Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Probiotic Supplementation Attenuates Increases in Body Mass and Fat Mass During High-Fat Diet in Healthy Young Adults

Kristin L. Osterberg[1,2], Nabil E. Boutagy[1,2,3], Ryan P. McMillan[1,3], Joseph R. Stevens[1,2], Madlyn I. Frisard[1,2,3], John W. Kavanaugh[1], Brenda M. Davy[1,2], Kevin P. Davy[1,2,3], and Matthew W. Hulver[1,2,3]

Objective: The objective was to determine the effects of the probiotic, VSL#3, on body and fat mass, insulin sensitivity, and skeletal muscle substrate oxidation following 4 weeks of a high-fat diet.
Methods: Twenty non-obese males (18-30 years) participated in the study. Following a 2-week eucaloric control diet, participants underwent dual X-ray absorptiometry to determine body composition, an intravenous glucose tolerance test to determine insulin sensitivity, and a skeletal muscle biopsy for measurement of in vitro substrate oxidation. Subsequently, participants were randomized to receive either VSL#3 or placebo daily during 4 weeks of consuming a high-fat (55% fat), hypercaloric diet (+1,000 kcal day^{-1}). Participants repeated all measurements following the intervention.
Results: Body mass (1.42 ± 0.42 kg vs. 2.30 ± 0.28 kg) and fat mass (0.63 ± 0.09 kg vs. 1.29 ± 0.27 kg) increased less following the high-fat diet in the VSL#3 group compared with placebo. However, there were no significant changes in insulin sensitivity or in vitro skeletal muscle pyruvate and fat oxidation with the high-fat diet or VSL#3.
Conclusions: VSL#3 supplementation appears to have provided some protection from body mass gain and fat accumulation in healthy young men consuming a high-fat and high-energy diet.

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Introduction

Obesity and type 2 diabetes are associated with dysbiosis of the gut microbiota (1). High-fat, high-energy diets cause a shift in gut bacterial communities and a modest increase in circulating endotoxin concentrations (2). Once in the circulation, endotoxin binds to the toll-like receptor 4 (TLR4) and activates several proinflammatory pathways (3). This modest elevation in circulating endotoxin, termed "metabolic endotoxemia" (2), has been implicated as a cause for the low-grade systemic inflammation observed in obesity and related metabolic diseases (2,4). Elevated circulating endotoxin has been shown to initiate obesity and insulin resistance in rodent models (4). In addition, elevated endotoxin concentrations have been associated with insulin resistance (4) and increased risk for incident type 2 diabetes in humans (5). Therefore, therapeutic modulation of the gut microbiota may be a promising strategy for prevention and treatment of metabolic diseases.

Some probiotic species, such as *Lactobacillus* and *Bifodobacterium*, may improve intestinal health by discouraging the growth of gram negative bacteria, reducing intestinal inflammation, and improving gut barrier integrity (6). In addition, small, but significant, body mass and fat mass loss has been reported in individuals with obesity following consumption of some probiotic strains (7,8). Interestingly, Hultson et al. (9) reported less body mass and fat mass gain and prevention of insulin resistance in young, healthy subjects consuming a single probiotic strain during 7 days of high-fat overfeeding. Whether these effects are sustained during longer periods of consuming a high-fat diet is unknown. Importantly, probiotics may have strain-specific anti-inflammatory effects (10), and probiotic mixtures may be more effective than single strains in modulating gut bacterial communities and improving immune function (11). In this regard, VSL#3, a commercial multispecies probiotic, has been reported to attenuate body weight gain, proinflammatory cytokines, and insulin resistance in mice fed a high-fat diet (12). Therefore, we tested the hypothesis that the multi-strain probiotic VSL#3 would attenuate the increase in body mass and fat mass with a high-fat diet in healthy, young, non-obese males. An exploratory aim of the present study was to determine if a more prolonged high-fat diet reduced insulin sensitivity and altered in vitro skeletal muscle metabolism and, if so, whether supplementation of VSL#3 would have a protective influence.

[1] Department of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, Virginia, USA. Correspondence: Matthew W. Hulver (hulvermw@vt.edu) [2] Fralin Translational Obesity Research Center, Virginia Tech, Blacksburg, Blacksburg, Virginia, USA [3] Metabolic Phenotyping Core, Virginia Tech, Blacksburg, Virginia, USA.

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Methods

Participants

Twenty healthy, non-obese (BMI <30 kg m^{-2}) males (18-30 years of age) volunteered to participate in this study. All participants were healthy and not taking any medications that might impact the dependent variables. Furthermore, none of the participants had taken antibiotics or probiotics within the past 6 months, were regular consumers of pre- or probiotics, or had a history of gastrointestinal disorders. Participants were required to meet the following criteria: fasting blood glucose <100 mg dL^{-1}, total cholesterol <200 mg dL^{-1} triglycerides <150 mg dL^{-1}, and resting blood pressure $<140/90$ mmHg. All participants were sedentary (≤ 2 days, 20 min day^{-1} of low-intensity physical activity) and consumed a diet containing $<40\%$ of total energy from fat as assessed from a 4-day food record. The Institutional Review Board at Virginia Polytechnic Institute and State University approved the study protocol. The purpose, risks, and benefits of participating in this study was explained to each participant prior to obtaining written consent.

Experimental design

We utilized a randomized, double-blind placebo-controlled design for the present study. Following screening, subjects consumed a eucaloric control diet (55% carbohydrate, 30% fat, 15% protein) for 2 weeks. The saturated fat content of the control diet was approximately 8% of total energy intake. Energy requirements were estimated based on height, weight, age, and activity level. Diets were planned using Nutrition Data System for Research (NDSR) software version 2012 (University of Minnesota) by a registered dietitian and a 7-day rotating menu was utilized. The individual macronutrients and saturated fat content of the diets were ± 5 g of the target values. The control diet was designed to maintain weight stability and to reduce the variability in the macronutrient composition of the diet and its impact on the gut microbiota among subjects prior to baseline. Additional food items in the form of 250 kcal modules with a macronutrient composition similar to the overall diet were provided to counter any trends in weight loss or gain during the control diet. After the 2-week control diet, the participants underwent baseline testing described in detail below. Subsequently, participants were randomized to receive either placebo or VSL#3 for the 4-week high-fat diet period. The participants reported to the Laboratory for Eating Behavior and Weight Management each morning in a fasted state and body mass was recorded. Breakfast was prepared each day by research staff and consumed under their supervision. Participants received a cooler with food for the remainder of the day to take with them. All unwashed containers along with any uneaten food was returned. Baseline testing measurements were repeated following the intervention.

The 4-week high-fat diet was designed to be comprised of 55% fat, 30% carbohydrate, and 15% protein. The saturated fat content of the high-fat diet was approximately 25% of total energy intake. The high-fat diet also provided an additional 1,000 kcal ($\sim$35%) above daily energy requirements. The extra calories were supplied in the form of a high-fat milk shake that also served as the vehicle for the placebo or VSL#3. The milk shake was composed of ice cream and coconut milk (463 g) and contained 65 g of saturated fat and 81 g of total fat. Two sachets of VSL#3 (450 billion bacteria per sachet) or two identical sachets of placebo (cornstarch) were blended into the milk shake. The strains of bacteria contained in VSL#3 are as follows: *Streptococcus thermophilus* DSM24731, *Lactobacillus acidophilus* DSM24735, *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM24734, *Lactobacillus paracasei* DSM24733, *Lactobacillus plantarum* DSM24730, *Bifidobacterium longum* DSM24736, *Bifidobacterium infantis* DSM24737, and *Bifidobacterium breve* DSM24732. Subjects consumed the entire milk shake with breakfast each day under the supervision of research staff.

Experimental testing

All testing took place at the Human Integrative Physiology Laboratory between the hours of 5:00 and 11:00 am. Participants fasted for 12 h, did not consume caffeine or alcohol, performed no vigorous physical activity for the prior 48 h, and were free from acute illness for the prior 2 weeks.

Measurements

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. Body composition was quantified by dual-energy X-ray absorptiometry (General Electric, Lunar Prodigy Advance, software version 8.10e Madison, WI).

Serum endotoxin was measured with a PyroGene™ Recombinant Factor C Endotoxin Detection Assay (Lonza, Walkersville, MD) with a minimal detection limit of 0.01 EU mL^{-1} (range = 0.01-10.0 EU mL^{-1}). Interleukin (IL) 6 and tumor necrosis factor-α (TNFα) was measured with a BioPlex® multiplex immunoassay (BioRad, Hercules, CA). LPS binding protein (LBP; Abnova, Taipei City, Taiwan) and soluble cluster of differentiation (sCD)14; (Minneapolis, MN) of the LPS-TLR4 signaling cascade were measured by ELISA as these proteins have been suggested to be more stable biological indicators of endotoxin exposure given the known difficulty of detecting circulating endotoxin (13,14). High-sensitivity C-reactive protein (hsCRP; R&D Systems, Minneapolis, MN) was also measured with a commercially available ELISA.

We recently reported that 5 days of a high-fat diet had no effect on insulin sensitivity or in vitro skeletal muscle metabolism in the fasted state (15). As such, an exploratory aim was to determine if a more prolonged high-fat diet reduced insulin sensitivity and altered in vitro skeletal muscle metabolism and, if so, whether supplementation of VSL#3 would have a protective influence. Whole body insulin sensitivity was estimated using Bergman's minimal model (MINMOD Millenium software) using a modified frequently sampled intravenous glucose tolerance test (IVGTT) (16) as previously described (17). The acute insulin response to glucose (AIRg), glucose effectiveness (S_G), and the disposition index (DI) which is the product of S_I and AIRg were also estimated to provide a more comprehensive assessment of glucose homeostasis. Plasma glucose was measured using a YSI Glucose Analyzer (Yellow Springs, OH). Serum insulin concentrations were measured using the Immulite 1000 immunoassay analyzer (Siemens, Deerfield, IL).

Skeletal biopsies were performed using a modified Bergström needle (Cadence, Staunton, VA) with suction as previously described (17). Skeletal muscle samples were homogenized and palmitate and pyruvate oxidation rates were determined as previously described (18). Pyruvate oxidation was used to assess the activity of pyruvate dehydrogenase (PDH), the enzyme that catalyzes the oxidation of

TABLE 1 Subject characteristics before and after the 4-week high-fat diet

Variable	Placebo ($N = 11$)		VSL#3 ($N = 9$)		*P* values		
	Pre high fat	Post high fat	Pre high fat	Post high fat	Group effect	Time effect	Time × treatment effect
Age (years)	22.9 ± 0.9	-	22.4 ± 1.4	-			
Height (m)	1.8 ± 0.03	-	1.7 ± 0.02	-			
BMI (kg m^{-2})	23.2 ± 0.6	24.0 ± 0.5	23.9 ± 0.9	23.9 ± 0.7*	0.77	0.00	0.09
Body mass (kg)	74.6 ± 2.6	77.0 ± 2.6	74.0 ± 4.0	75.1 ± 2.3*#	0.77	0.00	0.09
Body fat mass (kg)	13.6 ± 1.9	15.0 ± 1.9	16.3 ± 3.0	16.9 ± 3.0*#	0.49	0.00	0.046
Body fat (%)	18.3 ± 2.5	19.5 ± 2.3*	21.2 ± 2.4	21.6 ± 2.3*	0.47	0.00	0.09
Lean mass (kg)	58.28 ± 2.8	59.15 ± 2.7*	55.05 ± 1.6	55.79 ± 1.8*	0.35	0.00	0.75

Values are expressed as mean ± SE.
* denotes a significant time effect.
# denotes a time x treatment effect.

pyruvate and the provision of glucose-derived acetyl CoA to the TCA. [1-^{14}C]-pyruvate oxidation was assessed in a similar manner to fatty acid oxidation with the exception that pyruvate was substituted for BSA-bound palmitic acid. All samples were run in triplicate and data were normalized to total protein content and expressed in nmol/mg protein/h.

Stool samples were collected from all subjects in sterile containers (Commode collection systems), delivered to the laboratory within 24 h of collection, and immediately stored at −80°C. At the conclusion of the study, DNA was extracted from samples using the QIAamp Fast Stool DNA Mini Kit (QIAGEN). Two of the nine VSL#3 bacterial species (*Streptococcus thermophiles* and *Lactobacillus acidophilus)* were selected to be amplified and quantified with the use of previously described 16 S rDNA primers (Integrated DNA Technologies, Coralville, IA) and quantitative real time PCR (qPCR) (19,20). Target gene expression fecal bacteria was normalized to the ribosomal subunit 16 S DNA levels. Relative quantification of target genes were calculated using the ΔΔCT method. Derivation of the ΔΔCT equation has been described in Applied Biosystems User Bulletin no. 2 (P/N 4303859). All samples were run in triplicate and expressed as target gene DNA/16 S DNA in arbitrary units.

Statistical analysis

Our primary outcome was change in body fat mass. We powered our study based on previous studies involving high-fat-diet-induced body mass gain in humans (21-23). With 20 subjects, 80% power and alpha = 0.05 we anticipated being able to detect a 1.0 kg (*Cohen's d* = 1.2) difference in the magnitude of change in body fat mass in VSL#3 compared with control following the high-fat diet. Data were analyzed using SPSS software (IBM SPSS, 22 Armonk, NY). Repeated measures analysis of variance was used to compare subject characteristics and dependent variables over time between the two groups. Tukey's post-hoc analyses were performed for multiple comparisons. One-tailed, independent *t* tests were used to compare the magnitudes of change from baseline in dependent variables. Significance was set *a priori* at $P < 0.05$. All values are presented as mean ± SE.

Results

Subject baseline characteristics

Subject characteristics are shown in Table 1. There were no differences in any of the subject characteristics between VSL#3 and placebo at baseline (all $P > 0.05$). There were no significant differences between the groups in total energy and macronutrient intake, saturated fat intake, or dietary fiber intake (data not shown). Total energy and macronutrient composition of the control and high-fat diets are shown in Table 2. By design, the high-fat diet was higher in total energy, fat, and saturated fat, and lower in carbohydrate and fiber (all $P < 0.001$).

Body mass and composition

Body mass and composition were similar in the two groups at baseline (Table 1). However, the magnitude of increase in body mass (1.42 ± 0.42 kg vs. 2.30 ± 0.28 kg, $P = 0.043$) (Figure 1A) and fat mass (0.63 ± 0.09 vs. 1.29 ± 0.27 kg, $P = 0.023$) (Figure 1B) with

TABLE 2 Mean energy and macronutrient composition of the control and high-fat diet

Diet condition	Energy (kcal day^{-1})	Protein (g day^{-1})	CHO (g day^{-1})	Fat (g day^{-1})	SFA (g day^{-1})	Fiber (g day^{-1})
Control	2,903 ± 78	108 ± 3.0	408 ± 11.0*	98 ± 4.0	28 ± 1.0	18 ± 1.0*
High fat	3,947 ± 79*	125 ± 3.0*	282 ± 6.0	256 ± 5.0*	140 ± 2.0*	14 ± 1.0

Values are expressed as mean ± SE.
CHO = carbohydrates; SFA = saturated fatty acids.
*$P < 0.01$.

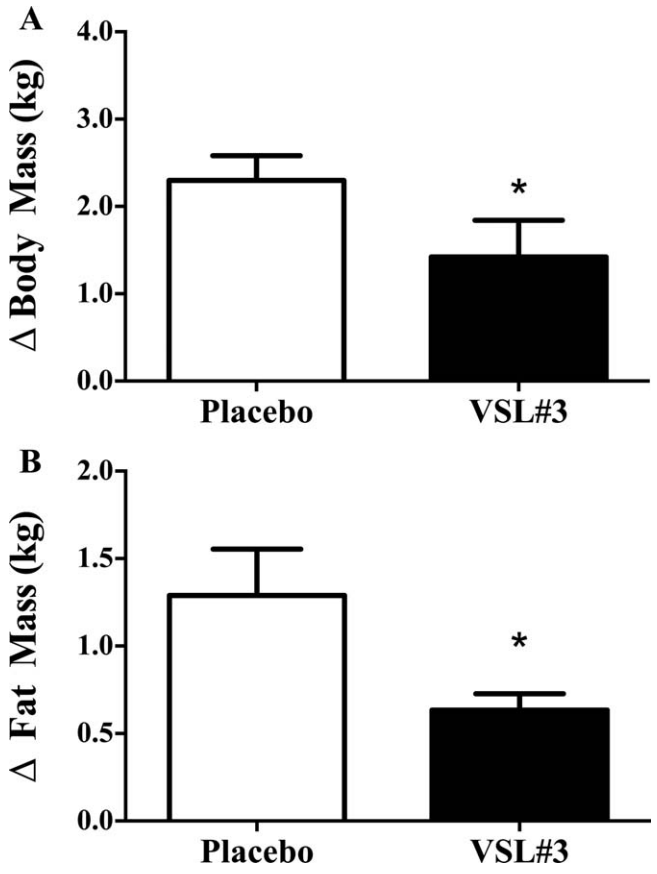

Figure 1 Magnitude of change in (**A**) body mass and (**B**) body fat mass following high-fat feeding. *$P < 0.05$. Values are expressed as mean ± SE.

the high-fat diet was smaller in VSL#3 compared with placebo group, respectively. Fat-free mass increased ($P = 0.001$) in both groups; however, the magnitude of increase attributed to fat-free mass was similar (42.1% ± 10.5% vs. 44.7% ± 7.6%, $P = 0.84$) in the VSL#3 and placebo groups, respectively (Table 1).

Inflammatory cytokines

Circulating proinflammatory biomarkers at baseline and following the high-fat diet in the two groups are shown in Table 3. There were no differences between VSL#3 and placebo in any of these variables at baseline. Neither endotoxin ($P = 0.914$), sCD14 ($P = 0.170$), LBP ($P = 0.598$), nor the LBP/sCD14 ratio ($P = 0.814$) changed following the high-fat diet in either the VSL#3 or placebo groups. IL6 tended to increase ($P = 0.056$) following the high-fat diet in both the VSL#3 (20.2% ± 11%) and placebo groups (52% ± 38%). TNFα increased ($P = 0.007$) following the high-fat diet in both the VSL#3 (16.4% ± 6%) and placebo groups (13% ± 7%); however, the magnitude of change in circulating TNFα was not different ($P = 0.447$) between groups. hsCRP did not change following the high-fat diet in either the VSL#3 or placebo groups ($P = 0.129$).

Insulin sensitivity and in vitro skeletal muscle substrate oxidation

Fasting glucose and insulin concentrations were similar at baseline in VSL#3 and placebo groups (Table 4). Insulin sensitivity, AIRg, Sg, and DI were not different between the groups at baseline. There was no effect of high-fat diet or VSL#3 treatment on any of the IVGTT variables (Table 4). In addition, there were no differences between groups in total (CO_2 + ASM production), or incomplete (ASM production) skeletal muscle fatty acid oxidation, or pyruvate oxidation at baseline nor were these variables affected by high-fat feeding or VSL#3 treatment (Table 5).

Fecal probiotic enrichment

There were no differences in the fecal enrichment of the probiotic species *Streptococcus thermophiles* ($P = 0.609$) *and Lactobacillus acidophilus* ($P = 0.718$) between the groups at baseline. Following the high-fat diet, S*treptococcus thermophiles* was significantly enriched in both groups ($P = 0.002$); however the magnitude of change in fecal bacterial enrichment was greater ($P = 0.02$) in VSL#3 compared with the placebo group (Figure 2A). Similarly, *Lactobacillus acidophilus* was enriched over time ($P = 0.041$); however there was a significant time × treatment interaction ($P = 0.40$), indicating that the VSL#3 group had greater fecal bacterial enrichment compared with the placebo group following the high-fat diet (Figure 2B).

TABLE 3 Circulating proinflammatory biomarkers before and after the 4-week high-fat diet

	Placebo ($N = 11$)		VSL#3 ($N = 9$)		*P* values		
Variable	Pre high fat	Post high fat	Pre high fat	Post high fat	Group effect	Time effect	Time × treatment
Endotoxin (EU)	6.2 ± 0.3	6.8 ± 0.3	6.6 ± 0.3	6.7 ± 0.3	0.47	0.91	0.28
sCD14 (ng mL^{-1})	1743.6 ± 137.5	1624.2 ± 69.3	1690.5 ± 54.0	1601.0 ± 72.0	0.66	0.17	0.96
LBP (μg mL^{-1})	15.0 ± 0.9	15.8 ± 0.7	16.5 ± 1.1	15.1 ± 1.0	0.67	0.59	0.21
LBP/sCD14 (U)	0.01 ± 0.001	0.01 ± 0.0005	0.01 ± 0.0008	0.01 ± 0.0005	0.86	0.81	0.37
IL-6 (pg mL^{-1})	11.3 ± 1.2	14.8 ± 2.8	11.9 ± 1.7	13.6 ± 1.6	0.77	0.06	0.84
TNFα (pg mL^{-1})	12.8 ± 1.2	14.0 ± 1.0	12.1 ± 0.6	13.8 ± 0.5*	0.69	0.01	0.45
hsCRP† (ng mL^{-1})	1.4 ± 0.4	1.6 ± 0.4	2.0 ± 0.5	1.7 ± 0.2	0.58	0.13	0.23

Values are expressed as mean + SE.
sCD14 = soluble cluster of differentiation 14; LBP= lipopolysaccharide binding protein; IL-6 = interleukin 6; TNFα = tumor necrosis factor alpha; hsCRP = high-sensitivity C-reactive protein.
† Values were log transformed.

TABLE 4 Insulin sensitivity before and after the 4-week high-fat diet

Variable	Placebo ($N = 11$)		VSL#3 ($N = 9$)		*P* values		
	Pre high fat	Post high fat	Pre high fat	Post high fat	Group effect	Time effect	Time × treatment
Fasting glucose (mmol L^{-1})	4.0 ± 0.4	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.2	0.64	0.82	0.72
Fasting insulin (mIU mL^{-1})	5.2 ± 1.4	4.2 ± 0.8	6.1 ± 1.5	5.9 ± 1.4	0.17	0.44	0.14
SI [(mU L^{-1}) min^{-1}]	5.8 ± 0.9	5.9 ± 0.6	5.9 ± 1.3	5.5 ± 0.9	0.79	0.64	0.91
AIRg [(mU L^{-1}) 10 min^{-1}]	526 ± 72	562 ± 83	481 ± 102	523 ± 90	0.73	0.23	0.092
DI	2604 ± 300	2916 ± 479	2160 ± 293	2718 ± 471	0.46	0.26	0.73
Sg (percent min^{-1})	0.03 ± 0.003	0.04 ± 0.007	0.26 ± 0.003	0.04 ± 0.009	0.67	0.09	0.62

Values are expressed as mean ± SE.
SI = insulin sensitivity, AIRg = acute insulin response, DI = disposition index, Sg = glucose effectiveness.

Discussion

The major finding of the present study was that VSL#3 treatment attenuated body mass and fat mass gain following a high-fat diet compared with placebo. However, neither the high-fat diet nor VSL#3 treatment altered circulating endotoxin concentrations, pro-inflammatory cytokines, insulin sensitivity, or skeletal muscle substrate metabolism.

Our findings are consistent with prior observations that rodents fed VSL#3 (12) and other single strain probiotic supplements (24-27) gained less body mass and fat mass during high-fat feeding compared to controls (12). Our findings are also consistent with recent observations (9) that young, healthy subjects consuming a single probiotic strain (*Lactobacillis casei Shirota*) gained less body and fat mass during a high-fat diet compared with placebo.

Some strains of probiotics, such as from the Lactobacillus genus (7,8,28-30) have been reported to be efficacious in promoting body mass loss in obese humans. However, the mechanisms by which probiotics may contribute to body mass loss or attenuate body mass gain are poorly understood. Our study was not designed to address this issue but there are several plausible mechanisms.

First, Jumpertz et al. (31) reported that overfeeding, which produced rapid changes in gut microbial communities, resulted in a reduction in nutrient absorption in non-obese humans. As such, it is possible that VSL#3 supplementation altered the composition of the gut microbiota in a manner that led to reduced energy harvesting and, in turn, led to less body and fat mass gain. Second, Ogawa et al. (29) reported that *Lactobacillus gasseri SBT2055* supplementation suppressed pancreatic lipase activity, promoted fecal fat excretion and reduced energy absorption. Thus, although *Lactobacillus gasseri SBT2055* was not one of the VSL#3 probiotic strains, it is possible that fat mass increased less in the present study through a reduction in lipid absorption (26,29) following the probiotic treatment. Finally, VSL#3 may have increased energy expenditure and whole body fat oxidation (32) via GLP-1 secretion (12), increased angiopoietin-like protein-4 (inhibitor of lipoprotein lipase) (33), and/or increased sympathetic nervous system activity to white and brown adipose tissue (34). Future studies will be necessary to determine the mechanism(s).

Circulating endotoxin, LBP, sCD14, the LBP/sCD14 ratio, and circulating proinflammatory cytokines did not change with the high-fat diet (with the exception of TNFα) or VSL#3 treatment. In contrast, Laugerett et al. (13) reported that 56 days of overfeeding (760 kcal day^{-1} with 70 g of fat) did not increase circulating endotoxin or IL-6 in the fasted state; however, the LBP/sCD14 ratio increased with overfeeding. We have recently shown that a 5-day high-fat diet increases fasting serum endotoxin concentration in healthy, non-obese, young men (15). As such, it is possible that 4-6 weeks of a high-fat, high-energy diet results in adaptations that lead to the detoxification of endotoxin. Longer term studies are needed to

TABLE 5 Skeletal muscle substrate oxidation before and after the 4-week high-fat diet

Variable	Placebo ($N = 11$)		VSL#3 ($N = 9$)		*P* values		
	Pre high fat	Post high fat	Pre high fat	Post high fat	Group effect	Time effect	Time × treatment
Total palmitate oxidation [(nmol mg $protein^{-1}$) hr^{-1}]	8.1 ± 1.0	6.4 ± 0.7	6.02 ± 0.9	5.09 ± 0.4	0.08	0.09	0.62
ASM [(nmol mg $protein^{-1}$) hr^{-1}]	7.4 ± 1.0	5.8 ± 0.7	5.5 ± 0.8	5.0 ± 0.4	0.85	0.15	0.47
PDH activity [(nmol mg $protein^{-1}$) hr^{-1}]	157.5 ± 24.7	133.4 ± 14.8	157.2 ± 21.5	142.6 ± 22.7	0.85	0.35	0.81

Values are expressed as mean ± SE.
ASM = acid soluble metabolites; PDH = pyruvate dehydrogenase.

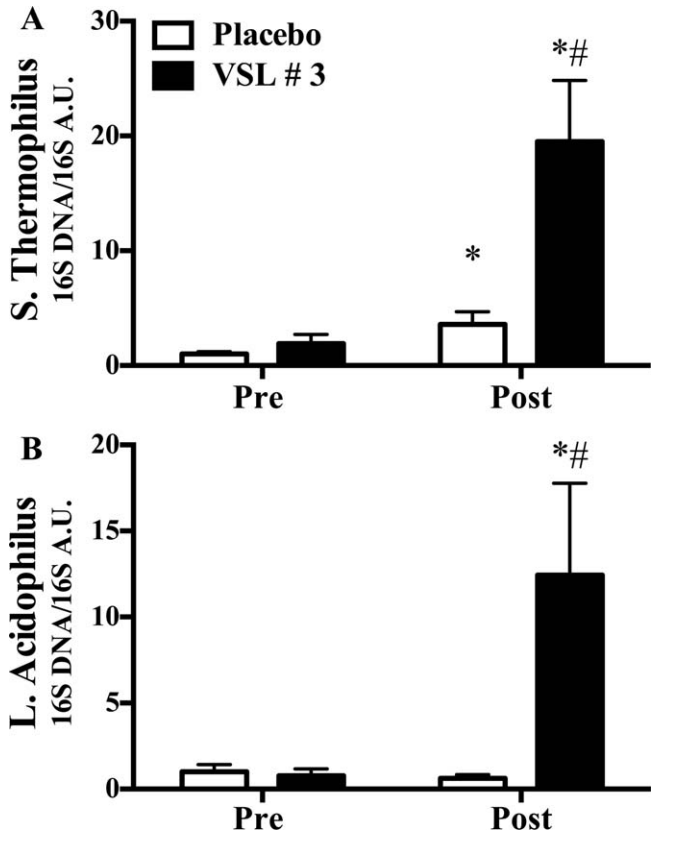

Figure 2 (**A**) Fecal *Streptococcus thermophiles* and (**B**) fecal *Lactobacillus acidophilus* 16 S DNA before and after the high-fat diet with placebo or VSL#3 treatment. *Diet effect, $P < 0.05$; #Interaction effect, $P < 0.05$. Values are expressed as mean ± SE. S. Thermophiles = *Streptococcus thermophiles*; L. acidophilus = *Lactobacillus acidophilus;* A.U. = arbitrary units.

determine if these protective mechanisms persist or become less effective over time.

We hypothesized that supplementation with VSL#3 would attenuate decrements in insulin sensitivity with a high-fat diet by reducing circulating endotoxin and the activity of proinflammatory pathways. In contrast to a previous report (9), insulin sensitivity was not altered by the high-fat diet or VSL#3 treatment in the present study. Our observation is consistent with some (22,35) but not all (23,36-38) previous studies. Nevertheless, the reason(s) for this discrepancy is unclear. We may have been underpowered and/or the IVGTT used in the present study may not have been sensitive enough to detect small changes over the course of the intervention. However, the ability to detect reductions in insulin sensitivity with high-fat feeding does not appear to depend on the method of measurement as decrements have been detected with less sensitive methods (23). Furthermore, some studies utilizing the euglycemic hyperinsulinemic clamp technique have not detected reductions in insulin sensitivity with a high-fat diet (22). At least one previous study (36) has coupled reductions in physical activity with a high-fat diet making comparisons with our study difficult. Future studies will be necessary to better understand how insulin sensitivity is influenced by a high-fat diet.

We recently demonstrated that 5 days of a high-fat diet had no effect on in vitro skeletal muscle metabolism in healthy young males studied in the fasted state (15). As such, we pursued the possibility in the present study that a more prolonged period of consuming a high-fat diet would be necessary to elicit dysregulated skeletal muscle metabolism that mimics that observed in obesity (18). In contrast, the 4-week high-fat diet in the present study failed to alter in vitro skeletal muscle metabolism in the fasted state. Parenthetically, in our previous study (15) we demonstrated that 5 days of a high-fat diet attenuated the increase in skeletal muscle homogenate glucose oxidation in the fasted-to-fed transition. These observations occurred in the absence of any change in insulin sensitivity which is consistent with some (39,40) but not all (9) previous studies of similar duration. Importantly, our observations in skeletal muscle homogenates were associated with an increase in fasting endotoxin concentrations and altered dynamics of the phospho-p38 to total-p38 ratio during the fasting-to-fed transition following the 5-day high-fat diet. Taken together, these observations suggest that future studies may need to focus on understanding how a high-fat diet influences skeletal muscle metabolism in the fasting-to-fed transition and potential interventions should focus on the latter instead of on the fasting state in isolation.

There are some limitations of the present study that should be considered. First, our sample size was relatively small so we may have been underpowered to detect significant changes in some of our secondary outcomes (e.g., insulin sensitivity and skeletal muscle substrate metabolism). Second, we included only healthy, young, male participants. Therefore, it is unknown whether VSL#3 would attenuate the increase in body and fat mass during a high-fat diet in females or in older or less healthy populations. Third, we did not assess compliance to our instructions to participants to maintain their current level of physical activity during the study. Thus, it is possible that the subjects were not compliant with our request and the greater weight loss observed in the VSL#3 group was due, at least in part, to increased physical activity. However, we believe it is unlikely that only the VSL#3 group was non-compliant or spontaneously increased their physical activity level. Finally, we did not attempt to identify specific probiotic strains that may have contributed to the attenuated increase in body and fat mass with the high-fat diet. Future studies will be necessary to address this important issue.

In conclusion, the major finding from the present study is that supplementation with the probiotic VSL#3 provides some protection from body and fat mass gain with a high-fat diet in healthy, young males. The mechanism(s) responsible are unclear. As such, future studies will be necessary to provide this insight. Neither the high-fat diet nor VSL#3 treatment influenced insulin sensitivity or skeletal muscle substrate oxidation. Future studies are needed to determine the amount and type of dietary fat as well as the duration of high-fat diets on insulin sensitivity and in vitro skeletal muscle metabolism. Future interventions may need to target skeletal muscle metabolism in the fasting-fed transition rather than in the fasting state in isolation. O

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