

Early Skeletal Muscle Adaptations to Short-Term High-Fat Diet in Humans Before Changes in Insulin Sensitivity

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Objective: The purpose of this investigation was to understand the metabolic adaptations to a short-term (5 days), isocaloric, high-fat diet (HFD) in healthy, young males.

Methods: Two studies were undertaken with 12 subjects. Study 1 investigated the effect of the HFD on skeletal muscle substrate metabolism and insulin sensitivity. Study 2 assessed the metabolic and transcriptional responses in skeletal muscle to the transition from a fasted to fed state using a high-fat meal challenge before and after 5 days of the HFD.

Results: Study 1 showed no effect of a HFD on skeletal muscle metabolism or insulin sensitivity in fasting samples. Study 2 showed that a HFD elicits significant increases in fasting serum endotoxin and disrupts the normal postprandial excursions of serum endotoxin, as well as metabolic and transcriptional responses in skeletal muscle. These effects after 5 days of the HFD were accompanied by an altered fasting and postprandial response in the ratio of phosphorylated- to total-p38 protein. These changes all occurred in the absence of alterations in insulin sensitivity.

Conclusions: Our findings provide evidence for early biological adaptations to high-fat feeding that proceed and possibly lead to insulin resistance.

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Introduction

High-fat diet (HFD)–induced obesity is associated with a modest elevation in circulating endotoxin concentrations (termed metabolic endotoxemia) and insulin resistance in rodents (1–3). We (4) have previously reported that lipopolysaccharide suppresses skeletal muscle homogenate fatty acid oxidation and increases glucose oxidation in rodents. However, whether a HFD increases circulating endotoxin and produces dysregulated skeletal muscle substrate metabolism in nonobese humans is unknown. Therefore, the purposes of this investigation were to determine whether a short-term HFD elicited metabolic endotoxemia in nonobese humans and adversely affected whole-body insulin sensitivity and skeletal muscle substrate metabolism when transitioning from a fasting to fed state.

Methods

Experimental design

Twelve college-aged (mean, 21 ± 1 year), nonobese [mean body mass index (BMI), 22.3 ± 3.9 kg/m²] males volunteered for the

study. They were free from overt disease and not taking any medications. All were sedentary (<2 days/week for <20 min/day), nonsmoking, and weight stable (± 2 kg) for the previous 6 months. The study protocol was approved by the institutional review board at Virginia Tech (Blacksburg, VA).

Study 1. Subjects ($n = 6$) consumed a lead-in control diet [55% carbohydrate, 15% protein, and 30% fat (11% SFA)] that was isocaloric to their habitual diet for 1 week before the HFD. Subsequently, subjects were provided a HFD for 5 days. The composition of the HFD was 30% carbohydrate, 15% protein, and 55% fat (25% SFA) and designed to be isocaloric to the lead-in control diet. Subjects reported to our metabolic kitchen daily to eat breakfast, receive remaining meals for the day, and to have body weight measured. A skeletal muscle biopsy from the vastus lateralis muscle and a 3-h intravenous glucose tolerance test (IVGTT) were performed in the postabsorptive state (10- to 12-h fast) before and after the HFD (study time line is provided in Supporting Information).

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Author contributions: AA analyzed data, interpreted data, and wrote the manuscript. KH recruited subjects, conceived the study design, and carried out experiments. RM conceived and carried out experiments. KO contributed to the study design and planned/provided all meals to the subjects. NB and BD contributed to the study design and edited the document. MF, KD, and MH conceived the study design and experiments and contributed to editing of the document. All authors gave final approval of the submitted and published versions.

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Study 2. Using the identical feeding paradigm as in study 1, a separate group of subjects ($n = 6$) consumed a high-fat meal consisting of 880 kcal [63% fat (10% SFA), 25% carbohydrate, and 12% protein] before and after the HFD. Muscle biopsies were collected before (10- to 12-h fast) and at 4 h after the high-fat meal challenge. A study time line is provided in Supporting Information.

General procedures

Extended methods are provided in Supporting Information. Body weight and height were measured on a digital scale and stadiometer, respectively. Body composition was determined by DEXA (Prodigy Advance, GE Healthcare, Madison, WI). Whole-body insulin sensitivity was assessed in study 1 using the IVGTT (5) (MINMOD Millennium Software) as previously described (6,7) and by the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) in both studies 1 and 2 (8). Serum endotoxin concentrations were determined using the PyroGene Recombinant Factor C endotoxin detection assay (Lonza, Basel, Switzerland).

Dietary assessment

Energy requirements were estimated based on height, weight, age, and activity level (9). A research dietitian instructed volunteers to accurately report food and beverage intake and reviewed all records with the participants for accuracy and sufficient detail. Food intake records were analyzed with the Nutritionist Pro Diet Analysis Software (Axxya Systems, Stafford, TX).

Skeletal muscle biopsies and homogenate preparation

Biopsies samples were taken with suction from the vastus lateralis muscle under local anesthesia (1% lidocaine) using a modified Bergström needle as described previously (7). Skeletal muscle homogenates were prepared and measures of glucose oxidation and enzyme activities [phosphofructokinase (PFK), citrate synthase (CS), and β -hydroxyacyl CoA dehydrogenase (β -HAD)] were performed as previously described (4,10).

mRNA extraction, qRT-PCR, and western blotting

Samples were placed in Trizol (Invitrogen, Carlsbad, CA), snap-frozen in liquid nitrogen, and subsequently stored at -80°C until analysis. RNA was extracted using an RNeasy Mini Kit (Qiagen) and DNase I treatment (Qiagen, Valencia, CA). qRT-PCR was performed using an ABI PRISM 7900HT Sequence Detection System instrument and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Primers and 5# FAM-labeled TaqMan probes were purchased as pre-validated assays (ABI). Relative quantification of target genes was calculated using the ΔCT method (Applied Biosystems User Bulletin no. 2 P/N 4303859). All samples were run in triplicate and expressed as target gene mRNA/cyclophilin B mRNA in arbitrary units. Muscle used for western blotting was placed in mammalian lysis buffer (50 mM Tris-HCL, 1 mM EDTA, 150 mM NaCL, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, 0.01% Igepal CA) with protease and phosphatase inhibitor cocktail (Thermo Scientific, Waltham, MA), snap-frozen in liquid nitrogen, and subsequently stored at -80°C until analysis. Proteins (30 μg) were separated using a 10% Criterion-Tris- HCL gel (Bio-Rad, Hercules, CA) and subsequently transferred to PVDF membrane (Bio-Rad, Hercules, CA).

TABLE 1 Subject characteristics before and after acute HFD

Variable	Study 1 ($n = 6$)		Study 2 ($n = 6$)	
	Pre	Post	Pre	Post
Body weight (kg)	70.2 $\pm$ 3.4	70.0 $\pm$ 3.2	67.2 $\pm$ 5.0	67.2 $\pm$ 5.1
Body fat (%)	17.0 $\pm$ 2.7	16.7 $\pm$ 2.8	16.7 $\pm$ 3.2	17.5 $\pm$ 3.3
Insulin sensitivity (S_i)	12.6 $\pm$ 3.9	13.6 $\pm$ 5.8		
HOMA-IR ($\text{mU} \cdot \text{mmol/L}^2$)	3.53 $\pm$ 0.91	2.30 $\pm$ 0.72 ^a	3.93 $\pm$ 0.63	4.92 $\pm$ 1.04
Glucose (mmol/L)	5.04 $\pm$ 0.01	4.69 $\pm$ 0.26	4.68 $\pm$ 0.17	4.82 $\pm$ 0.06

All values are expressed as mean $\pm$ SEM.

S_i , insulin sensitivity; HOMA-IR, homeostatic model assessment for insulin resistance

^aData missing for one subject.

Blots were probed with primary antibodies against phospho-p38 and total p38 (Cell Signaling, Danvers, MA; 1:1,000) followed by anti-rabbit or anti-mouse secondary antibodies (Jackson Immuno Research Laboratories, West Grove, PA; 1:10,000). Proteins were visualized using Super-Signal Chemiluminescent Substrate (Pierce, Rockville, IL) and a ChemiDoc XRS Imaging System (BioRad, Hercules, CA). p38 protein was normalized to GAPDH (Santa Cruz Biotechnology, Dallas, TX; 1:1,000).

Statistical analysis

All statistics were run using Prism 6 (GraphPad Software, San Diego, CA). A paired t -test was used to determine differences in the resulting means from assays performed in the fasted state before and after the HFD (Study 1). A two-way repeated measures analysis of variance followed by a Sidak's multiple comparison test was used to compare changes in variables of interest in response to the meal challenge before and after the HFD (study 2). All data are presented as mean $\pm$ standard error of the mean (SEM) and considered significant at $P \leq 0.05$.

Results

Body weight and body fat percentage did not change with the HFD in study 1 or 2 ($P > 0.05$) (Table 1). Whole-body insulin sensitivity did not change after the HFD in study 1 or 2 (both $P > 0.05$) (Table 1).

There was a significant increase in fasting endotoxin concentrations after the HFD in study 1 and 2 (Figure 1A). In study 2, we observed a significant 2.5-fold increase ($P < 0.05$) in postprandial endotoxin concentrations at 1 h after the consumption of the high-fat meal challenge before the HFD, which was not evident after the HFD (Figure 1B). In response to the meal challenge after the HFD, serum endotoxin did not change from fasting levels (Figure 1B).

There were no changes ($P > 0.05$) in glucose oxidation or any enzyme activities in the homogenates in response to the HFD in study 1 (data not shown).

Glucose oxidation was increased ($P < 0.05$) after the meal challenge before the HFD but not after (Figure 1C). There was a modest, but

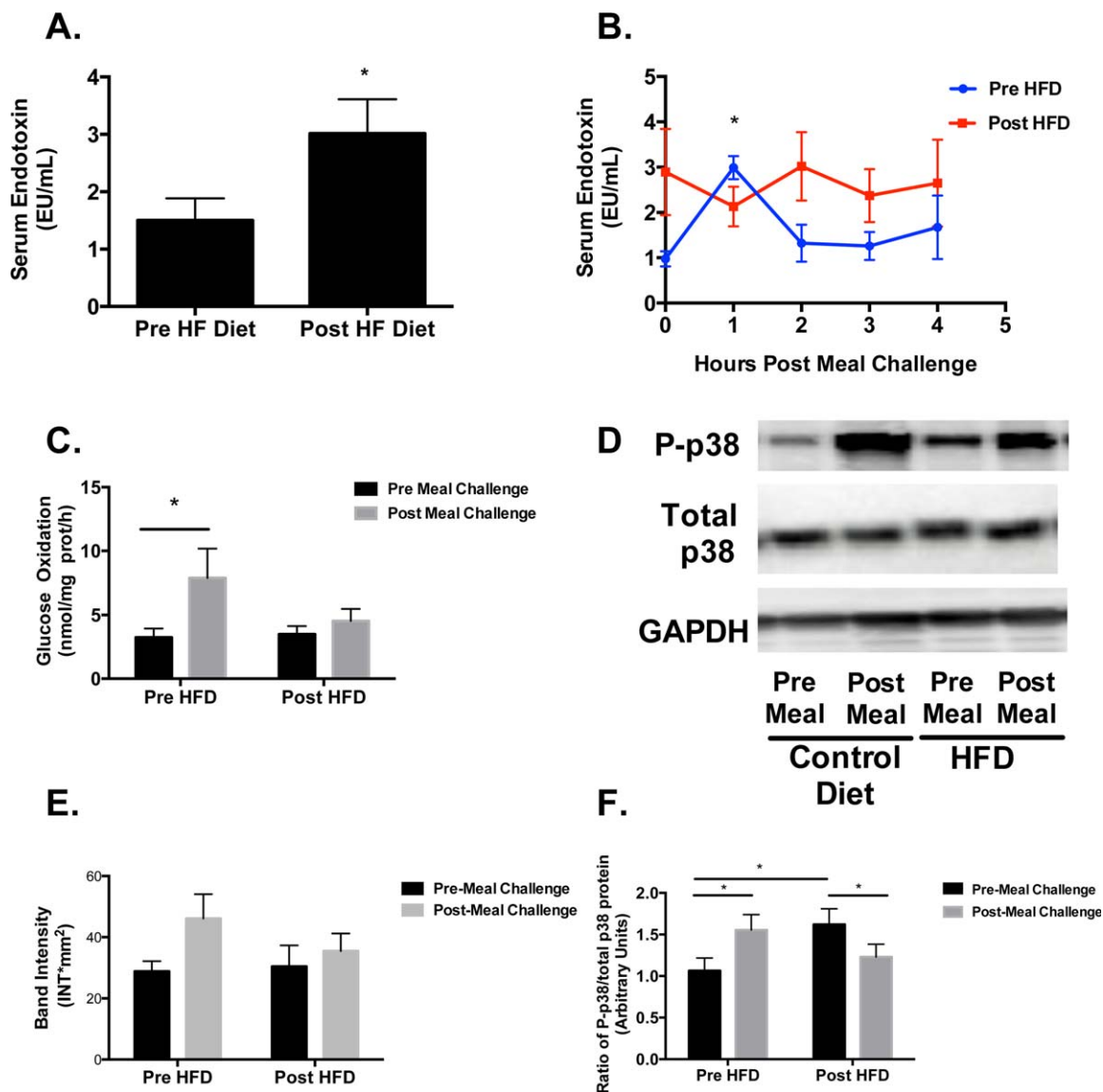


Figure 1 Fasting and postprandial endotoxin, skeletal muscle homogenate glucose oxidation, and p38 protein. (A) Fasting serum endotoxin concentrations were significantly higher ($P < 0.05$) after HFD for studies 1 and 2 combined. (B) Postprandial endotoxin was significantly increased ($P < 0.05$), relative to baseline, 1 h post-meal challenge before HFD, but not after. (C) Glucose oxidation in skeletal muscle homogenates was significantly increased ($P < 0.05$) 4 h post-meal challenge before HFD, but not after. (D, E) p38 MAPK phosphorylation was increased, although nonsignificant, 4 h post-meal challenge before HFD, but not after. (F) There was a significant HFD $\times$ meal challenge interaction ($P < 0.001$) for phospho- to total-p38 protein ratio, which was significantly increased ($P < 0.01$) under fasting conditions after HFD, and in response to meal challenge before HFD, but decreased after. All data are expressed as mean $\pm$ SEM. * $P < 0.05$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nonsignificant, increase in the phosphorylation of p38 in response to the meal challenge, which was not evident after the HFD (Figure 1D and E). When comparing the phospho- to total-p38 protein ratio, there was a significant diet $\times$ meal challenge interaction ($P < 0.001$) in that the ratio was significantly increased ($P < 0.05$) under fasting conditions after the HFD, increased in response to the meal before the HFD, and then decreased in response to the meal after the HFD (Figure 1F). A similar pattern of increases in response to meal challenge before the HFD was observed with PGC1 α , PGC1 β , NADH

dehydrogenase, and PPAR δ mRNA; this response was blunted in all targets after the HFD (Figure 2A–D).

Discussion

The major finding of the present study is that 5 days of an isocaloric HFD increases postabsorptive endotoxin concentrations and attenuates the increase in skeletal muscle homogenate glucose oxidation

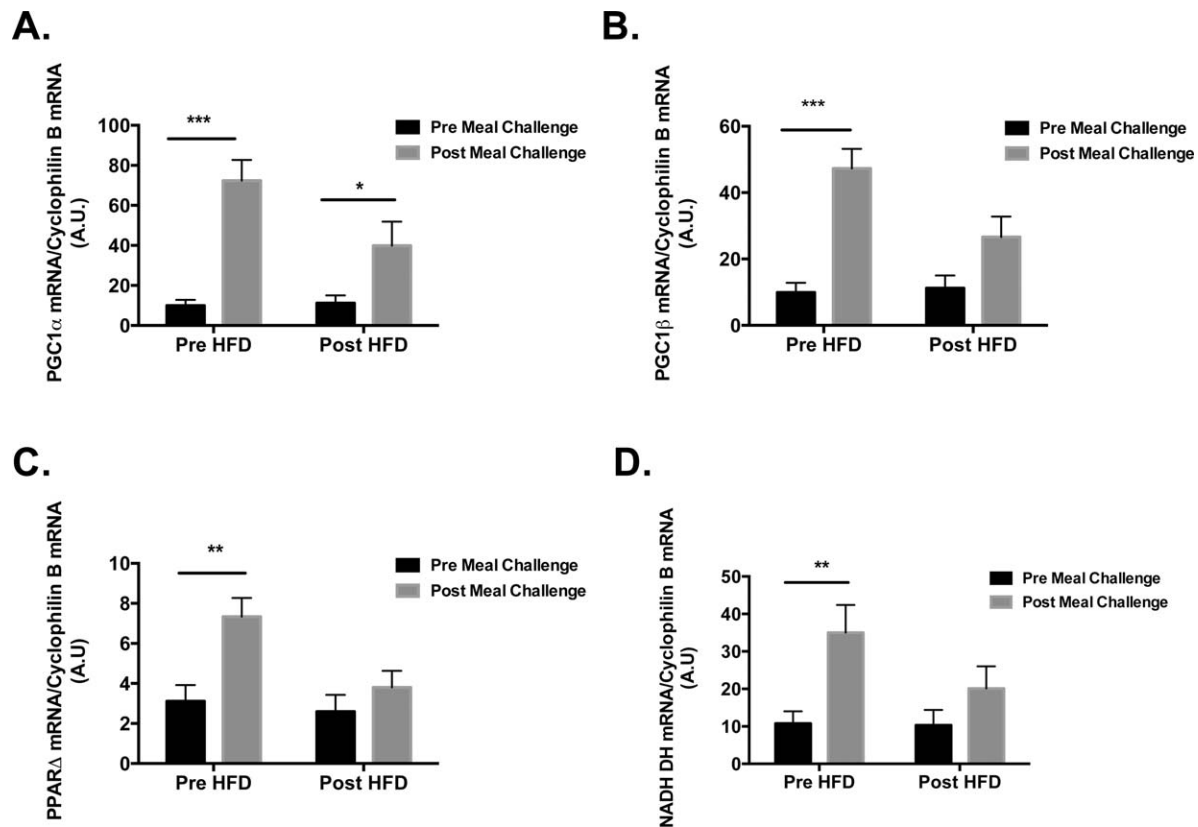


Figure 2 Metabolic mRNA targets in skeletal muscle. mRNA concentrations of (A) PGC1- α , (B) PGC1- β , (C) NADH dehydrogenase, and (D) PPAR α were significantly increased in skeletal muscle 4 h post-meal challenge before HFD; all increased significantly less after HFD. All data are expressed as mean $\pm$ SEM. * P < 0.05, ** P < 0.01, *** P < 0.001.

observed during the fasting-to-fed transition. Consistent with the latter observations, the increased phospho- to total-p38 protein ratio and expression of key metabolic genes during the fasting-to-fed transition were also attenuated after the HFD. Importantly, the observed dysregulation of skeletal muscle substrate metabolism occurred in the absence of changes in insulin sensitivity.

The increase in fasting and postprandial endotoxin concentrations is consistent with prior reports in animals (2,11) and humans (12-17). However, our study is the first to demonstrate that circulating endotoxin concentrations are increased after 5 days of an isocaloric HFD in healthy humans. Moreover, this “metabolic endotoxemia” was associated with a blunted postprandial increase in skeletal muscle homogenate glucose oxidation.

There are three isoforms of p38 expressed in skeletal muscle, p38- α , - β , and - γ (18). p38- α is the isoform primarily involved in initiating TLR4-mediated inflammatory signaling (18) and p38- γ has been demonstrated to promote PGC1- α gene transcription (19). Importantly, PGC1- α transcription is an important molecular event regulating mitochondrial oxidative metabolism (20). To our knowledge, we are the first to report in humans these dynamic responses by p38 MAPK in response to acute (a single meal) and chronic (5 days of HFD) feeding. Based on the robust induction of oxidative mRNA targets in response to the meal challenge, we are speculating the

increase in the phospho-p38 to total-p38 ratio is a due to increased phosphorylation of p38- γ . The heightened phospho-p38 to total-p38 ratio observed in fasting samples after 5 days of the HFD is speculated to represent changes in p38- α , which falls in line with the higher serum endotoxin levels. Although we acknowledge that the small number of subjects was a limitation to this study, we also believe these data are novel and compelling and warrant future studies to determine which p38 isoforms are responsible for this observation.

In conclusion, we are the first to demonstrate that, in the absence of changes in whole-body insulin sensitivity, a short-term HFD increased fasting circulating endotoxin concentrations while resulting in an attenuated increase in circulating endotoxin and skeletal muscle homogenate glucose oxidation during the fasting-to-fed transition. In addition, a short-term HFD alters the dynamics of the phospho-p38 to total-p38 protein ratio under fasting conditions and during the fasting-to-fed transition. Taken together with the smaller increase in mRNA expression, these findings suggest a potential mechanism for the observed dysregulated skeletal muscle substrate metabolism after the fasting-to-fed transition. Importantly, this dysregulated skeletal muscle substrate metabolism was observed before measurable changes in whole-body insulin sensitivity. **O**

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