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Serum endotoxin, gut permeability and skeletal muscle metabolic adaptations following a short term high fat diet in humans

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ABSTRACT

Background: Our previous work demonstrated that a short-term high fat diet (HFD) increased fasting serum endotoxin, altered postprandial excursions of serum endotoxin, and led to metabolic and transcriptional responses in skeletal muscle in young, healthy male humans.
Purpose: The purpose of the present study was to determine if a short-term high fat diet: 1) increases intestinal permeability and, in turn, fasting endotoxin concentrations and 2) decreases postprandial skeletal muscle fat oxidation.
Methods: Thirteen normal weight young adult males (BMI 23.1 ± 0.8 kg/m^2, age 22.2 ± 0.4 years) were fed a control diet (55% carbohydrate, 30% fat, 9% of which was saturated, 15% protein) for two weeks, followed by 5 days of an isocaloric HFD (30% carbohydrate, 55% fat, 25% of which was saturated, 15% protein, isocaloric to the control diet). Intestinal permeability (via four sugar probe test) was assessed in the fasting state. Both before and after the HFD, a high fat meal challenge (HFM, 820 kcal, 25% carbohydrate, 63% fat, 26% of which was saturated, and 12% protein) was administered. After an overnight fast, blood samples were collected before and every hour for 4 h after the HFM to assess endotoxin, and other serum blood measures. Muscle biopsies were obtained from the vastus lateralis before and 4 h after the HFM in order to assess substrate oxidation (glucose, fatty acid and pyruvate) using radiolabeled techniques. Insulin sensitivity was assessed via intravenous glucose tolerance test. Intestinal permeability, blood samples and muscle biopsies were assessed in the same manner before and following the HFD.
Main findings: Intestinal permeability was not affected by HFD ($p > 0.05$), but fasting endotoxin increased two fold following the HFD ($p = 0.04$). Glucose oxidation and fatty acid oxidation in skeletal muscle homogenates significantly increased after the HFM before the HFD (+97%, and +106% respectively) but declined after the HFM following 5 days of the HFD (−24% and +16% respectively). Fatty acid suppressibility of pyruvate oxidation increased significantly after the HFM (+32%) but this physiological effect was abolished following 5 days of the HFD (+7%). Insulin sensitivity did not change following the HFD.
Conclusion: These findings demonstrate that in healthy young men, consuming an isocaloric HFD for 5 days increases fasting endotoxin, independent of changes in gut permeability. These changes in endotoxin are accompanied by a broad effect on skeletal muscle substrate metabolism including increases in postprandial fat oxidation. Importantly, the latter occurs independent of changes in body weight and whole-body insulin sensitivity.

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1. Introduction

The pathophysiology of obesity and Type 2 Diabetes Mellitus (T2DM) is associated with alterations of the gut microbiota in animal models [1–7] and humans [6,8–10]. Lipopolysaccharide (LPS), or endotoxin is the major glycolipid component of the outer membrane of gram-negative bacteria that comprise approximately 70% of the total bacteria in the gut [11]. Lysis of gram-negative bacteria leads to the sustained transport of endotoxin into the systemic circulation, and subsequent activation of a family of pattern recognition receptors called toll-like receptors (TLR). Activation of TLR initiates a well-characterized immune response and inflammatory signaling pathway via activation of nuclear factor kappa-β (NF-kβ) leading to systemic inflammation, and a host of chronic inflammatory conditions which are associated with obesity, T2DM, and other diseases [12,13]. Endotoxin induced inflammation may therefore provide a mechanistic link between gut microbiota and metabolic disease.

The consumption of a high fat/high sugar, westernized diet leads to changes in the composition/activity of the gut microbiota, which increases circulating endotoxin, inducing a state of metabolic endotoxemia [1,2,14,15]. Metabolic endotoxemia is associated with the development of a low-grade chronic inflammatory state, obesity, and insulin resistance in rodents [1,2]. Endotoxemia is associated with an increased prevalence and incidence of T2DM [16]. In humans, fasting endotoxin concentrations are higher in prediabetic and T2DM compared with normoglycemic individuals [8,17].

We recently reported that a short-term (5 days) high fat diet (HFD) increased fasting endotoxin concentrations, altered postprandial excursions of serum endotoxin, and led to metabolic and transcriptional responses in skeletal muscle in young, healthy volunteers [18]. Specifically, glucose oxidation and several transcriptional targets of metabolism and inflammatory signaling increased in response to a meal challenge before but not following a short-term high fat diet. We have also previously reported that LPS, at concentrations similar to those observed during metabolic endotoxemia, alters substrate preference of skeletal muscle to favor glucose over that of fatty acids in both cultured myotubes and rodents [19]. Accordingly, we tested the hypotheses that a short-term high fat diet would increase intestinal permeability and, in turn, increase fasting concentrations of endotoxin. To better understand the relationship between endotoxin and skeletal muscle substrate oxidation in humans, we also studied the oxidation of glucose, pyruvate, and fatty acids in skeletal muscle samples collected under fasting and postprandial conditions prior to and following short term high fat diet. We hypothesized that elevated endotoxin following 5 days of high fat diet would be accompanied by decreased postprandial fatty acid oxidation.

2. Methods

2.1. Subjects

Thirteen normal weight (72.1 kg ± 3.2, BMI 23.1 kg/m^2 ± 0.9) and sedentary (≤ 2 days, 20 min/day of low-intensity physical activity) males (age 22.2 ± 1.6 years) served as participants for the study. All were weight stable (< ± 2.5 kg) for six months prior to enrollment, non-smokers with no history or family history of cardiometabolic disease, habitual intake <40% total fat and 15% saturated fat, not taking medications known to affect dependent variables, blood pressure < 140/90 mmHg, fasting glucose <100 mg/dL, LDL cholesterol <130 mg/dL, total cholesterol <200 mg/dL, and triglycerides <250 mg/dL. The Virginia Polytechnic Institute and State University Institutional Review Board approved all study procedures. Participants were informed of all procedures, benefits and any potential risks associated with the study before written consent was obtained.

2.2. Experimental design

Following screening, participants began a two-week lead-in period of controlled feeding (control diet). The prepared meals consisted of 55% carbohydrates (CHO), 30% fat (9% saturated fat), and 15% protein. Following the lead-in period, participants consumed an isocaloric to energy needs HFD for 5 days. The HFD was comprised of 55% fat (25% saturated fat), 30% CHO, and 15% protein. Participants completed a high-fat meal (HFM) challenge [820 kcal (~30% total daily energy intake), 52 g CHO (25%), 24 g protein (12%), 58 g fat (63%, ~26% saturated fat)], before and following the 5-day HFD. After an overnight fast, muscle biopsies were taken immediately prior to, and 4 h after the HFM for assessment of skeletal muscle metabolic response and adaptation (see Fig. 1 for study design).

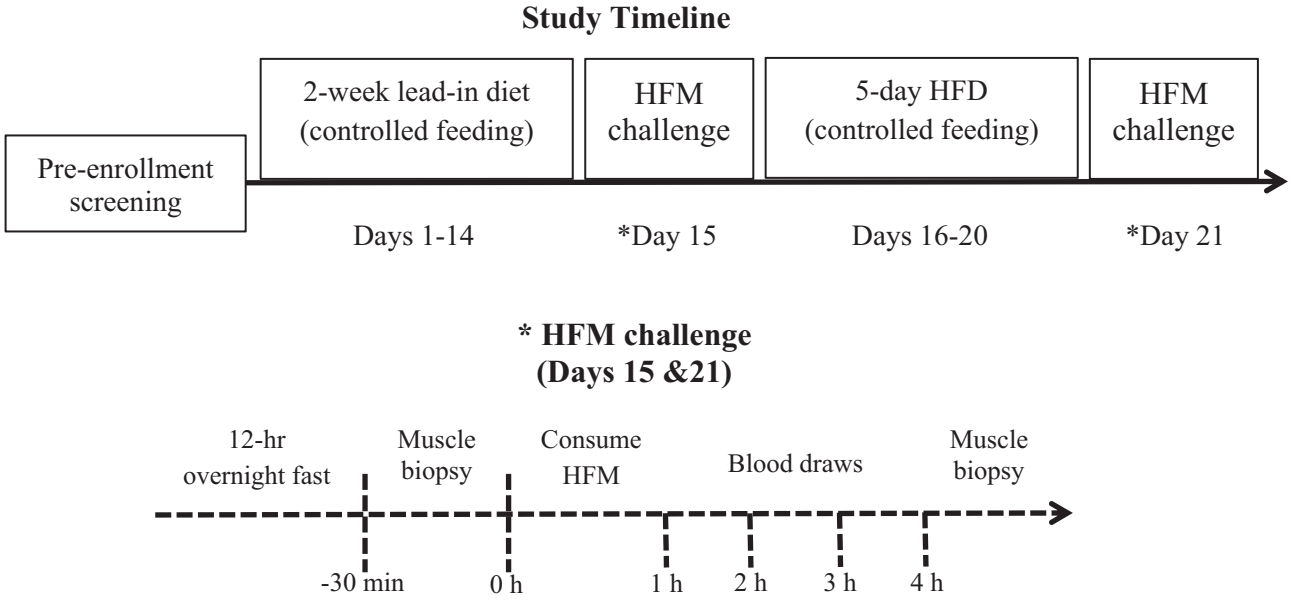

Fig. 1. Schematic of research design. Full study timeline and HFM challenge timeline for the day before the HFD begins (Day 15) and the day following the HFD (Day 21).

Table 1
MS/MS transitions for detection of sugar probes and internal standard ($^{13}C_6$-glucose).

Compound	Retention time (min)	MW (g mol^{-1})	Parent $[M + H]^+$ (m/z)	Daughter (m/z)	Cone voltage (V)	Collision energy (eV)
Sucralose	0.24	396.238	395.238	358.9705	42	10
Mannitol	0.38	182.1748	181.1748	88.8979	28	14
Sucrose	0.43	342.3319	341.3319	178.959	38	12
Lactulose	0.46	342.3319	341.3319	160.934	12	8
$^{13}C_6$-glucose	0.39	186.2596	185.2596	91.8909	18	8

2.3. Controlled feeding procedures

Four-day food intake records were used to confirm that habitual diets contained <40% of total calories from fat. After being trained on proper reporting techniques (using food models and measurement devices clarifying serving sizes) by a research dietitian, participants recorded food intake for three weekdays and one weekend day. The research dietitian, using the three-pass method, reviewed habitual diet records with the participant [20]. The food intake was analyzed using Nutrition Data System for Research (NDS-R) software version 2014 (University of Minnesota) by a trained diet technician. Energy requirements for each participant were estimated using the Institute of Medicine equation based on height, weight, age, and activity level [21]. Both the control diet and HFD were administered on a seven-day cycle of menus consisting of meals and snacks with two optional snack modules (250 kcals). Diets were planned by a research dietitian using NDS-R software. The two-week lead-in (controlled feeding) and five-day HFD period required participants to consume planned meals. The diets provided 3 g of fiber per 500 kcal (± 5 g/day). All meals were prepared in a metabolic kitchen in the Laboratory for Eating Behaviors and Weight Management. Participants ate breakfast while supervised in the laboratory each morning and were provided with a cooler containing food for the remainder of the day. Participants were weighed each day prior to breakfast to ensure weight stability. A trend of >1.0 kg weight loss or gain was offset by adding or subtracting 250 kcal food modules with the same macronutrient composition as the overall diet. All uneaten items and unwashed containers were returned to the metabolic kitchen and weighed to monitor compliance. Participants were instructed to only consume those foods and beverages provided (except water) and were asked each morning if they consumed anything other than what was provided.

2.4. High fat meal challenge

The purpose of the HFM challenge was to characterize the fasted to fed transition period before and following the HFD. Participants arrived at the laboratory following a 12-hour overnight fast. Upon arrival, they were interviewed to ensure protocol compliance after which their first biopsy was taken from the vastus lateralis muscle. Biopsies were taken before and 4 h after a HFM. Participants were required to consume the HFM within 10 min. Following the initial biopsy, an intravenous catheter was inserted in an antecubital vein for baseline and hourly blood sampling. Participants remained seated and awake for the duration of the meal challenge; movies, reading, and homework were permitted. The biopsies taken before and following the meal were obtained from different legs.

2.5. Anthropometrics

Body weight was measured to the nearest ±0.1 kg on a digital scale (Model 5002, Scale-Tronix, White Plains, NY). Height was measured to the nearest ±0.1 cm using a stadiometer (Model 5002, Scale-Tronix, White Plains, NY). Body composition (total fat and fat-free mass) was analyzed by dual-energy x-ray absorptiometry (General Electric, Lunar Digital Prodigy Advance, software version 8.10e Madison, WI).

2.6. Biochemical measurements

Four sugar probes were employed to assess gut permeability [22] as described previously [23,24], assessing permeability in all regions of the gut (see Table 1). Urine was collected in two pooled samples: a 0–5 h sample representative of gastric and small intestinal permeability (collected during the visit), and a 6–24 h sample representative of colonic permeability (collected after the visit) [25,26]. Urine was collected in 24 h collection containers with 5 mL 10% thymol in methanol (w/v) to inhibit bacterial growth. Excretion (quantified by UPLC-MS/MS) was calculated as a percentage of total sugar dose recovered as described previously [23]. Data acquisition, processing, and quantification of the sugar probe data was performed using Waters MassLynx v4.1 software. Serum endotoxin concentrations were determined using the PyroGene Recombinant Factor C endotoxin detection assay (Lonza, Basel, Switzerland). Serum free fatty acid concentrations were determined using the free fatty acids half-micro test assay (Roche Diagnostics, Penzberg, Germany). Serum triglyceride concentrations were determined using the Triglyceride-GPO reagent set assay (Teco Diagnostics, Anaheim, CA). An insulin-augmented frequently sampled intravenous-glucose-tolerance test (IVGTT) was used to assess whole-body insulin sensitivity as described previously [27].

2.7. Skeletal muscle biopsies

Biopsies were taken from the vastus lateralis muscle using a suction-modified Bergström-type needle (Cadence, Staunton, VA) technique [27,28] as described previously [29,30]. Tissue samples were immediately placed in ice cold PBS to remove blood and connective tissue. Muscle tissue collected was prepped and stored as previously described [31].

2.7.1. Substrate metabolism measurement

Substrate oxidation in vastus lateralis muscle was measured using radio-labeled fatty acid ([1-^{14}C]- palmitic acid) from Perkin Elmer (Waltham, MA), specifically measuring $^{14}CO_2$ production and ^{14}C-labeled acid-soluble metabolites (ASM) as previously described [18]. Fatty acid suppressibility of pyruvate oxidation (supPO) was assessed by measuring [1-^{14}C]-pyruvate in the presence or absence of 0.5% non-labeled bovine serum albumin (BSA) bound-palmitic acid (100 μM) as previously described [31]. A decrease in pyruvate oxidation in the presence of free fatty acids was indicative of increased supPO, and thus suggestive of a preference for fatty acid as the oxidative substrate over that of pyruvate. No change or an increase in pyruvate oxidation in the presence of free fatty acids was indicative of no change or a decrease in supPO, and thus a preference for pyruvate as an oxidative substrate.

2.7.2. Enzyme activity analysis

Citrate Synthase (CS) activity was assessed by measuring the reduction of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) from the formation of Coenzyme A (CoASH) over time. Malate Dehydrogense (MDH) activity was measured spectrophotometrically at 340 nm at 37 °C. For the determination of β-hydroxyacyl-CoA dehydrogenase (BHAD), oxidation of NADH to NAD was measured. All enzyme activity analyses were performed as previously described [29,32].

Table 2
Participant characteristics before and following the HFD.

Variable (n = 13)	Before	Following
Age (yrs)	22.2 ± 0.4	–
Height (m)	1.77 ± 0.02	–
Weight (kg)	72.1 ± 3.2	71.2 ± 2.9
BMI (kg/m^2)	23.1 ± 0.9	23.0 ± 0.8
Body fat mass (kg)	16.6 ± 2.1	16.3 ± 2.0
Body fat (%)	22.0 ± 1.7	21.4 ± 1.7
Fat free mass (kg)	54.2 ± 1.7	54.5 ± 1.9

All data are expressed as mean ± SEM.

2.8. Statistics

Two-way repeated measures analysis of variance was used to determine differences in meal responses before and following the HFD. Multiple comparisons were performed using a Tukey post-hoc analysis. Independent *t*-tests were used to compare percent change in protein levels between before and after high fat meal time points, before and following a HFD. Paired t-tests were used to compare intestinal permeability before and following a HFD. Relationships among variables of interest were assessed using Pearson Product Moment correlation analysis. Data that did not follow a normal distribution were $\log_{10}$ transformed. All data are expressed as means ± standard error of the mean (SEM). The significance level was set a priori at $p < 0.05$.

Sample size/power calculations were based on the number of subjects needed to detect statistically ($p < 0.05$) and physiologically significant differences in the magnitude of change in substrate oxidation in response to the high fat diet. We aimed for a >80% power to detect significant changes for an effect size as small as 0.50. Following an interim analysis of included participants, the study was ended due to a sufficient number of subjects (n = 13) for the intended outcome.

3. Results

Participant characteristics are shown in Table 2. There were no significant changes in weight, BMI, body fat mass, or lean mass following the HFD. The energy and macronutrient content of the lead-in (control) diet, and HFD are presented in Table 3.

There was no significant change in gastroduodenal, intestinal, or colonic permeability following the HFD (Table 4). Fasting endotoxin concentrations increased (1.2 ± 0.1 vs. 2.3 ± 0.4 Eu/mL, p = 0.04) following the HFD, but postprandial serum endotoxin concentrations area under the curve did not change following the HFD (9.88 vs 10.79 Eu/ml, p = 0.66, Fig. 2).

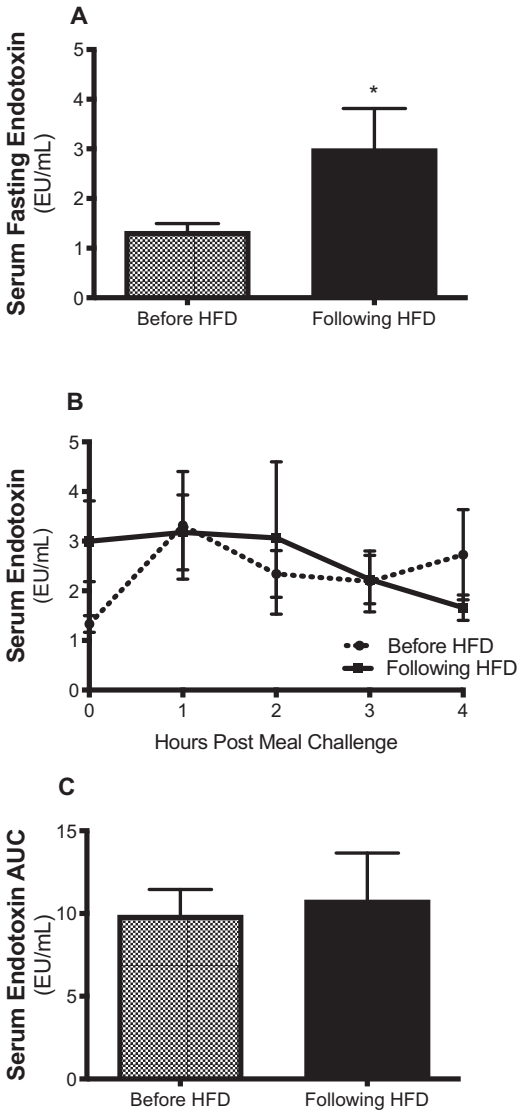

Fig. 2. Fasting and postprandial serum endotoxin concentrations before and following high fat diet. Blood was taken at baseline and every hour for four hours after the meal challenge and was analyzed by an endotoxin detection assay kit to determine differences before and following the HFD. (A) Fasting serum endotoxin is higher following a HFD ($p = 0.04$) (B, C) Serum endotoxins show some variation before and after the HFD although not significant (before 9.9 EU/mL vs after 10.8 EU/mL). All data are expressed as mean ± SEM. **Significant difference found.*

Table 3
Energy and macronutrient content of the lead-in and HFD.

Condition	Energy (kcal/day)	Protein (%kcal)	CHO (%kcal)	Fat (%kcal)	SFA (%kcal)
Lead-in	2756 ± 74	15.2 ± 0.05	55.7 ± 0.06	30.1 ± 0.06	8.6 ± 0.1
High fat	2737 ± 95	14.8 ± 0.02	30.1 ± 0.17	55.4 ± 0.06	24.8 ± 0.1

All data are expressed as mean ± SEM.

Table 4
Gut permeability before and following the HFD.

Variable (n = 13) (values expressed as excretion ratio)	Time of collection	Before HFD	Following HFD	p-Value
Gastroduodenal permeability	0–5 h	0.058 ± 0.011	0.075 ± 0.016	0.13
Small intestinal permeability	0–5 h	0.035 ± 0.005	0.056 ± 0.008	0.084
	6–24 h	0.15 ± 0.0204	0.11 ± 0.014	0.22
Colonic permeability	0–5 h	0.45 ± 0.092	0.52 ± 0.122	0.66
	6–24 h	0.62 ± 0.107	0.55 ± 0.067	0.48

All data are expressed as mean ± SEM.

Table 5
Substrate oxidation and oxidative enzyme activities in fasting and fed conditions before and following the HFD.

	Before HFD Fasted	Before HFD Fed	Following HFD Fasted	Following HFD Fed
Glucose oxidation[c,d,e] (nmol/mg protein/hr)	4.5 ± 0.7	7.3 ± 1.1	6.2 ± 0.7	4.6 ± 0.5
Fatty acid oxidation[b,c,d,e] (nmol/mg protein/hr)	7.4 ± 1.0	12.8 ± 1.4	8.2 ± 1.1	8.4 ± 1.1
Pyruvate oxidation[a,b,d,e] (nmol/mg protein/hr)	427.6 ± 33.4	444.1 ± 36.4	386.9 ± 35.5	289.8 ± 21.2
FA suppressibility of pyruvate oxidation[b,c,d,e]	1.4 ± 0.1	1.8 ± 0.2	1.5 ± 0.1	1.6 ± 0.1
CS[c,e] (μmol/mg protein/min)	105.6 ± 14.0	143.3 ± 20.2	104.3 ± 12.9	81.7 ± 13.1
MDH[c,e] (μmol/mg protein/min)	1760.9 ± 144.0	2004.1 ± 89.3	1589.9 ± 154.0	1440.1 ± 93.4
BHAD (μmol/mg protein/min)	53.9 ± 6.0	47.8 ± 7.7	52.9 ± 6.7	35.3 ± 4.0

CS = citrate synthase; MDH = malate dehydrogenase; BHAD = 3-hydroxyacyl-CoA dehydrogenase. All data are expressed as mean ± SEM.
[a] HFD effect (significance $p < 0.05$).
[b] HFM challenge effect (significance $p < 0.05$).
[c] HFD/HFM challenge interaction (significance $p < 0.05$).
[d] Change in postprandial oxidation after HFD (significance $p < 0.05$).
[e] Percent change in response to HFM challenge (significance $p < 0.05$), PO $p = 0.09$. See Fig. 3.

In an effort to better understand the adaptive response in skeletal muscle to transient changes in dietary macronutrient composition, we assessed substrate oxidation in skeletal muscle homogenates from samples collected under both fasting and postprandial conditions. There were no significant changes in fasting skeletal muscle glucose oxidation (GO), fatty acid oxidation (FAO), pyruvate oxidation (PO), and supPO following the HFD (data not shown). A significant HFD x HFM interaction for GO ($p = 0.002$), FAO ($p = 0.04$), and supPO ($p = 0.03$) was observed (no interaction for PO) (Table 5). The observations suggest that making such measures in fasting samples only are less informative than those collected under dynamic circumstances such as postprandial conditions.

Both glucose and fatty acid oxidation were significantly increased following the HFM before the HFD but this response was attenuated after the HFD. Postprandial GO, FAO, PO and supPO increased before the HFD, but the increase was significantly attenuated following the HFD (Table 5). The percentage change in GO ($p = 0.003$), FAO ($p = 0.04$), PO ($p = 0.09$) and supPO ($p = 0.01$) was smaller following the HFD (Fig. 3). Postprandial GO increased before the HFD (+96.9% ± 36.3) but not following (−24.3% ± 4.5, $p = 0.003$) (Fig. 3A).

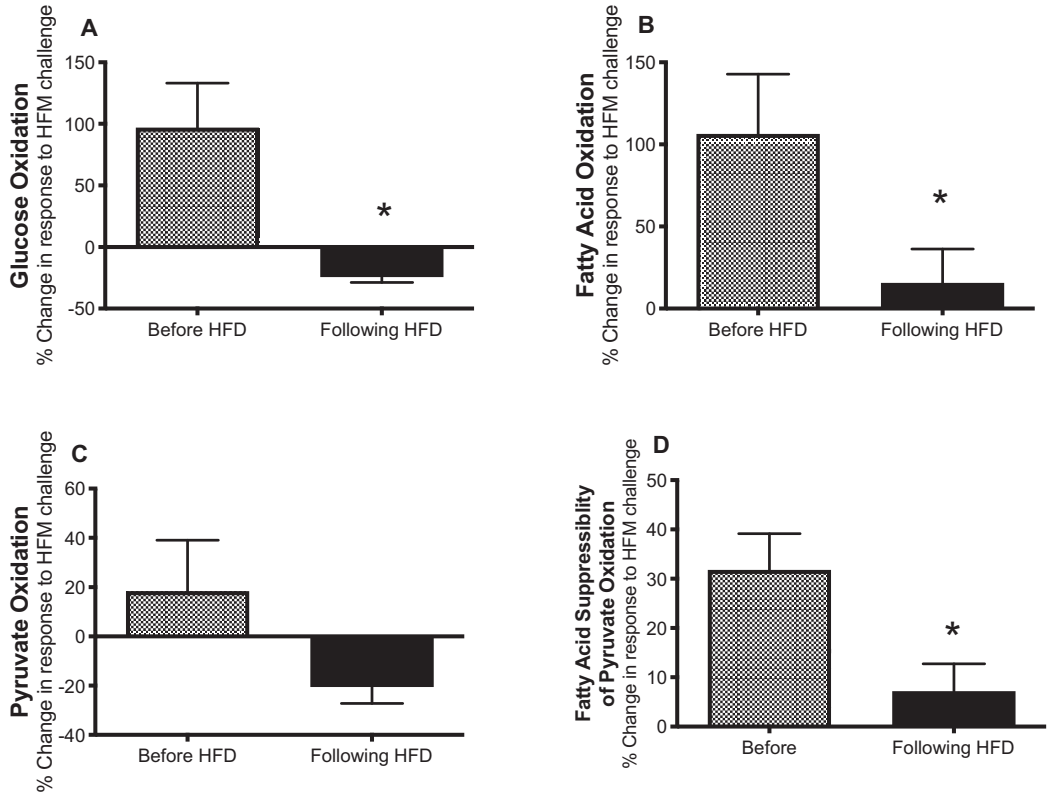

Fig. 3. Skeletal muscle substrate metabolism before and following the high fat diet. Substrate oxidation was measured using radiolabeled substrates in muscle homogenates. Five days of isocaloric HFD disrupted postprandial substrate oxidation in skeletal muscle. The percentage change in (A) GO ($p = 0.003$), (B) FAO ($p = 0.04$), (C) PO ($p = 0.09$) and (D) supPO ($p = 0.01$) was smaller following the HFD. All data are expressed as mean ± SEM. **Significant difference found.*

Postprandial FAO increased before the HFD by 106.3% ± 36.6, but this effect was blunted to 15.6% ± 20.8 ($p = 0.04$) following the HFD (Fig. 3B). Postprandial PO before the HFD was 18.3% ± 20.7 and following the HFD, it was −20.42% ± 6.8 ($p = 0.09$) (Fig. 3C). There was a significant attenuation in fatty acid suppressed pyruvate oxidation in the fasted to fed transition following the HFD. Postprandial supPO before the HFD was 31.8% ± 7.4, and following the HFD, +7% ± 5.6 ($p = 0.01$) (Fig. 3D). These data highlight that the short term high fat diet had a blunting effect on our in vitro measures of substrate oxidation, which one could speculate is due to high fat diet-induced post-translational modifications in the skeletal muscle that retained in our homogenate systems. These data also highlights the utility of this controlled feeding model for future studies to better understand diet-induced post-translational modifications important for metabolic adaption to diet.

Postprandial serum free fatty acids AUC was higher following the HFD than before the HFD (Fig. 4A & B, $p = 0.03$). Fasting triglycerides concentrations declined (0.85 ± 0.11 vs. 0.53 ± 0.06 mmol/L, $p < 0.001$), and the postprandial triglycerides AUC was significantly lower following the HFD (Fig. 4C & D, $p = 0.01$, 5.82 ± 2.5 vs 4.23 ± 0.5 mmol/L/h). Fasting glucose (4.6 ± 0.2 vs. 4.5 ± 0.2 mmol/L), insulin (6.3 ± 2.7 vs. 6.5 ± 2.5 μIU/mL) and free fatty acids (481 ± 84 vs. 462 ± 69 μmol/L) concentrations did not change (all $p > 0.05$) following the HFD.

Estimated insulin sensitivity (5.6 ± 0.7 vs. 4.78 ± 06 × 10^{-4} min^{-1}/(μLU/mL)) did not change following the HFD. Additionally, there was no change in disposition index (DI), acute insulin response to glucose (AIRg), or glucose effectiveness (Sg), all derived from the MINMOD (data not shown).

Similar to substrate oxidation, significant HFD × HFM interactions for CS and MDH activity were found (Table 5). In addition, both CS and MDH activity increased significantly after the HFM before the HFD; however, the activity of both decreased after the HFM (both $p < 0.05$) following the HFD. BHAD did not change ($p > 0.05$) with the HFD or HFM.

4. Discussion

4.1. Intestinal permeability and endotoxins

A major finding of the present study was that 5 days of HFD in healthy participants resulted in an approximately 2-fold increase in fasting serum endotoxin concentrations. The increase in serum endotoxin concentration observed in the present study is consistent with prior studies in animal models [2,33–35], our previous study [18], and others in humans [14,36,37] that demonstrate that serum endotoxin concentration was significantly higher following a HFD. Increased gut permeability has been linked to elevated circulating endotoxin concentrations [38,39]. However, in contrast to our hypothesis, intestinal permeability did not significantly change following the HFD. As such, it is possible that other mechanisms contributed to the increase in serum endotoxin.

Endotoxins may enter circulation via very low density lipoproteins [40], high density lipoproteins or chylomicrons [40–42], which may have contributed to intestinal transport of endotoxin during the postprandial period. This would seem unlikely to impact colonic transport of endotoxins or fasting endotoxin concentrations, where and when intestinal lipoprotein transport would be minimal, respectively. Moreover, it is not clear how endotoxin binding to lipoproteins affects the measurement of serum endotoxins, and if this is confounding. Additionally, elevated fasting endotoxin concentrations and the flat postprandial excursion is inconsistent with a role for lipoprotein transport given their expected postprandial rise and fall.

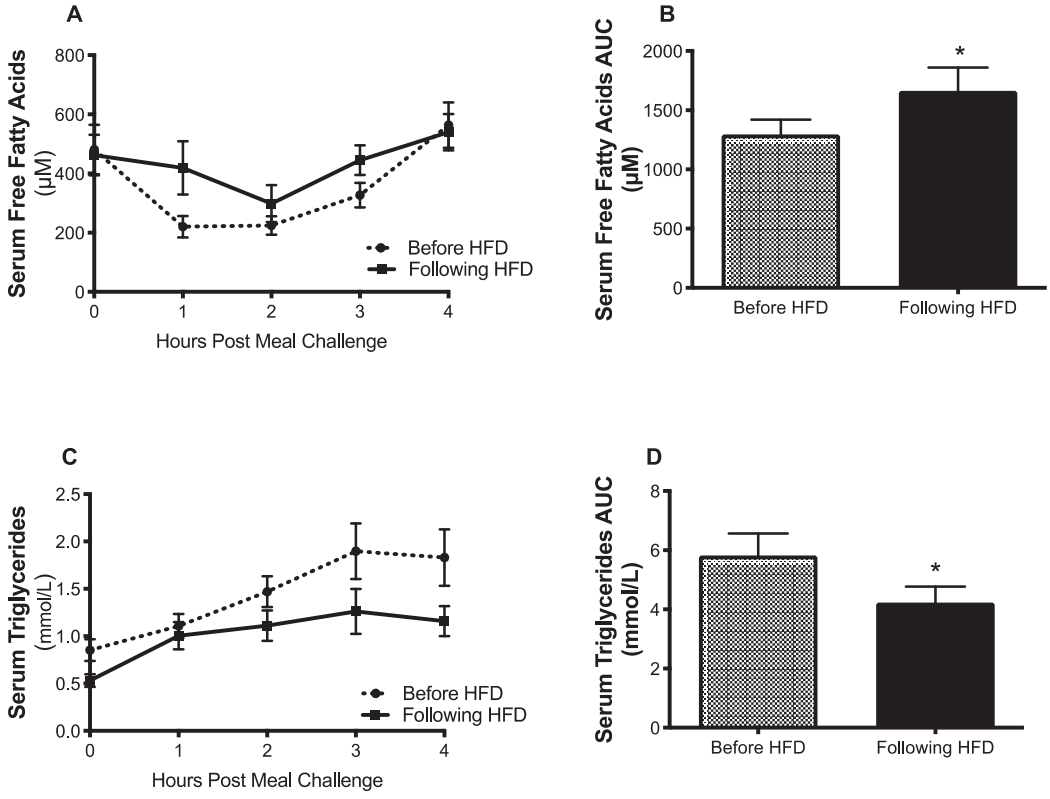

Fig. 4. Fasting and postprandial serum FFA and triglyceride concentrations before and following high fat diet. Blood was taken at baseline and every hour for four hours after the meal challenge and was analyzed by assay kits to determine differences before and following the HFD. (A, B) Serum free fatty acids were elevated postprandially following the HFD across the entire postprandial period ($p = 0.03$). (C, D) Serum triglycerides were lower postprandially following the HFD ($p = 0.01$). All data are expressed as mean ± SEM. **Significant difference found.*

Of further interest, two factors controlling endotoxin absorption are permeability and the concentration gradient (inside vs. outside the gut barrier). A potential explanation is that gut endotoxin concentrations changed, but permeability didn't. Passive absorption via paracellular mechanisms is driven by a concentration gradient. Flow occurs in an attempt to normalize the concentration differences on both sides of the barrier. If the gradient changes, flux through the barrier will change even if permeability does not.

Contrary to our hypothesis, gut permeability did not change as a result of short term high fat feeding. This is counter to studies of obese mouse models that have observed changes in permeability, although with longer intervention periods (up to 12 weeks) [2,43,44]. Additionally, others have reported no change in intestinal permeability in genetically obese mice [45]. It is also likely that anatomical and physiological differences between species are contributing factors to our disparate finding. Previous studies of obese humans have shown associations with intestinal dysbiosis and permeability [46–48]. Our observations are consistent with the work from Morales et al. showing 7 days of a HFD had no effect on gut permeability using the same methodology described herein [49]. Our findings confirm and expand on their study as the high fat feeding intervention described here was a controlled feeding intervention under energy balance conditions. A recent review from Rohr and colleagues examined the effects of high fat diet on intestinal permeability and highlighted gut dysbiosis influences markers of inflammation and permeability [50], but made no reference to such in healthy humans in the context of short term changes in dietary fat content.

The impact of small changes in intestinal or colonic permeability on serum endotoxin appearance is not known. While we have observed elevated serum endotoxin under fasting conditions and attenuated postprandial excursions in the current and a previous study using the identical feeding model in humans [18], the mechanisms remain unclear.

4.2. Substrate oxidation and oxidative enzymes

Previous work from our group has shown that LPS, at concentrations consistent with metabolic endotoxemia, resulted in increased GO and suppressed FAO in skeletal muscle cell lines and mouse models [19,29]. As stated above, we have also reported an increase in serum endotoxin in humans under fasting conditions and during the postprandial period following a high fat meal following 5 days of high fat feeding [18]. While there was no obvious relationship in the current study between serum endotoxin and preference for glucose as an oxidative substrate in human skeletal muscle, we have observed an interesting adaptive response in skeletal muscle of humans during the transition from a diet of moderate/high CHO content to one of low CHO and high fat. Our results are not consistent with our previous findings in rodents and skeletal muscle cell lines that LPS suppresses fatty acid oxidation, as we did not observe changes in samples collected under fasting conditions, when we found a significant increase in serum endotoxin. Also, the attenuation of both glucose and fatty acid oxidation under postprandial conditions occurred when endotoxin excursions were altered. It is possible that the changes in substrate metabolism are a reflection of skeletal muscle adaptation to dietary change, and thus a confounding factor in discerning the effects of altered serum endotoxin concentrations on skeletal muscle substrate metabolism.

The subjects in our study were healthy, normal weight, and experienced no changes in weight, body composition, or insulin sensitivity. However, we observed blunted postprandial changes in substrate oxidation and oxidative enzyme activities. There is evidence from obese individuals and those with T2DM of suppressed skeletal muscle fatty acid oxidation and oxidative enzyme activities [51,52]. As such, our observations suggest adverse effects, yet we have no evidence to support this assumption. Perhaps our findings have captured the normal metabolic response (in healthy humans) to a shift in macronutrient composition of diet from moderate high CHO/moderate fat content to low CHO/high fat content. While we did not measure triacylglycerol and glycogen content or enzyme activities associated these pathways due to limited sample, it is possible that these actions were upregulated in our sample of healthy humans as they adapted to a lower CHO/high fat diet.

In the current study, we also employed an assay to assess substrate preference (fatty acids vs. pyruvate) by measuring the ability for palmitate to suppress pyruvate oxidation in muscle homogenates. To our knowledge, this is the first study to use fatty acid suppressed pyruvate oxidation as a measure of substrate preference in skeletal muscle, and moreover, during the fasted-fed transition. Our observation of the reduction in fatty acid suppressed pyruvate oxidation during the fasted-fed transition (~30% to <10%, Fig. 3D) aligns with the reduced fatty acid oxidation measures under the same conditions, and supports the notion that TAG synthesis may be favored. Our observations also suggest there may be post-translational modifications (acetylation, OGlyNAc, phosphorylation, or other) that are retained in the homogenate system to affect substrate oxidation and enzyme activity. Taken together, these data provide evidence for the utility of our controlled feeding model as a tool to better characterize metabolic adaptation in humans under a variety of conditions.

We observed that fatty acids were less effective at suppressing pyruvate oxidation following the HFD, which contradicts previous reports in whole body changes in fat oxidation following a high fat diet [53–55]. The contradiction may reflect methodological differences, as the current study measured substrate oxidation in skeletal muscle homogenates, which may not be representative of whole body substrate oxidation. These observations highlight the importance of better understanding how metabolically active tissues such as liver, adipose tissue, and skeletal muscle adapt to diets of differing macronutrient composition and how these adaptations influence whole body substrate oxidation.

As reported, we did not observe any change in whole body insulin sensitivity, as measured via IVGTT, in response to the short term high fat diet. However, we did observe difference in postprandial excursions of serum free fatty acids (higher) and triacylglycerols (lower) following the high fat diet. One could speculate that the blunted postprandial suppression of free fatty acids following the high fat diet is reflective of modest diet-induced insulin resistance at the level of adipose tissue. However, this would need to be confirmed using the hyperinsulinemic-euglycemic clamp, which was beyond the scope of our study. The reduction in serum triacylglycerols is perhaps suggestive of increased clearance, which may have been an adaptive response to the short term high fat diet at the level of lipoprotein lipase. These observations warrant further interrogation of site specific insulin sensitivity and triacylglycerol clearance with transient changes in dietary macronutrient concentrations.

4.3. Strengths, limitations

The current study was an isocaloric controlled feeding trial where the meals were provided by our metabolic kitchen. Healthy young males acted as their own controls and remained weight stable indicating compliance throughout the study. In addition, muscle biopsies were analyzed immediately following the collection to determine substrate oxidation at the level of the skeletal muscle. Among these strengths, there are also limitations with the study.

The HFD used in the present study was not associated with changes in gut permeability. An ideal assay has yet to be determined for the measurement of endotoxins, and likewise gut permeability [38,42], and thus, the urine sugar probe test may have lacked the sensitivity to detect modest changes [56–59]. Future studies might employ a measure of plasma levels of glucagon-like peptide-2 (GLP-2) which has been shown to detect gut barrier function [60] or other assays such as bacteria related assays, histological approaches or different biomarkers as described by Bischoff et al. [38]. Also, future studies will be needed to

determine the relationships between intestinal lipoprotein transport, gut permeability, and serum endotoxin appearance in humans.

The types of fatty acids in the diet were not manipulated in the present study. Saturated fat was the most abundant fat in the diet consistent with that of a western diet. Manipulation of other types of fatty acids in the HFD may have different outcomes than the present study. In addition, we measured substrate level metabolism in skeletal muscle, and the impact or relation to substrate oxidation and metabolic switching at the whole body level is yet to be determined.

5. Conclusion

Previous observations of increased serum endotoxin and altered postprandial excursion following short term high fat diet in young healthy male humans were confirmed; however, no differences in gut permeability were detected. We also did not observe preferential oxidation of glucose over that of fatty acids in skeletal muscle samples collected under conditions of elevated serum endotoxin. We did observe disparate patterns of fasted-fed transition measures of substrate oxidation and enzyme activities in skeletal muscle, demonstrating a normal adaptive response to short term changes in dietary macronutrient composition. Thus, we suggest that our five day HFD feeding model may be an appropriate tool for understanding the post-translational signals that govern metabolic adaptation to dietary change.

Future studies involving metabolic characterization of participants and adaptations to dietary interventions are needed. Collectively, these data may provide important new insight regarding the impact of the prevailing diet on skeletal muscle metabolism and, potentially, its regulation in health and disease.

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Author contributions

MWH, KPD, BMD, APN conceived and designed the research; SMB, RPM, NEB, MDT, ATS, KLO collected the data; SMB, RPM, MWH, KPD, APN analyzed the data; SMB, RPM, MWH interpreted the results of the data; SMB, APN prepared figures and tables; SMB, MWH, KPD drafted the manuscript. SMB, RPM, NEB, MDT, APN, BMD, KPD, MWH edited, revised and approved the final version of the manuscript.

Declaration of competing interest

Authors declared no conflict of interest.

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