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Diet-Induced Obesity Causes Insulin Resistance in Mouse Brown Adipose Tissue

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Abstract

Objective—Diet-induced obesity (DIO) causes several pathophysiological changes in adipose tissue. Increased inflammation reduces white adipose tissue (WAT) insulin sensitivity and contributes to the development of diabetes. However, little is known about how DIO alters the function of brown adipose tissue (BAT), an organ that consumes calories by β 3-adrenergic receptor (AR)-mediated thermogenesis and helps regulate energy balance.

Methods—To test the effects of DIO on BAT, we fed 6 week-old C57BL/6 mice either a normal chow diet (NCD) or a high-fat diet (HFD). After 16 additional weeks, we measured body fat, WAT and BAT mRNA expression, glucose tolerance, and rates of glucose uptake in response to insulin and the β 3-AR agonist mirabegron.

Results—Compared with NCD, HFD increased body fat and impaired glucose tolerance. Both WAT and BAT had higher mRNA levels of markers of inflammation, including TNF α and F4/80. Insulin signaling in BAT and WAT was reduced, with decreased Akt phosphorylation. Diet-normalized BAT glucose uptake rates were lower in response to mirabegron.

Conclusions—These results support a model in which DIO leads to BAT inflammation and insulin resistance, leading to a broader impairment of BAT function.

Keywords

Brown Adipose Tissue; Insulin resistance; Obesity; β 3-Adrenergic Receptor Agonist

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Introduction

There are two distinct functions for adipose tissue. White adipose tissue (WAT) stores energy, while brown adipose tissue (BAT) and the related beige adipocytes contribute to energy expenditure through activation of the thermogenic tissue-specific uncoupling protein 1 (UCP1) (1). When energy intake exceeds energy expenditure, WAT accumulates and can eventually lead to obesity. Diet-induced obesity (DIO) has been consistently shown to cause resistance to insulin-mediated glucose uptake in liver, muscle, and WAT (2–5). Preliminary evidence suggests that this process may also occur in BAT (6), but this process has not been demonstrated at the molecular level. Recently it was discovered that a β 3-adrenergic receptor (AR) agonist can stimulate human BAT (7), making it a potential therapeutic treatment for obesity. It is therefore critical to determine the physiological parameters affecting signal transduction underlying BAT thermogenesis. In this study, we used a mouse model to determine if DIO causes BAT insulin resistance at the molecular level. We then assessed whether DIO affects the ability of insulin or a β 3-AR agonist to stimulate BAT glucose uptake.

Methods

Mice and diets

Animal care and study protocols were approved by the Animal Care Committee of the Joslin Diabetes Center (JDC). Beginning at 6 weeks of age, C57BL/6J male mice from Jackson Labs were fed either chow diet (Research Diet Inc, 10% kcal fat, 70% carbohydrate) or high-fat diet (HFD) (Research Diets Inc., 60% kcal fat, 20% carbohydrate). At 16 weeks, mice were transferred to JDC and maintained on either normal chow diet (NCD), with 21% kcal from fat, or HFD, with 60% kcal from fat. All mice had free access to food and water and were kept in temperature-controlled facilities at 23°C on a 12-hour light/dark cycle for 8 weeks. Body composition was measured at 22 weeks of age by DXA (GE Lunar PIXImus 2 Series Densitometer).

Immunoblotting and Histology

Immunoblotting was carried out as described (8) with antibodies listed in Table S1. Densitometry of the bands was quantified using ImageJ software. Interscapular BAT (iBAT) and perigonadal WAT (pgWAT) were fixed in Bouin's fixative, embedded in paraffin, and stained with hematoxylin and eosin at the Harvard Medical School Histology Core (<http://www.dfhcc.harvard.edu/core-facilities/rodent-histopathology-pathology/>).

Quantitative RT-PCR

RNA was extracted from iBAT and pgWAT tissue using Qiazol and RNeasy mini-kit following manufacturer's protocols (Qiagen) and reverse transcribed using High Capacity cDNA kit (Applied Biosystems). 2.5 μ L of 4 ng/ μ L cDNA was used in a 10 μ L reaction with intercalating SYBR Green dye (Bio-Rad) and quantified using the ABI Prism 7900 sequence detection system. Values were normalized to TATA-binding protein (TBP). Primers (IDT) are listed in Table S2.

Insulin Tolerance and Glucose Uptake Rate Assays

A separate group of 36 mice was raised as above. At age 26 weeks, mice were fasted for four hours and then anesthetized with 0.3 $\mu\text{g}/\mu\text{L}$ Avertin (2,2,2-tribromoethanol)(Sigma-Aldrich) dissolved in PBS, at 10 $\mu\text{L}/\text{g}$. Mice were given a retro-orbital injection of 2-deoxy-D-[1,2- $\text{C}^{14}(\text{N})$]glucose (10 $\mu\text{Ci}/30\text{g}$ of body weight) as previously described (8) with one of three treatments: 1 $\mu\text{L}/\text{g}$ body weight of 10% DMSO/saline (vehicle), 0.75 mU/g body weight of insulin diluted in vehicle, or 0.1 $\mu\text{g}/\text{g}$ body weight mirabegron (Santa Cruz) dissolved in vehicle. Mice were sacrificed by cervical dislocation, and iBAT, pgWAT, inguinal WAT (ingWAT), and skeletal muscle (gastrocnemius) were resected. Tissues were snap-frozen in liquid nitrogen and stored at -80°C . The amount of glucose radiotracer was determined as described (9).

Statistical Analyses

Data are expressed as the mean \pm SEM and were compared using independent *t*-tests or two-way ANOVA, with *P* values <0.05 considered statistically significant.

Results

DIO changed iBAT lipid content and mRNA expression

Compared to the NCD, mice fed 16 weeks of a HFD had higher body weight, fat percentage, and impaired fasting glucose and glucose tolerance (Figure S1a–d). In particular, the iBAT from HFD mice was larger, both in absolute terms, and when normalized to body weight (Figure 1a–b). At the cellular level, pgWAT from DIO mice showed adipocytes that were larger and had an abundance of crown-like structures consistent with macrophage infiltration, and the brown adipocytes had larger lipid droplets (Figure 1c–f).

The histological changes were corroborated by mRNA expression profiling. Leptin, a measure of white adipocyte triglyceride stores, was expressed ten-fold higher in the pgWAT compared to iBAT, and DIO led to higher levels in both tissues, reflecting a shift toward a white adipocyte-like phenotype in iBAT (Figure 1g). Expression of UCP1, the marker of brown adipocytes, was several orders of magnitude higher in iBAT compared to pgWAT, and DIO led to higher expression in pgWAT but not iBAT (Figure 1h). In both pgWAT and iBAT, DIO produced higher expression levels of the macrophage markers F4/80 and CD68 (Figure 1i and S2) as well as the inflammatory cytokines genes $\text{TNF}\alpha$, IL6, and MCP1 (Figure 1j–l). For all five markers of inflammation, the relative expression levels were higher in pgWAT compared with iBAT by 10- to 100-fold.

HFD impaired iBAT insulin signaling

In mice fed a NCD, both the pgWAT and iBAT demonstrated insulin dose-dependent increases in phosphorylation of Akt and ERK, two kinases central to insulin receptor signal transduction, with Akt responsible for glucose uptake (10). In response to HFD, insulin signaling through both Akt and ERK was significantly blunted in iBAT (Figure 2a–b).

A different set of mice fed NCD or HFD for 16 weeks were fasted for 4 hours and then injected with ^{14}C -deoxy-D-glucose mixed with vehicle, insulin, or mirabegron, a $\beta 3$ -AR

agonist. Thirty minutes after injection with either vehicle or mirabegron, there was no change in blood glucose levels, but they were significantly lower with administration of insulin (Figure 2c). DIO did not affect the change in blood glucose after injection with vehicle or mirabegron, but insulin was not as effective at lowering blood glucose in HFD-treated mice (Figure 2d).

To quantify the effect of DIO on the specific insulin- and β 3-AR agonist-responsive tissues, we also measured the rates of glucose uptake in iBAT, pgWAT, ingWAT, and skeletal muscle after treatment with vehicle, insulin, and mirabegron. Rates were higher in response to insulin in all four tissues, but the β 3-AR agonist mirabegron significantly increased glucose uptake only in iBAT (Figure 3a–d). To compare the effects of diet on the fold change of insulin- and mirabegron-mediated glucose uptake, we normalized the rates in each diet to what was seen in the mice treated with vehicle. In all four tissues, DIO reduced the rates of glucose uptake, but they were significantly lower only in the pgWAT ($P=0.02$) and skeletal muscle ($P=0.05$). In contrast, mirabegron-induced glucose uptake was impaired only in iBAT ($P=0.01$) (Figure S3a–d). Thus, iBAT was comparatively resistant to DIO-associated decreases in insulin-mediated glucose uptake.

Discussion

Despite the extensive body of work showing how DIO produces insulin resistance in liver, muscle, and WAT, very little is known about its effects on BAT. Given the multiple roles that human BAT may have in regulating energy balance and metabolism (11, 12), it has become essential that we better understand its unique molecular physiology. In this study, we found that DIO causes resistance to insulin signaling in both of the principal PI3K and ERK signaling pathways, and our findings suggest the process was mediated by inflammation within the BAT itself.

Recently it was shown that DIO increased WAT inflammation, but BAT was resistant to macrophage infiltration (13), suggesting that BAT may also be comparatively resistant to DIO-mediated insulin resistance. Our results are in fact consistent with these findings; specifically, expression of inflammatory markers was 10–100 fold lower in iBAT compared to pgWAT, regardless of diet. In this study, the mice consumed a higher percentage of dietary fat for a longer duration, which led to higher fasting glucose levels and a greater degree of glucose intolerance. Others have reported similar findings to ours in the setting of HFD with the same percentage of dietary fat (14). Thus, our results demonstrate that iBAT is indeed more resistant to DIO-induced inflammation than WAT depots, but inflammatory changes eventually are evident in iBAT when subjected to a higher burden of fat calories.

A limitation of this study is that while DIO led to insulin resistance in iBAT, we do not yet know the entire signaling pathway by which it occurred. Potential sources include both direct and indirect effects of inflammatory mediators on the insulin-signaling pathway (15, 16), and future studies should be directed toward defining the underlying processes and the potential effects on energy homeostasis. In addition, our data suggest that DIO may also impair β 3-AR signaling (10, 17). If indeed β 3-AR signaling is impaired in iBAT in response to DIO, this could reduce thermogenesis as has been implicated in human models of BAT

function (18, 19). A more pronounced effect may be seen with lower doses of mirabegron, and a dose-response assessment will better-characterize the β 3-AR effect.

In summary, we show that DIO causes BAT insulin resistance and reduced glucose uptake in a mouse model, which is associated with increased tissue inflammation. DIO-induced changes in BAT may also reduce adrenergic signaling, which could impair its ability to help prevent obesity and metabolic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Importance Questions

What is already known about this subject?

- Diet induced obesity (DIO) causes inflammation and insulin resistance in muscle, liver, and white adipose tissue, but little is known about its effect on brown adipose tissue (BAT).
- Mirabegron is the first β 3-adrenergic receptor (AR) agonist found to activate human BAT.

What does your study add?

- DIO causes insulin resistance in BAT.
- The insulin resistance is likely from increased BAT inflammation.
- Insulin resistance in BAT may also be accompanied by reduced β 3-AR-mediated glucose uptake, which suggests that DIO-induced insulin resistance in BAT leads to a broader impairment of BAT function.

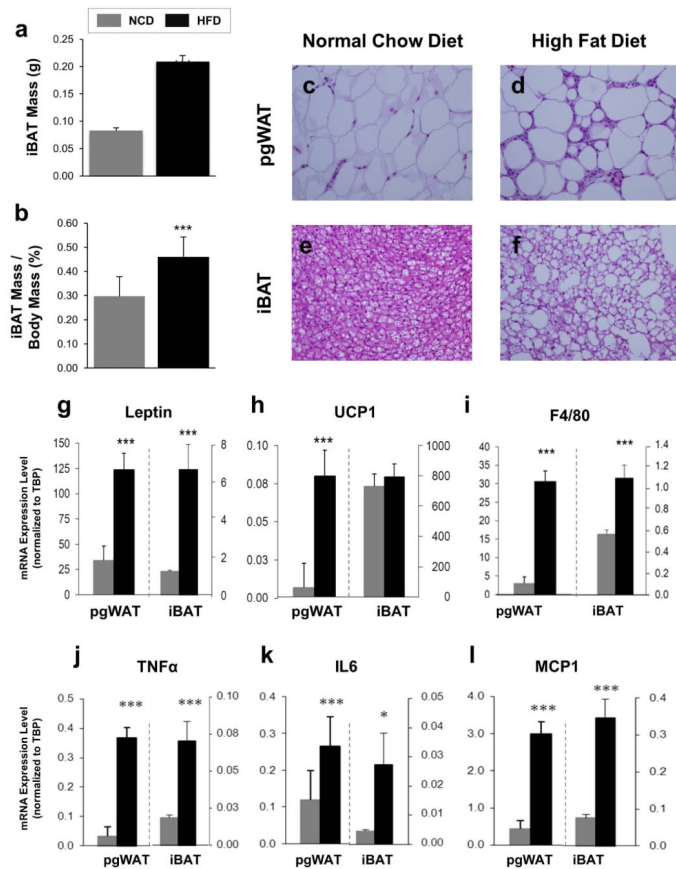


Figure 1. Effects of DIO on pgWAT and iBAT Anatomy and mRNA Expression

(a) iBAT mass was recorded after sacrifice. (b) iBAT mass was normalized by dividing by whole body mass. Hematoxylin and eosin stained sections of perigonadal WAT from mice who ate (c) NCD or (d) HFD and iBAT from mice who ate (e) NCD or (f) HFD; shown at 400X magnification. mRNA expression analysis was performed on pgWAT and iBAT taken from C57BL/6J male mice fed NCD or HFD for 18 weeks. Expression levels are shown as averages normalized to TBP for the following genes (g) leptin; (h) UCP1; (i) F4/80; (j) TNF α ; (k) IL6; (l) MCP1. n=12 per group. * $P < 0.05$, *** $P < 0.001$.

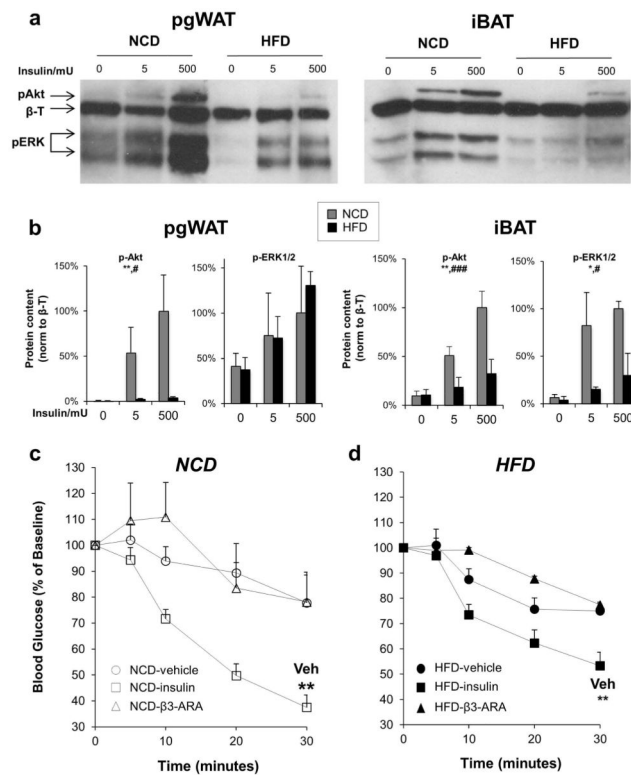


Figure 2. High fat diet reduces insulin signaling in pgWAT and iBAT

(a) Representative immunoblots of phospho-Akt (S473) and phospho-ERK in pgWAT and iBAT from mice on NCD or HFD for 18 weeks treated via IVC injection with vehicle, 5 mU insulin, or 500 mU insulin for 5 minutes before tissue harvest. β-Tubulin (β-T) was used as a loading control. Data shown are representative of three independent experiments. (b) Densitometry of p-Akt (n=4) and p-ERK (n=2). Two-way ANOVA $*P < 0.05$, $**P < 0.01$ for comparing NCD and HFD, and $\#P < 0.05$, $###P < 0.001$, for comparing insulin doses. Blood glucose levels were determined after injection of vehicle (circles), 0.75 mU/kg insulin (squares), 0.1 μg/g body weight of the β3-adrenergic agonist mirabegron (triangles) in mice fed (c) NCD (white symbols) or (d) HFD (black symbols). n=5–6 per treatment. $**P < 0.01$, compared with vehicle.

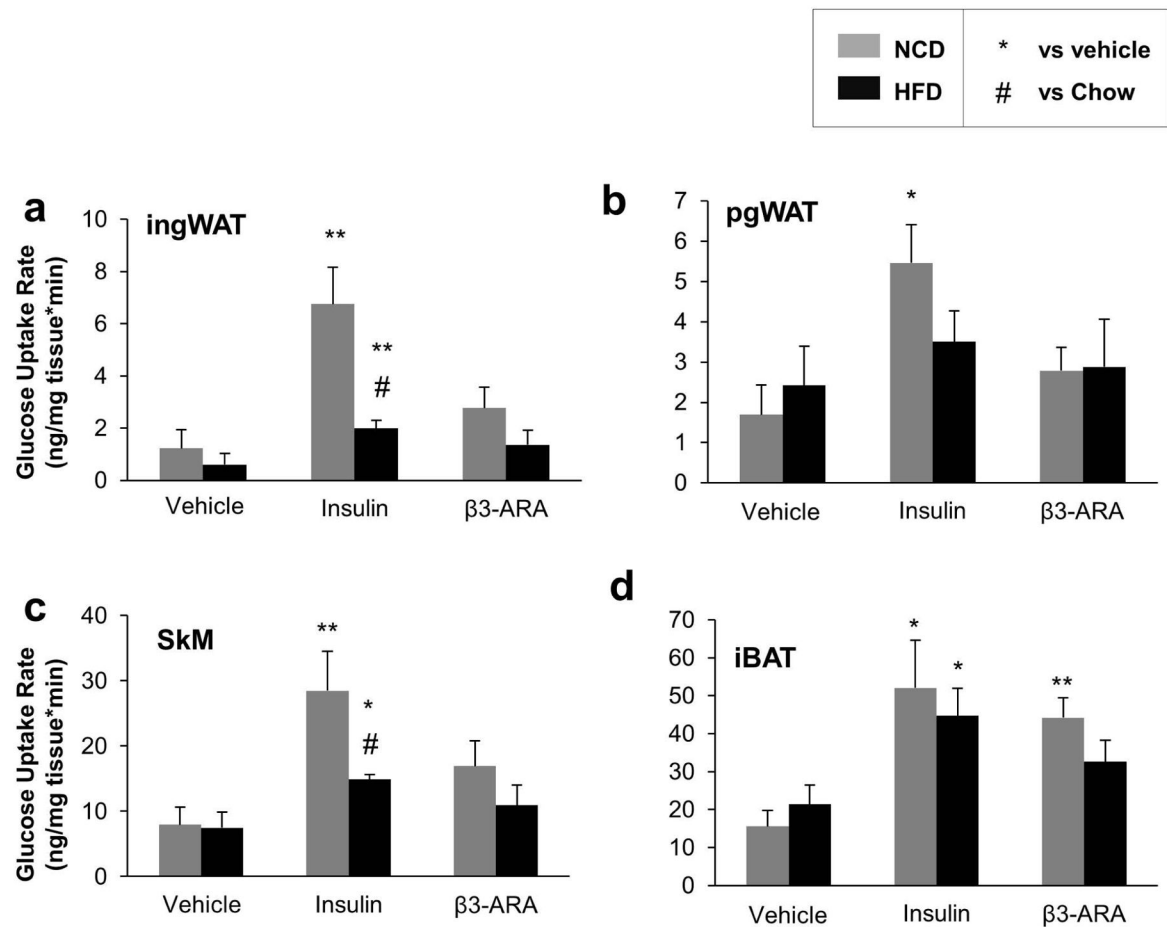


Figure 3. Tissue Glucose Uptake Rates in Mice Fed NCD or HFD

Rates of ^{14}C -2-deoxy glucose uptake were determined in (a) ingWAT, (b) pgWAT, (c) skeletal muscle (SkM), and (d) iBAT after a retro-orbital injection of tracer with one of three treatments: vehicle, 0.75 mU/kg insulin, or 0.1 $\mu\text{g/g}$ body weight of the β 3-adrenergic agonist mirabegron (β 3-ARA). $n=5-6$ per group. * $P < 0.05$, ** $P < 0.01$ vs. vehicle.