Palmitate acutely induces insulin resistance in isolated muscle from obese but not lean humans

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STUDY 31

Introduction

This study investigates how saturated fat differently affects overweight and lean individuals' insulin sensitivity.

Conclusions

This study shows impaired insulin sensitivity from saturated fat exposure to muscle cells derived from overweight individuals, but does not show the same effects in leaner individuals.

Amendments

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Palmitate acutely induces insulin resistance in isolated muscle from obese but not lean humans

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Thrush AB, Heigenhauser GJ, Mullen KL, Wright DC, Dyck DJ. Palmitate acutely induces insulin resistance in isolated muscle from obese but not lean humans. *Am J Physiol Regul Integr Comp Physiol* 294: R1205–R1212, 2008. First published February 27, 2008; doi:10.1152/ajpregu.00909.2007.—Exposure to high fatty acids (FAs) induces whole body and skeletal muscle insulin resistance. The globular form of the adipokine, adiponectin (gAd), stimulates FA oxidation and improves insulin sensitivity; however, its ability to prevent lipid-induced insulin resistance in humans has not been tested. The purpose of this study was to determine *1*) whether acute (4 h) exposure to 2 mM palmitate would impair insulin signaling and glucose transport in isolated human skeletal muscle, *2*) whether muscle from obese humans is more susceptible to the effects of palmitate, and *3*) whether the presence of 2 mM palmitate + 2.5 μg/ml gAd (P+gAd) could prevent the effects of palmitate. Insulin-stimulated (10 mU/ml) glucose transport was not different, relative to control, following exposure to palmitate (−10%) or P+gAd (−3%) in lean muscle. In obese muscle, the absolute increase in glucose transport from basal to insulin-stimulated conditions was significantly decreased following palmitate (−55%) and P+gAd (−36%) exposure (control vs. palmitate; control vs. P+gAd, $P < 0.05$). There was no difference in the absolute increase in glucose transport between palmitate and P+gAd, indicating that in the presence of palmitate, gAd did not improve glucose transport. The palmitate-induced reduction in insulin-stimulated glucose transport in muscle from obese individuals may have been due to reduced Ser Akt (control vs. palmitate; P+gAd, $P < 0.05$) and Akt substrate 160 (AS160) phosphorylation (control vs. palmitate; P+gAd, $P < 0.05$). FA oxidation was significantly increased in muscle of lean and obese individuals in the presence of gAd ($P < 0.05$), suggesting that the stimulatory effects of gAd on FA oxidation may not be sufficient to entirely prevent palmitate-induced insulin resistance in obese muscle.

fatty acids; glucose transport; fat oxidation; adiponectin receptor isoform 1; adiponectin; diabetes

IT IS WIDELY CONSIDERED THAT abnormal muscle fatty acid (FA) metabolism, leading to the accumulation of intramuscular lipids, may be an important factor in the development of insulin resistance. Recent research suggests that reactive lipid species, such as long-chain fatty acyl-CoA, diacylglycerol, and ceramide, play a causative role in the progression of insulin resistance (9, 10, 34). Acutely, the infusion of a lipid emulsion for several hours (4, 13, 16, 34) can induce whole body insulin resistance. Exposure to high circulating FA for 4–6 h can impair skeletal muscle insulin signaling in rodents (34) and humans (16) and ex vivo in isolated rodent soleus muscle (1, 27). However, we are unaware of any study that has examined the direct effects of FA on insulin-signaling and glucose transport in an ex vivo isolated human muscle preparation, thereby avoiding the systemic effects associated with lipid infusion. The metabolic effects of high FA on skeletal muscle have also not yet been compared between lean and obese humans.

Adiponectin is a 30-kDa adipokine that improves insulin sensitivity by affecting liver and skeletal muscle glucose and lipid metabolism (11, 29, 32). Adiponectin circulates in plasma primarily in a high molecular weight multimeric form, and its concentration is reduced in obesity (14). However, it is the truncated globular form of adiponectin (gAd) that has potent metabolic effects in skeletal muscle (11, 29, 33). Skeletal muscle expresses predominantly the adiponectin receptor isoform 1 (AdipoR1) to which gAd specifically binds (31). The regulation of AdipoR1 expression in human muscle is controversial; mRNA content has been demonstrated to be both unchanged (23) and increased in obesity (3, 11) and decreased in individuals with a family history of type 2 diabetes (12). However, AdipoR1 protein content has not yet been compared in skeletal muscle of lean and obese humans.

gAd elicits its insulin-sensitizing effects through increased insulin-stimulated glucose uptake and FA oxidation (7, 32) and increased GLUT4 translocation (8) in skeletal muscle. These effects are likely mediated, in part, through the phosphorylation/activation of AMP-activated protein kinase (AMPK) (11, 29, 32). The stimulatory effects of gAd on AMPK activation are blunted in muscle from obese humans with concomitant reductions in the stimulation of FA oxidation and glucose transport (7, 11). It is uncertain whether the insulin-sensitizing effects of gAd are impaired in the presence of high palmitate or whether gAd can prevent palmitate-induced insulin resistance.

The objectives of the present study were to determine whether *1*) 4 h of exposure to high concentrations of palmitate would impair insulin signaling, glucose transport, and FA oxidation in isolated human rectus abdominus muscle; *2*) whether muscle derived from obese subjects was more susceptible to FA-induced insulin resistance than from lean subjects; *3*) whether the presence of gAd in the incubation medium could prevent/blunt the insulin desensitizing effects of palmitate; and *4*) finally, AdipoR1 protein content was measured in muscles from lean and obese subjects, as this had not previously been reported and could potentially explain differences in gAd action in skeletal muscle from lean and obese humans.

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Intro

Intro: Abnormal muscle fat metabolism (within the muscle cells) leads to accumulation of fats within the muscle cells, it is believed, and this contributes to insulin resistance. Labs have indicated that a number of different types of fat molecules (like diacylglycerols and ceramides) play a causative role in insulin resistance. Exposure to fats by blood infusion over hours or days leads to reduced insulin sensitivity (greater insulin resistance). High circulating free fats in the blood stream increases insulin resistance in animal models, as well as in humans.

Adiponectin is a hormone released by fat cells and improves insulin sensitivity (reducing insulin resistance) in muscle and in liver (by increasing fat oxidation and glucose uptake). It usually circulates in a globular form and is then truncated/cut to an active form, which can affect muscle by binding the adiponectin receptor. Adiponectin increases insulin sensitivity by increasing insulin stimulated glucose (blood sugar) uptake into the cells - this is possible through the activation of the AMPK molecule. Adiponectin's ability to activate AMPK is reduced in obese individuals.

We hypothesized that palmitate would induce insulin resistance to a greater degree in muscle from obese individuals and that this would be at least partially corrected by gAd. We also hypothesized that AdipoR1 protein content would be reduced in skeletal muscle from obese individuals and that this may, in part, explain the reduced responsiveness to gAd in obese muscle.

METHODS

Subjects. Nineteen lean (body mass index <26 kg/m^2, mean 24.0 $\pm$ 0.6 kg/m^2) and 16 obese (body mass index >29 kg/m^2, mean 35.4 $\pm$ 2.1 kg/m^2) women participated in this study. Subjects were admitted to McMaster Medical Centre in Hamilton, ON, for abdominal hysterectomies or other abdominal surgeries. Subjects gave informed written consent prior to participating in this study. This study adhered to the principles of the declaration of Helsinki Code and was approved by McMaster University and University of Guelph ethics committees. All subjects had maintained the same body mass for the past year, were nondiabetic, nonsmokers, and sedentary. Subjects were screened for any medications (e.g., to control blood pressure or lipids, glucose tolerance, etc.) or diseases that might interfere with the results of the study.

Sample preparation. Following an overnight fast (12–18 h) general anesthesia was induced with a short-acting barbiturate and maintained by a fentanyl and rocuronium volatile anesthetic mixture. Venous blood samples were collected in a 5-ml sodium-heparinized tube during anesthesia. A skeletal muscle biopsy ($3 \times 2 \times 1$ cm) from the rectus abdominus was excised at resting length perpendicular to the direction of the muscle fibers and placed immediately into gassed, modified, ice-cold Krebs-Henseleit buffer (KHB) containing 8 mmol/l glucose for transport to the laboratory (~5 min). In the same ice-cold buffer solution, 6–11 muscle strips weighing ~25 mg were separated from the muscle sample and clamped at resting length. Any additional tissue not used for incubations was immediately frozen in liquid nitrogen and stored for the analysis of AdipoR1.

The experimental protocol is shown in Fig. 1. Muscle samples were placed immediately in gassed (95%O_2-5% CO_2) preincubation KHB containing 8 mmol/l glucose and 4% BSA (FA free), for 20 min in a shaking water bath at 30°C. Muscle samples were then transferred into incubation buffers of control (same as preincubation buffer, control); 2 mM palmitate or 2 mM palmitate + 2.5 μg/ml gAd (P+gAd; recombinant human gAd; cat. no. 450-21; Peprotech, Ottawa, ON, Canada) and incubated for 4 h. This concentration of palmitate is the upper physiological limit observed in humans and can induce insulin resistance in 4–6 h in isolated rodent muscle (1, 27). The supraphysiological concentration of gAd used has previously been shown to evoke a significant increase in glucose transport and FA metabolism in isolated rodent and human muscle (7, 29, 32). Incubations were conducted in 20-ml glass scintillation vials, and all buffers were pregassed and maintained at a constant temperature of 30°C in a shaking water bath. Muscles were regassed after 2 h of incubation. A low concentration of insulin was included in all incubation (control, palmitate, P+gAd) and experimental (basal and insulin-stimulated glucose transport, FA oxidation, insulin signaling, muscle viability) buffers (14.3 μU/ml) to maintain viability (30).

Muscle viability. In preliminary experiments, ATP and phosphocreatine contents were measured spectrophotometrically (2) to confirm muscle viability (7). Muscle samples were frozen in liquid nitrogen immediately following excision (0 min) or following 4 h of incubation. Muscle ATP (0 min, 20.8 $\pm$ 1.7; 4 h, 18.3 $\pm$ 1.7 μmol/g dry wt) and phosphocreatine (0 min, 56.0 $\pm$ 2.3; 4 h, 52.4 $\pm$ 5.4 μmol/g dry wt) content was maintained throughout the incubation.

Glucose transport. Six muscle strips were used to measure basal (without insulin) and insulin-stimulated (insulin, 10 mU/ml) glucose (3-*O*-methylglucose) transport, following each of the three conditions (control, palmitate, P+gAd). The procedure for assessing glucose transport has previously been described in more detail (24, 30). Briefly, following the initial 4-h incubation, muscle strips were transferred into 2 ml of pregassed KHB containing 8 mM glucose and 32 mM mannitol in the absence or presence of insulin (10 mU/ml; maintained in all subsequent steps) in a gently shaking water bath. Muscle strips were washed (2 × 10 min) with glucose-free KHB containing 4 mM pyruvate and 36 mM mannitol. Muscle strips were subsequently incubated for 20 min (insulin) or 40 min (basal) in KHB containing 4 mM pyruvate, 8 mM 3-*O*-[^{3}H]methyl-D-glucose (0.8 μCi/ml) and 32 mM [^{14}C]mannitol (0.3 μCi/ml). Glucose transport was determined as the accumulation of intracellular 3-*O*-[^{3}H]methyl-D-glucose.

FA metabolism. FA metabolism was assessed during the final hour of the 4 h incubation period. Briefly, muscles incubated in either palmitate or P+gAd were transferred to a 20-ml glass scintillation vial containing 2 ml of the same incubation buffer, but with the addition of 0.5 μCi/ml of [1-^{14}C]palmitate (Amersham, Oakville, ON, Canada) to measure exogenous palmitate oxidation and the incorporation of palmitate into endogenous lipid pools as previously described (25).

Western blot analysis. Following 4 h of incubation in either control, palmitate, or P+gAd, muscle samples were transferred to a vial containing the same incubation buffer with the addition of 10 mU/ml of insulin for 10 min to measure phosphorylation and/or protein content with insulin stimulation. Muscle samples were then blotted, cut free of clamps, weighed, and immediately frozen in liquid N_2 until further analysis. Akt phosphorylated at Ser473 (Ser Akt; Santa Cruz Biotechnology, Santa Cruz, CA), Akt phosphorylated at the Thr308

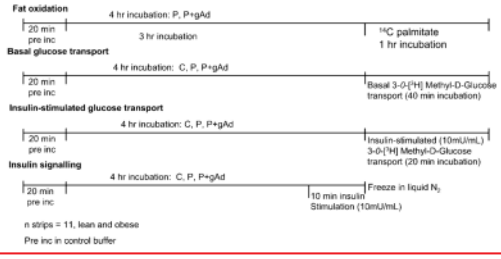

Fig. 1. Experimental protocol. C, control, P, palmitate; gAd, globular adiponectin; pre-inc, preincubation.

Study Design/Figure 1

Researchers recruited 19 lean (24 BMI) and 16 overweight (35 BMI) women for the study. All participants were weight stable for the last year.

Participants came to the laboratory fasted (12-18 hours) and had muscle biopsies taken, then the researchers plated these muscle cells on dishes and took measurements of fat utilization (fat oxidation), blood sugar uptake (glucose transport), insulin induced glucose transport, as well as insulin signaling in these cells without manipulation, then incubated with either nothing (control), 2mM palmitate (saturated fat), or palmitate (saturated fat + adiponectin) for 4 hours (this is the upper physiological level of palmitate found in the blood, but a supraphysiological concentration of adiponectin).

(Thr Akt; Santa Cruz Biotechnology), unphosphorylated Akt (Santa Cruz Biotechnology), and phosphorylated Akt substrate 160 (AS160) at Thr642 (P-AS160; Medicorp, Montreal, QB, Canada) was measured. Thirty to fifty milligrams of nonincubated (untreated) muscle was stored in liquid nitrogen for the analysis of AdipoR1 (ABCAM, Cambridge, MA).

Muscle samples were homogenized as previously described (28). Fifty micrograms of the tissue lysate for insulin-signaling proteins and 60 μg for AdipoR1 were solubolized in 4× Laemmeli's buffer, boiled at 95°C for 5 min, and then resolved by SDS-PAGE. Lean and obese muscle samples were run on the same gels. Gels were wet transferred to polyvinylidene difluoride (insulin-signaling proteins) or nitrocellulose (AdipoR1: 200 mA/1.5 h) membranes. Insulin-signaling membranes were blocked for 3 h [2.5% BSA (Ser Akt, Thr Akt, Akt), 7.5% BSA (P-AS160)], and AdipoR1 membranes were blocked for 1 h (5% nonfat milk) all in Tris-buffered saline with 0.01% Tween. Membranes were incubated overnight at 4°C with primary antibodies (insulin signaling 1:500, AdipoR1; 1:1,000, in blocking buffer). Membranes were washed and then incubated for 1 h with the secondary antibody (insulin-signaling anti-rabbit, AdipoR1 donkey anti-goat horseradish peroxidase). Membranes were washed again, and proteins were detected using enhanced chemiluminescence method (Syngene Chemigenius2; PerkinElmer, Waltham, MA). Immunoreactive bands were quantified using densitometry (Gene Tools software, PerkinElmer). Equal loading was confirmed using nonspecific protein staining with Ponceau-S stain (Sigma Aldrich, Oakville, ON, Canada).

Blood biochemistry. Two hundred microliters of whole blood were added to 1 ml of ice-cold 0.6 mM perchloric acid and immediately centrifuged at 10,000 *g* for 2 min. The perchloric acid extract was frozen at −80°C for subsequent flourometric analysis of blood glucose (2). The remaining blood was centrifuged at 10,000 *g* for 2 min and aliquots of plasma were collected and frozen. Plasma insulin, adiponectin, and leptin were measured using commercially available radioimmunoassay kits (Linco Research, St. Charles, MO). Plasma free FA was measured using a commercially available colorimetric assay kit (NEFA C test kit; Wako, Richmond, VA).

Calculations and statistical analyses. All data are presented as means ± SE. Two-way repeated-measures ANOVA was performed to detect differences in basal vs. insulin-stimulated glucose uptake between incubation conditions (control vs. palmitate vs. P+gAd). A one-way ANOVA was used to analyze within-group (lean, obese) differences of Western blot analysis (control vs. palmitate vs. P+gAd). A Student-Newman-Keuls post hoc test was used to check significant differences revealed by the ANOVA. One-tailed paired *t*-tests were used for within group (palmitate vs. P+gAd in lean or obese) analysis of FA metabolism. A one-tailed, unpaired *t*-test was used for between-group (lean vs. obese) analysis of body composition, blood parameters, and AdipoR1 protein analysis. Significance was accepted at $P \leq 0.05$, and trends were marked in parentheses ($0.05 < P < 0.10$).

The homeostasis model of assessment was used to compare insulin resistance [HOMA-IR = (fasting glucose × fasting insulin)/22.5] between lean and obese, where a larger number indicates a greater level of insulin resistance (17, 22).

RESULTS

Subject characteristics. Subject characteristics are shown in Table 1. Obese individuals had significantly greater body mass and body mass index (+34%, and +32%, respectively, $P < 0.0001$) compared with lean individuals. Blood glucose and plasma free FA were not different between lean and obese individuals. Fasting plasma insulin and leptin were significantly higher in obese individuals (+33% and +67%, respectively, $P < 0.01$) compared with lean individuals, whereas plasma adiponectin was significantly lower (−42%, $P < 0.01$).

Table 1. *Subject characteristics*

Characteristics	Lean	Obese
Age, yr	47±2	47±2
Body mass, kg	61.0±2.5	94.8±6.3‡
BMI, kg/m^2	24.0±0.6	35.4±2.1‡
Fasting blood glucose, mmol/l	5.0±0.1	5.5±0.4
Fasting plasma insulin, pmol/l	22.2±1.0	33.2±4.8†
HOMA-IR	5.0±0.3	8.0±1.2*
Fasting free fatty acid, mmol/l	0.89±0.06	0.92±0.03
Fasting plasma adiponectin, μg/ml	13.0±0.5	7.5±1.5*
Fasting plasma leptin, ng/ml	12.2±2.6	36.6±5.4*

Data are means ± SE. BMI, body mass index; HOMA-IR, homeostasis model of assessment comparing insulin resistance. *Significantly different from lean, 1-tailed unpaired *t*-test ($P < 0.01$); †significantly different from lean, 1-tailed unpaired *t*-test ($P < 0.05$), ‡significantly different from lean, 1-tailed unpaired *t*-test ($P < 0.0001$).

The homeostasis model of assessment used to compare insulin resistance was significantly greater in obese individuals ($P < 0.05$), indicative of whole body insulin resistance in this population.

Skeletal muscle glucose transport. Basal glucose transport was not different following exposure to palmitate or P+gAd in lean or obese individuals (Fig. 2, *A* and *B*). Insulin-stimulated glucose transport was significantly increased compared with basal glucose transport under all conditions in both lean and obese individuals ($P < 0.001$; Figs. 2, *A* and *B*).

In lean individuals, there was no significant difference in insulin-stimulated glucose transport with exposure to palmitate (−10%) or P+gAd (+3%) compared with control (Fig. 3*A*). The absolute increase in glucose transport from basal to insulin-stimulated conditions (Fig. 2*C*) was not significantly different among the three incubation conditions in this population ($P = 0.66$).

In contrast, insulin-stimulated glucose transport was reduced by 20% in isolated muscle from obese individuals when exposed to palmitate (palmitate vs. control, $P = 0.003$). When gAd was also included (Fig. 2*B*), there was a trend toward a reduction in insulin-stimulated glucose transport compared with control (control vs. P+gAd, −12%, $P = 0.09$). The inclusion of gAd tended to increase insulin-stimulated glucose transport compared with palmitate (+15%), but this also did not reach significance (P+gAd vs. palmitate, $P = 0.07$). When glucose transport was expressed as an absolute increase in response to insulin, both palmitate and P+gAd conditions were significantly lower than control, (palmitate vs. control, −55%; P+gAd vs. control, −36%, $P \leq 0.05$). There was no significant difference in the absolute increase in glucose transport between palmitate and P+gAd in skeletal muscle from obese individuals (P+gAd vs. palmitate, $P = 0.3$). This indicates that palmitate induced insulin resistance in skeletal muscle from obese individuals, but the inclusion of gAd was unable to completely prevent this.

Insulin-signaling proteins. The effects of palmitate and P+gAd conditions relative to control on Ser- and Thr-phosphorylated Akt, unphosphorylated Akt, and P-AS160 following acute insulin stimulation in lean and obese individuals are shown in Fig. 3. Palmitate or P+gAd did not significantly affect insulin-signaling protein content or phosphorylation in muscle from lean individuals (total Akt, Thr Akt, Ser Akt, P-AS160; Fig. 3, *A*, *C*, *E*, and *G*, respectively). In skeletal

Table 1

The participants were the same age (middle aged, 47 years old), but had different BMI (higher on the obese individuals), higher fasting insulin (higher on the obese), higher insulin resistance (obese), lower adiponectin (obese), and higher leptin levels (obese).

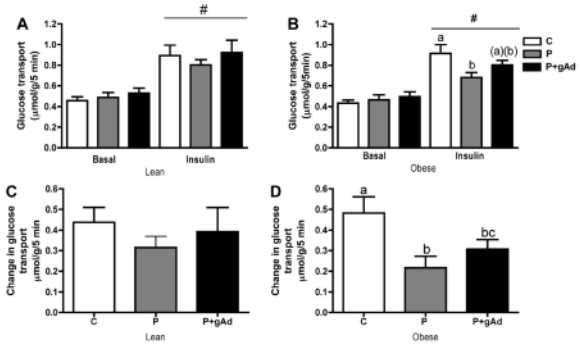

Fig. 2. Glucose transport in skeletal muscle of lean and obese humans. Effects of long-term exposure (4 h) of control, 2 mM palmitate, and 2 mM palmitate + 2.5 μg/ml gAd (P+gAd) on basal and insulin-stimulated glucose uptake in skeletal muscle of lean (n = 8; *A*) and obese (n = 8; *B*) humans. *C* and *D*: absolute change in glucose uptake in lean and obese skeletal muscle, respectively, in response to palmitate and P+gAd. Data was calculated by subtracting the respective rate of basal glucose uptake from insulin-stimulated glucose uptake. Basal and insulin-stimulated glucose transport was assessed over 40 and 20 min, respectively. Data is expressed over 5 min. Data are means ± SE. #All groups significantly different from basal. ^{a,b,c}Bars that have different letters are significantly different, $P \leq 0.05$; bars that share the same letter are not significantly different from each other. Trends are indicated in parentheses ($0.05 > P < 0.10$).

muscle from obese individuals, total Akt was not different following palmitate or P+gAd exposure relative to control (Fig. 3*B*). There was a nonsignificant reduction in Thr Akt phosphorylation following treatment with palmitate (−35%) and P+gAd (−37%) compared with control (Fig. 3*D*, P = 0.13). Ser Akt phosphorylation was significantly reduced (−58%) when exposed to palmitate in skeletal muscle of obese individuals (Fig. 3*F*, control vs. palmitate, $P < 0.001$). When gAd was included with palmitate, Ser Akt phosphorylation remained significantly reduced (−39%) compared with control (control vs. P+gAd, $P < 0.001$). In the presence of gAd, Ser Akt phosphorylation tended to be increased compared with palmitate (palmitate vs. P+gAd, P = 0.07). Phosphorylated AS160 (Fig. 3*H*) was also significantly reduced following exposure to palmitate (−32%) and P+gAd (−38%) in muscle from obese individuals (control vs. palmitate, control vs. P+gAd, $P < 0.05$).

Skeletal muscle FA metabolism. The effects of palmitate and P+gAd on FA metabolism are shown in Table 2 and Fig. 4. The inclusion of gAd resulted in increased palmitate oxidation (Fig. 4) and total palmitate uptake (Table 2) in muscle from both lean and obese individuals (Fig. 4, $P < 0.05$). There was no significant difference in FA incorporation into lipid pools in the presence of gAd in skeletal muscle of lean or obese individuals compared with palmitate (Table 2).

AdipoR1. AdipoR1 protein content was 20% lower in skeletal muscle of obese individuals; however, this was not significant (Fig. 5).

DISCUSSION

This study demonstrated that 4 h of 2 mM palmitate exposure can induce insulin resistance in isolated rectus abdominus muscle from obese but not lean individuals; this coincided with impaired Ser Akt and AS160 phosphorylation, which were also only apparent in the obese muscle. The inclusion of gAd did not restore insulin-stimulated glucose transport to control values or prevent the reduced phosphorylation of Ser Akt and AS160. In the presence of high palmitate, gAd was still able to stimulate FA oxidation in both lean and obese muscle, suggesting that the inability to restore insulin sensitivity was not fully a function of gAd resistance. It may be possible that under acute (4 h) conditions, either stimulating FA oxidation was insufficient, or the magnitude of the stimulation was not large enough to prevent palmitate-induced insulin resistance in obese individuals. There was a small, nonsignificant reduction (−20%) in AdipoR1 protein content in obese muscle compared with lean. It is uncertain whether this was a physiologically relevant reduction. Perhaps indicative of the change in AdipoR1 being nonrelevant was our observation that gAd was still able to stimulate FA oxidation, although this was slightly lower in obese than in muscle from lean individuals.

Isolated rectus abdominus muscle strips, as used in the present study, are a unique model that allows for the in vitro assessment of human muscle metabolism in isolation of systemic influences (15). Rectus abdominus muscle has a relatively even distribution of Type I and IIa fibers, with a small portion of Type IIb fibers, making it slightly less glycolytic but comparable to the more commonly used vastus lateralis muscle (26). Furthermore, citrate synthase activity is similar between rectus abdominus and vastus lateralis, which indicates a comparable oxidative capacity (7). It should be recognized that, as with any in vitro model, extrapolation to the in vivo condition must be cautious. Furthermore, other skeletal muscles/fiber types may not necessarily respond similarly.

Figure 2

The researchers are testing glucose uptake into the cells, with and without insulin stimulation, in lean and obese individuals. *C = Control media (no added saturated fat or adiponectin); P = Palmitate (saturated fat) addition to the cells; P+gAd: Palmitate + globular adiponectin addition to the cells.*

Primary Results

- Basal glucose uptake is the same for lean and obese.
- Glucose transport/uptake was increased with insulin stimulation.
- Glucose uptake was dampened by palmitate, but somewhat rescued by adiponectin (only in obese).

Take Away: Saturated fat (palmitate) reduces insulin mediated glucose uptake in obese derived muscle cells only (no such effect in lean derived muscle cells). However, adiponectin (at extremely high levels) recovers some of the insulin mediated glucose uptake in obese derived muscle cells only.

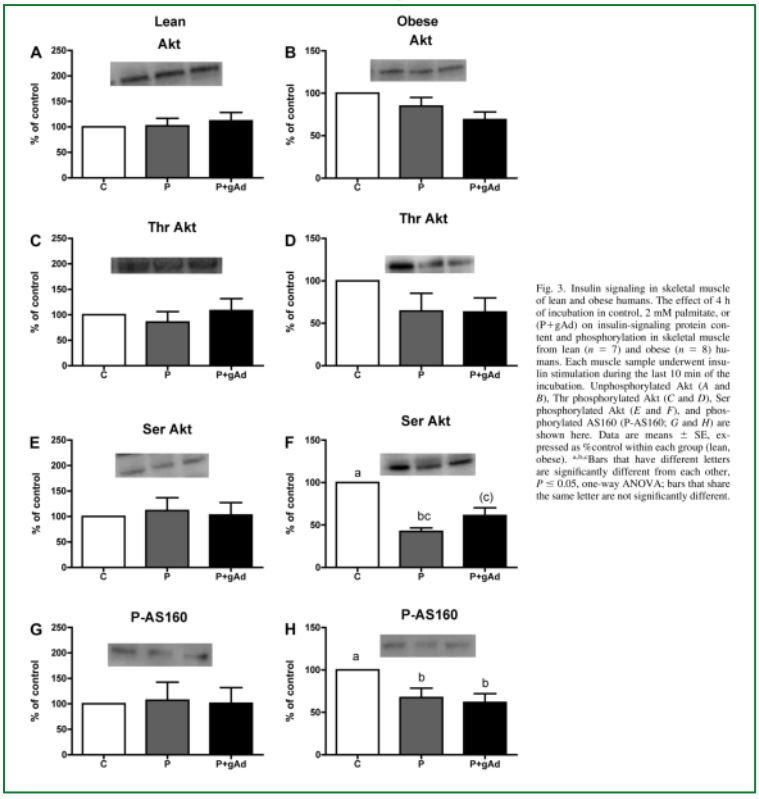

Fig. 3. Insulin signaling in skeletal muscle of lean and obese humans. The effect of 4 h of incubation in control, 2 mM palmitate, or (P+gAd) on insulin-signaling protein content and phosphorylation in skeletal muscle from lean (n = 7) and obese (n = 8) humans. Each muscle sample underwent insulin stimulation during the last 10 min of the incubation. Unphosphorylated Akt (*A* and *B*), Thr phosphorylated Akt (*C* and *D*), Ser phosphorylated Akt (*E* and *F*), and phosphorylated AS160 (P-AS160; *G* and *H*) are shown here. Data are means ± SE, expressed as %control within each group (lean, obese). ^{a,b,c}Bars that have different letters are significantly different from each other, $P \leq 0.05$, one-way ANOVA; bars that share the same letter are not significantly different.

Effect of palmitate and gAd on skeletal muscle glucose transport. The exposure of skeletal muscle to 2 mM palmitate for 4 h induced insulin resistance in skeletal muscle of obese, but not lean, individuals. Skeletal muscle of obese individuals is often characterized by impaired glucose transport (7, 19) and abnormal FA metabolism (20), including increased sarcolemmal FAT/CD36 content (6, 21) and increased lipid content (6, 18). Due to an increased tendency to take up and store FA, it is reasonable to hypothesize that this population is more susceptible to palmitate-induced insulin resistance, which is demonstrated by the findings of this study. It is perhaps surprising that we did not demonstrate a palmitate-induced decrease in insulin-stimulated glucose transport in muscle from lean individuals. However, it is possible that a longer exposure to palmitate, i.e., ≥6 h, would indeed have induced insulin resistance, which has previously been shown in isolated soleus

Figure 3

This data shows the expression levels of particular proteins/molecules that are key to the insulin signaling cascade within the muscle cells. Threonine and serine phosphorylation of Akt activate Akt. AS160 is a protein involved in the activation of the GLUT proteins that allow glucose into the cell from the media/blood (more phosphorylation at that location - threonine 642 - activates AS160 to allow more insulin signaling. *C = Control media (no added saturated fat or adiponectin); P = Palmitate (saturated fat) addition to the cells; P+gAd: Palmitate + globular adiponectin addition to the cells.*

Primary Results

- Total Akt levels are unaffected by palmitate.
- Phosphorylation of Akt at Threonine is unaffected by palmitate.
- Serine phosphorylation of Akt is reduced by palmitate, but somewhat recovered by adiponectin (in obese)
- AS160 phosphorylation is reduced by palmitate, but not recovered by adiponectin (in obese).

Note: There are no loading controls for these proteins (except the total Akt) - so, we are simply taking the researchers' word that the conditions were equally measured - this enough to dismiss this data entirely, if you do not believe the researchers.

Take Away: Insulin signaling is somewhat dampened by palmitate (saturated fat) in obese derived muscle cells only, with a partial recovery when adiponectin is exposed to the cells. No effect is experienced in lean derived muscle cells.

Table 2. *Fatty acid incorporation into DAG and TAG pools*

Fatty Acid Metabolism	Lean		Obese	
	Palmitate	P+gAd	Palmitate	P+gAd
Lipid Incorporation				
DAG	29.0±4.2	27.5±4.9	25.9±4.4	22.7±3.8
TAG	50.2±8.3	61.4±8.7	48.2±9.1	45.1±10.2
Total	81.1±12.3	81.7±13.2	75.4±12.1	67.5±14.2
Total palmitate uptake	108.9±10.4	162.8±21.3*	125.4±15.7	156.5±21.8*
Oxidation-to-Esterification Ratio				
OX/DAG	1.8±0.3	2.8±0.8	2.4±0.4	3.7±1.0
OX/TAG	1.0±0.1	0.9±0.1	1.4±0.2	2.4±0.4

Data are means ± SE in nmol/g; lean individuals, $n = 5$; obese individuals, $n = 8$. DAG, diacylglycerol; TAG, triacylglycerol; P+gAd, palmitate + globular adiponectin; OX, oxidation. Oxidation-to-esterification ratio: palmitate oxidation, nmol/g (shown in Figure 5): *P vs. P + gAd, $P < 0.05$, 1-tailed paired t-test.

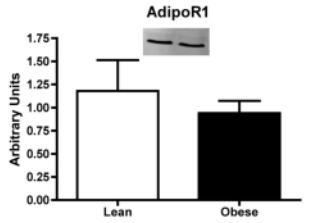

Fig. 5. Adiponectin receptor isoform 1 (AdipoR1) protein content in skeletal muscle from lean and obese humans. Skeletal muscle AdipoR1 receptor protein content in skeletal muscle from lean ($n = 8$) and obese ($n = 7$) humans. Data are means ± SE.

muscle from lean nondiabetic rodents (1). Nonetheless, the important finding of our study is that in a defined 4-h period, palmitate was able to induce insulin resistance in muscle from obese, but not lean, individuals.

An unexpected observation in this study was that insulin-stimulated glucose transport was not different between lean and obese individuals in the absence of palmitate. Certainly, it is well recognized that glucose transport may be reduced in skeletal muscle of obese individuals (7, 19). The pharmacological dose of insulin used in this study was chosen because it is known to maximally stimulate glucose transport in an isolated muscle preparation. Thus, we cannot dismiss the possibility that this maximal stimulation may have overcome any impairment in insulin-stimulated glucose transport, and that this impairment may have been evident in the presence of a lower, submaximal insulin dosage.

It has previously been shown that gAd stimulates glucose transport in skeletal muscle from lean and obese individuals and that this effect is additive to insulin (7). In the present study, the inclusion of gAd did not completely prevent palmitate-induced insulin resistance in obese muscle. The absolute increase in glucose transport from basal to insulin-stimulated conditions was slightly greater when gAd was included (P+gAd) compared with palmitate, but this remained significantly different from control, indicating that gAd did not restore insulin-stimulated glucose transport. In lean individuals, insulin-stimulated glucose transport was also unaffected by the presence of gAd.

In the present study, the stimulatory effects of gAd in the absence of palmitate were not measured due to the limited number of viable strips that could be procured for incubation from a given rectus abdominus sample. As a result, we cannot conclude whether gAd was able to independently stimulate glucose transport. However, it has previously been shown in rectus abdominus muscle strips that gAd increases basal and insulin-stimulated glucose transport (7). It is quite possible that the presence of a high palmitate concentration (P+gAd condition) may have prevented this additive effect, i.e., induced gAd resistance.

Effects of palmitate and gAd on insulin-signaling proteins. The present study demonstrated that exposure to palmitate significantly reduced Ser Akt phosphorylation (−58%) and tended to reduce Thr Akt phosphorylation (−35%) under insulin-stimulated conditions. This is in agreement with previous studies in rat soleus muscle (27) and C2C12 muscle cells (9). In accordance with the reduction in Akt phosphorylation, AS160 phosphorylation was also reduced by 35%, which has also been shown in rodent soleus muscle (1). In C2C12 myocytes exposed to palmitate, ceramide content increased and inhibited insulin signaling by stimulating the dephosphorylation of Akt (9), therefore preventing the phosphorylation of AS160. In the present study, the impaired insulin-stimulated glucose transport in muscle from obese individuals may also have been the result of palmitate-induced ceramide production, which prevented the phosphorylation of Akt and AS160. In skeletal muscle from lean individuals, palmitate exposure had

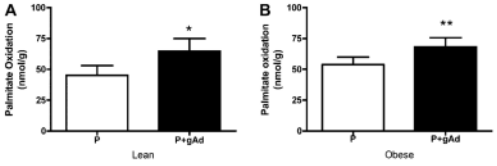

Fig. 4. Fatty acid oxidation in skeletal muscle of lean and obese humans. The effects of long-term exposure (4 h) of 2 mM palmitate and (P+gAd) on fatty acid oxidation in skeletal muscle of lean ($n = 5$; *A*) and obese ($n = 8$; *B*) humans. Data are means ± SE. *$P < 0.05$, significantly different from palmitate, 1-tailed paired t-test; **$P < 0.01$, significantly different from palmitate, 1-tailed paired t-test.

no significant effect on any of the insulin-signaling proteins that were measured, consistent with the lack of impairment in insulin-stimulated glucose transport. Due to tissue constraints, ceramide content and other lipids were not measured in this study, and so a definitive role of reactive lipid species cannot be determined.

Effects of palmitate and gAd on FA metabolism. gAd stimulates FA oxidation in human (7, 11) and rodent (24, 32) muscle. However, obesity may reduce the sensitivity of muscle to the acute stimulatory effects of gAd on AMPK activation and FA oxidation. For example, a greater concentration of gAd is required to stimulate FA oxidation in myotubes cultured from obese human muscle (11). Also, gAd-stimulated FA oxidation is blunted in isolated muscle from obese, compared with lean, humans (7). In addition to this, gAd fails to stimulate FA oxidation in isolated rat soleus muscle following 4 wk of high-fat feeding (24).

In the present study, gAd stimulated FA oxidation in the presence of high palmitate in muscle from both lean and obese individuals. This finding differs from our previous report (7) in which gAd-induced FA oxidation was significantly impaired in obese muscle. However, in our previous study, which used a 0.5 mM palmitate incubation medium, and a shorter period of exposure, gAd stimulated FA oxidation in skeletal muscle of lean and obese humans by 69% and 30%, respectively (7). In the present study, the relative increase in FA oxidation was much less: ~30% in lean, and ~20% in skeletal muscle from obese individuals. Indirectly, this suggests that the longer exposure to a high palmitate concentration may have reduced the stimulatory effects of gAd on FA oxidation.

The fact that palmitate-induced insulin resistance was not alleviated in obese muscle by gAd strongly suggests that the stimulatory effects of gAd on FA oxidation were insufficient to prevent insulin resistance. Whether a greater increase in palmitate oxidation would have proved beneficial is unknown. However, it should be noted that the stimulatory effect of gAd on palmitate oxidation was slightly reduced in obese (+20%) compared with lean (+30%) individuals; Accordingly, we did not observe any impairment in insulin-signaling proteins in the presence of palmitate, with or without gAd in muscle from lean individuals. Overall, the data indicates that obese muscle is more susceptible to the detrimental effect of palmitate, and this could not be rescued with gAd. Furthermore, our data suggests that high palmitate impairs the ability of gAd to independently stimulate glucose transport prior to any loss of stimulation of FA oxidation, i.e., acute development of gAd resistance.

AdipoR1. The regulation of AdipoR1 expression in human skeletal muscle is controversial. Findings of decreased, unchanged, and increased mRNA content in muscle from obese and type 2 diabetic individuals have been reported (3, 11). To the best of our knowledge, ours is the first study to examine AdipoR1 protein content in lean and obese human skeletal muscle. Here, we demonstrate a nonsignificant 20% reduction in AdipoR1 content in skeletal muscle from obese individuals. It is uncertain whether this modest reduction in AdipoR1 protein content in obese individuals translates into a physiological reduction in the stimulatory effects of gAd; however, it has previously been shown that the stimulatory effects of gAd are reduced in obesity (7). The fact that we were able to demonstrate gAd-induced stimulation of palmitate oxidation in obese muscle, albeit to a somewhat lesser extent than was observed in lean muscle, suggests that there may not have been a physiologically important reduction in this receptor. Unfortunately, this finding does not clarify the current controversy regarding the regulation of AdipoR1 in lean and obese muscle.

Perspectives and Significance

There are several important and novel findings of this study. First and most importantly, we demonstrated that in a 4-h period, 2 mM palmitate directly induces insulin resistance in isolated skeletal muscle from obese, but not lean, individuals. This is consistent with our observed palmitate-induced inhibition of AS160 and Akt phosphorylation in the obese group. Also of significance was the inability of gAd to restore skeletal muscle insulin-stimulated glucose transport in the presence of high palmitate in this group, despite a small, albeit significant, stimulation of palmitate oxidation. The accumulation of lipid species (ceramide and diacylglycerol) have not yet been measured in intact human muscle exposed to palmitate, and should be assessed as a potential mediator of lipid-induced insulin resistance in this model.

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