h incubation, the culture medium containing the adipose tissue minces was poured into ice-cold PBS and then filtered to remove the adipose tissue minces for storage at −80°C.

**Western blotting.** Clamp-frozen epididymal fat was homogenized in 2 volumes of ice-cold buffer containing cell lysis buffer (Bio-

Table 1. *Time course of high-fat diet-induced changes in body weight, epididymal fat pad mass, and plasma hormones and metabolites*

<table>
<thead>
<tr>
<th></th>
<th>2 Weeks</th>
<th></th>
<th>4 Weeks</th>
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<th>6 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Chow</td>
<td>High Fat</td>
<td>Chow</td>
<td>High Fat</td>
<td>Chow</td>
<td>High Fat</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>298.2±3.9</td>
<td>318.0±4.9*</td>
<td>367.1±6.2</td>
<td>410.7±8.6*</td>
<td>441.6±0.05</td>
<td>522.6±0.02*</td>
</tr>
<tr>
<td>Fat pad, g</td>
<td>3.0±0.2</td>
<td>5.3±0.3*</td>
<td>4.3±0.3</td>
<td>8.0±0.8*</td>
<td>5.6±0.4</td>
<td>16.9±1.8*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>112.2±2.0</td>
<td>113.6±3.4</td>
<td>106.7±5.6</td>
<td>115.7±5.2</td>
<td>103.1±8.1</td>
<td>122.7±4.3*</td>
</tr>
<tr>
<td>Insulin, μg/l</td>
<td>0.33±0.1</td>
<td>0.53±0.1*</td>
<td>0.45±0.1</td>
<td>1.38±0.3*</td>
<td>0.66±0.4</td>
<td>1.67±0.2*</td>
</tr>
<tr>
<td>Adiponectin, μg/ml</td>
<td>10.3±0.7</td>
<td>11.2±0.7</td>
<td>9.0±1.0</td>
<td>10.7±0.6</td>
<td>14.9±1.2</td>
<td>12.5±1.3</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.62±0.27</td>
<td>2.28±0.36*</td>
<td>0.67±0.17</td>
<td>4.38±0.73*</td>
<td>1.24±0.28</td>
<td>8.31±1.27*</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.28±0.05</td>
<td>0.47±0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8–14 rats per group. NEFA, nonesterified fatty acid; ND, no data available at this time point. Insulin, glucose, leptin, and adiponectin were measured after an overnight fast, whereas NEFA was measured in the fed state. *P < 0.05 vs. chow-fed group at the same time point.
Source) supplemented with protease inhibitor cocktail (Sigma) and phenylmethylsulfonyl fluoride. Homogenized samples were sonicated for 5 s and centrifuged for 15 min at 2,500 g at 4°C. The fat cake was carefully removed, and the protein concentration of the supernatant was determined using the BCA method. The CV for this assay is <5% in our laboratory. The protein content of CORE 1, COX IV, HSP60, eNOS, p-AMPK, and cytochrome c were determined by Western blot analysis as described previously (31, 32). Briefly, equal amounts of protein were separated on either 10% (CORE 1, HSP60, eNOS, p-AMPK) or 15% (COX IV, cytochrome c) gels. Proteins were wet-transferred to nitrocellulose membranes for 90 min at 200 mA/tank. Membranes were blocked in tris-buffered saline-0.01% Tween 20 (TBST) supplemented with 5% nonfat dry milk for 1 h at room temperature with gentle agitation. Membranes were incubated in TBST-5% nonfat dry milk supplemented with appropriate primary antibodies overnight at 4°C with gentle agitation. The following morning, blots were briefly washed in TBST and then incubated in TBST-1% nonfat dry milk supplemented with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Bands were visualized using ECL plus and captured using a Typhoon Imaging system (GE Health Care). Imagequant software was used to quantify relative band intensities. β-Actin was used as an internal control. In preliminary experiments we found that 6 wk of high-fat feeding had no effect on the protein content of β-actin in epididymal adipose tissue (3.21 ± 0.12 chow fed, 3.48 ± 0.23 high-fat fed, arbitrary densitometric units n = 6).

**Real-time PCR.** RNA was isolated from epididymal adipose tissue using an RNeasy kit according to the manufacturer’s instructions. One
mitochondrial DNA copy number. Total DNA was isolated from epididymal adipose tissue by use of a DNeasy kit. Genomic DNA samples were treated with RNase to remove contaminating RNA. Relative mitochondrial DNA (mtDNA) copy number was measured by determining the ratio of a mtDNA target sequence (mitochondrial D-loop) to the expression of a nuclear target sequence (β-actin) by determining the ratio of a mtDNA target sequence (mitochondrial D-loop) to the expression of a nuclear target sequence (β-actin) expression in adipose tissue. Six weeks of high-fat feeding did not result in decreases in AMPK phosphorylation or total protein content. Likewise, eNOS protein in epididymal adipose tissue was not decreased. High-fat feeding for 6 wk did not lead to increases in protein carbonyl formation or MDA, markers of oxidative stress (Fig. 3).

Effect of high-fat feeding on body weight, fat pad mass, and blood measurements. Significant differences in body mass and epididymal fat pad mass between control and high-fat-fed rats were evident at all time points (table 1). Fasting glucose levels were slightly elevated following 6 wk of high-fat feeding. High-fat feeding lead to increases in fasting insulin and leptin levels at all time points examined while having no effect on plasma adiponectin concentrations. NEFA concentrations were elevated in high-fat-fed rats following 6 wk of feeding.

RESULTS

Effect of high-fat feeding on body weight, fat pad mass, and blood measurements. Significant differences in body mass and epididymal fat pad mass between control and high-fat-fed rats were evident at all time points (table 1). Fasting glucose levels were slightly elevated following 6 wk of high-fat feeding. High-fat feeding lead to increases in fasting insulin and leptin levels at all time points examined while having no effect on plasma adiponectin concentrations. NEFA concentrations were elevated in high-fat-fed rats following 6 wk of feeding.

High-fat diet-induced reductions in adipose tissue mitochondrial content. High-fat feeding for 6 wk led to ~30–50% reductions in the protein content of various markers of mitochondrial content, CORE I, COX IV, and cytochrome c in epididymal adipose tissue (Fig. 1). No changes were seen following either 2 or 4 wk of high-fat feeding. In addition to reductions in constituents of the respiratory chain, we saw decreases in the mitochondrial chaperone protein HSP60. Similarly 6 wk of high-fat feeding led to reductions in relative mtDNA copy number and the expression of PGC-1α (Fig. 2).

High-fat diet-induced changes in AMPK signaling, eNOS protein content, and oxidative stress. Having determined the earliest time point in which reductions in adipose tissue mitochondrial content with high-fat feeding occurs, we wanted to determine whether these changes were associated with parallel decreases in AMPK and eNOS, reputed mediators of mitochondrial biogenesis in white adipose tissue. Six weeks of high-fat feeding did not result in decreases in AMPK phosphorylation or total protein content. Likewise, eNOS protein in epididymal adipose tissue was not decreased. High-fat feeding for 6 wk did not lead to increases in protein carbonyl formation or MDA, markers of oxidative stress (Fig. 3).

Palmitate decreases PGC-1α expression in adipose tissue. Epididymal adipose tissue minces were incubated in M-199 supplemented with or without 500 μM palmitate for 24 h. This treatment led to a ~50% reduction in PGC-1α expression. Hyperinsulinemia (24-h incubation with 2 mU/ml insulin) had no effect on PGC-1α expression (data not shown). The palmitate-induced reductions in PGC-1α expression was associated with decreases in COX IV and CORE I protein content (Fig. 4).
Time course of high-fat diet-induced alterations in glucose homeostasis. High-fat feeding led to impairments in glucose clearance and an exaggerated insulin response to an intraperitoneal glucose challenge in rats fed a high-fat diet for 4 and 6 wk (Fig. 5). The glucose-insulin AUC index (integrated AUC) can be used as a surrogate of whole body insulin action (6, 26) with increases in this value representing a worsening of systemic insulin action. As seen in Fig. 3. High-fat feeding does not result in decreases in AMPK phosphorylation (A), AMPKα protein content (B), endothelial NO synthase (eNOS) protein content (C), or increases in thiobarbituric acid-reactive substances (TBARS; D) and lipid peroxides (E) in rat epididymal adipose tissue. Data are presented as means ± SE for 6–11 samples per group. For Western blot data, high-fat-fed values are expressed relative to chow-fed control values at the same time point. Representative blots are shown for each protein of interest and for β-actin, used as an internal loading control.
DISCUSSION

Adipose tissue is increasingly being recognized as an important player in the regulation of whole body glucose metabolism. Of interest to the study of type 2 diabetes, a strong association between reductions in adipose tissue mitochondrial content and systemic insulin resistance has been reported. Data from these investigations have been generated primarily using adipose tissue or adipocytes from genetically modified rodents such as db/db mice and fa/fa rats (4, 13, 29, 30). Unfortunately, due to the marked insulin resistance and hyperglycemia present in these animals, it is difficult to identify the specific mechanisms that trigger reductions in adipose tissue mitochondrial content and whether decreases in adipose tissue mitochondria are a potential initiating event in the pathogenesis of systemic insulin resistance and glucose intolerance. With these points in mind, we sought to determine how quickly reductions in adipose tissue mitochondrial content occurs with high-fat feeding, a common approach used to cause abdominal obesity and insulin resistance in rodents.

Compared with chow-fed controls, 6 wk of high-fat feeding led to ∼30–50% reductions in the content of mitochondrial respiratory-chain proteins CORE I, COX IV, and cytochrome c.

Fig. 5. High-fat feeding causes impaired glucose tolerance and reductions in whole body insulin action. Area under the curve (AUC) calculations for the glucose and insulin responses to an intraperitoneal glucose tolerance test following 2, 4, or 6 wk of high-fat feeding are shown for 3–8 animals per group. The glucose-insulin AUC index was used as a marker of whole body insulin action and is the product of the glucose and insulin AUCs. *P < 0.05 vs. the corresponding chow-fed value at the same time point.

Fig. 4. Palmitate decreases the expression of PPARγ coactivator-1α (PGC-1α; A) and mitochondrial proteins (B) in epididymal adipose tissue ex vivo. Data are presented as means ± SE for 4–6 samples per group. For Western blot data, representative blots are shown for each protein of interest and for β-actin, used as an internal loading control. *P < 0.05.

5C, high-fat feeding led to marked increases in the glucose-insulin AUC index.
Likewise, we found similar reductions in mtHSP60, a chaperone protein involved in mitochondrial protein folding that has been previously shown to be decreased in epididymal adipose tissue from ob/ob (30) and db/db mice (19). Confirming our Western blot data, we found reductions in relative mtDNA copy number, a marker of the number of mitochondria per cell. Importantly, our data demonstrate that 6 wk of high-fat feeding is the earliest time point in which reductions in adipose tissue mitochondrial protein content is evident. The magnitude of this reduction is similar to what has been reported in db/db mice and fa/fa rats (4, 13, 29, 30), yet develops in the absence of prolonged hyperglycemia and hyperinsulinemia.

PGC-1α is a transcriptional coactivator and master regulator of mitochondrial biogenesis (8). Reductions in PGC-1α expression have been reported in insulin-resistant rodents (23, 29) and in obese and type 2 diabetic subjects (1, 25). Likewise, the induction of mitochondrial biogenesis in adipose tissue by thiazolidinediones is accompanied by increases in PGC-1α expression (1, 30). In accord with these findings, we found decreases in PGC-1α expression concurrent with reductions in adipose tissue mitochondrial marker proteins. These results are temporally consistent with the notion that reductions in PGC-1α expression could be involved in mediating high-fat diet-induced decreases in adipose tissue mitochondrial proteins.

The regulation of PGC-1α expression and mitochondrial biogenesis in adipose tissue is a complex process involving multiple factors. In models of severe insulin resistance, positive effectors of mitochondrial biogenesis in adipose tissue such as AMPK (21) and eNOS (29) are reduced. On the other hand negative modulators of mitochondrial content (2), such as oxidative stress, are increased in adipose tissue from diabetic mice (11). In the current investigation, we assessed whether changes in these variables occurred concurrently with the initial reductions in markers of mitochondrial content caused by high-fat feeding. Following 6 wk of high-fat feeding, a time point in which markers of mitochondrial content were reduced, AMPK phosphorylation and eNOS content were unchanged. Consistent with a recent report in high-fat-fed mice (16), we did not find any differences in protein carbonyl or MDA content in adipose tissue from high-fat-fed rats. Collectively our results suggest that alterations in AMPK, eNOS, and oxidative stress are not initiating events in the process mediating decreases in adipose tissue mitochondrial protein content.

Given these results, we sought to identify potential mechanisms that could help explain high-fat diet-induced reductions in adipose tissue mitochondrial content. In our high-fat feeding model rats maintain normal glucose levels but are both hyperinsulinemic and hyperlipidemic. Given the fact that palmitate has previously been shown to reduce the expression of PGC-1α in skeletal muscle cells (5, 7), we wanted to determine if the ex vivo treatment of rat adipose tissue with palmitate could reproduce the effects of high-fat feeding on adipose tissue PGC-1α expression and markers of mitochondrial content. We found that palmitate, but not high insulin levels, led to reductions in PGC-1α expression and COX IV and CORE I protein content. These findings, in combination with the observed increases in NEFA levels following high-fat feeding, suggest a potential causal role for fatty acids in the downregulation of adipose tissue PGC-1α expression and mitochondrial content.

To determine the relevance of the observed reductions in adipose tissue mitochondrial proteins to the development of insulin resistance, we measured changes in glucose homeostasis following 2, 4, and 6 wk of high-fat feeding. Contrary to our hypothesis, yet consistent with what has been reported in skeletal muscle (2), we found that reductions in adipose tissue mitochondrial content occurred after the development of high-fat diet-induced insulin resistance. These findings are temporally inconsistent with the notion that reductions in adipose tissue mitochondrial content are a causal, initiating event in the development of impaired glucose homeostasis. Rather, the results argue that reductions in adipose tissue mitochondrial function, independent of changes in mitochondrial content, could precede and be linked to the development of systemic insulin resistance. Furthermore, our results do not rule out the possibility that reductions in adipose tissue mitochondrial content may be associated with a further worsening of systemic insulin resistance.

This investigation is the first to characterize the temporal relationships between reductions in adipose tissue mitochondrial content, the potential mediators of this process, and changes in whole body glucose homeostasis in a model of diet-induced obesity. We found that reductions in adipose tissue mitochondrial content are associated with reductions in PGC-1α expression and that these effects can be reproduced ex vivo by treating adipose tissue organ cultures with palmitate. The most important finding of this study is that alterations in PGC-1α and mitochondrial markers occurred after the onset of impaired glucose homeostasis. Although the present findings in rat epididymal fat pads cannot necessarily be extrapolated to visceral fat depots in humans, our results argue against reductions in adipose tissue mitochondrial content being an initiating event in the development of impaired glucose homeostasis.

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