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Short-Term Overfeeding with Dairy Cream Does Not Modify Gut Permeability, the Fecal Microbiota, or Glucose Metabolism in Young Healthy Men

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Abstract

Background: High-fat diets (HFDs) have been linked to low-grade inflammation and insulin resistance.

Objective: The main purpose of the present study was to assess whether acute overfeeding with an HFD affects insulin sensitivity, gut barrier function, and fecal microbiota in humans.

Methods: In a prospective intervention study, 24 healthy men [mean ± SD: age 23.0 ± 2.8 y, body mass index (in kg/m^2) 23.0 ± 2.1] received an HFD (48% of energy from fat) with an additional 1000 kcal/d (as whipping cream) above their calculated energy expenditure for 7 d. Insulin sensitivity (hyperinsulinemic euglycemic clamp), gut permeability (sugar and polyethylene glycol absorption tests, plasma zonulin), and gut microbiota profiles (high-throughput 16S rRNA gene sequencing) were assessed before and after overfeeding, and 14 d after intervention. Additionally, inflammation markers such as high-sensitivity C-reactive protein, lipopolysaccharide-binding protein, leptin, high-molecular-weight adiponectin, calprotectin, regulated on activation normal, T cell expressed and secreted (RANTES), and monocyte chemoattractant protein-1 were measured in plasma by ELISA. Finally, lipid parameters were analyzed in serum by a laboratory service.

Results: Although participants gained 0.9 ± 0.6 kg ($P < 0.001$) body weight, overnutrition was not associated with a significant change in insulin sensitivity (M value and glucose disposal). Overfeeding for 7 d resulted in elevated serum total (10.2%), LDL (14.6%) and HDL (14.8%) cholesterol concentrations ($P < 0.01$). In contrast, fasting plasma triglyceride significantly declined (29.3%) during overfeeding ($P < 0.001$). In addition, there were no significant changes in inflammatory markers. Urine excretion of 4 sugars and polyethylene glycol, used as a proxy for gut permeability, and plasma concentration of zonulin, a marker of paracellular gut permeability, were unchanged. Moreover, overfeeding was not associated with consistent changes in gut microbiota profiles, but marked alterations were observed in a subgroup of 6 individuals.

Conclusions: Our findings suggest that short-term overfeeding with an HFD does not significantly impair insulin sensitivity and gut permeability in normal-weight healthy men, and that changes in dominant communities of fecal bacteria occur only in certain individuals. The study was registered in the German Clinical Trial Register as DRKS00006211. *J Nutr* 2018;148:77–85.

Keywords: high-fat diet, overfeeding, insulin sensitivity, hyperinsulinemic euglycemic clamp, inflammation, gut barrier function, gut permeability, zonulin, intestinal microbiota, lipopolysaccharide-binding protein

Introduction

Western diets are generally characterized by a high energy density with an excessive supply of fat and carbohydrates. Chronic caloric overload in combination with a lack of physical activity often leads to weight gain, chronic inflammation, insulin resistance, and subsequently metabolic disorders such as type

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2 diabetes, particularly in those with a genetic predisposition (1, 2). Low-grade systemic inflammation has been implicated in the development and maintenance of chronic metabolic diseases (3). In recent years, the gut microbiota and impaired gut barrier function have come into focus as potential factors associated with low-grade inflammation (4). High-fat diets (HFDs) have been shown to induce alterations in gut bacterial communities in mice (5, 6), and such changes have been shown by some studies to be associated with elevated plasma endotoxin concentrations, which is a marker for inflammation both in mice (7, 8) and in humans (9). Other studies in humans have shown that LPS concentrations are elevated not only postprandially after a high-fat bolus (10–13), but also 4 and 8 wk after high-fat consumption (14, 15), suggesting increased gut permeability and bacterial translocation. It has also been shown that an HFD rich in saturated fatty acids leads to increased colonic permeability (16). Increased gut permeability is mainly associated with diseases such as celiac disease (17) and inflammatory bowel disease (18). In recent years, a few studies have reported that an increased gut permeability in obese individuals leads to an increased influx of bacterial products (19–21). However, up to now, it is unclear whether acute overfeeding is affecting gut permeability. Therefore, the aim of this study was to investigate whether and how acute overfeeding affects insulin sensitivity, gut permeability, and fecal microbiota composition.

Methods

The study protocol was approved by the ethical committee of the Faculty of Medicine of the Technical University of Munich in Germany (approval no. 5499/12). The study was registered in the German Clinical Trial Register as DRKS00006211. Written informed consent was obtained from all participants before inclusion.

Study participants. Twenty-five healthy, non-smoking, lean men aged 19–31 y were recruited on a voluntary basis between 2013 and 2014 at the campus of the Technical University of Munich in Freising, Germany. The participants' eligibility was assessed with a detailed screening questionnaire including their medical history. Exclusion criteria were: BMI (in kg/m^2) >25, metabolic diseases including familial lipid disorders and other defined diagnoses of lipid disorders, smoking, acute infections, severe diseases (e.g., cancer), treatment with oral anticoagulants or antithrombotic medication, intestinal surgery, or intestinal diseases. One volunteer withdrew due to hypoglycemia during the clamp.

Study design. **Figure 1** summarizes the study design of this single-arm, prospective intervention study. The duration of the study was 4 wk divided into 3 time periods. During the first week (day 0), participants were instructed to keep their usual eating habits. Thereafter, volunteers were subjected to the 7-d overfeeding program (day 7). All participants received the same HFD adapted to their individually measured and calculated total energy requirements. Based on their respective resting metabolic rate (RMR), total energy requirement was calculated by multiplying RMR by a physical activity level factor of 1.5 to achieve individual-specific total energy expenditure. A surplus of 1000 kcal/d was provided by the addition of dairy cream, more precisely whipping cream (341 mL/d), selected due to its high content of SFAs. The macronutrient composition of the HFD was 48 energy percent (EN%) from fat (mainly SFAs), 34 EN% from carbohydrates, and 18 EN% from protein. To ensure compliance, participants received breakfast, lunch, and dinner in the Core Facility for Human Studies of the ZIEL—Institute for Food and Health, Freising, Germany. Subjects were monitored to make sure they completely consumed the energy-enriched meals provided. In addition, subjects were only allowed to drink mineral or tap water or coffee without milk during the week of overfeeding. Finally, during the 2 wk following intervention (day 21), participants were instructed to return to their usual eating habits.

Assessment of diet and physical activity. During the first and third study periods, participants were asked to record their dietary intake in standard forms. The energy content and macronutrient composition of the food were calculated using OptiDiet Plus software (version 5.1.2.046, GOE mbH).

Throughout the study, participants were instructed to minimize physical activity, which was monitored using an accelerometer (ActiGraph GT3X+; ActiGraph Corp.). Participants were asked to wear the accelerometer on their dominant hip during each day of the study. Physical activity was analyzed using ActiLife software (version 6.8.2; ActiGraph Corp.).

Phenotyping. Anthropometric parameters (height, weight, waist-to-hip ratio) were measured in a standardized manner between 0800 and 0900 following an overnight fast. BMI was calculated by dividing body weight in kilograms by height in meters squared (kg/m^2). Body weight and composition were measured using a Tanita BC-418 MA III body composition analyzer (Tanita Corp.). RMR was measured using a canopy hood (COSMED Quark RMR). Systolic and diastolic blood pressure and pulse rate were assessed in a sitting position after 5 min of rest.

Blood sampling and biochemical analyses. Blood samples were collected in the fasting state. Lipid parameters (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides), liver enzymes [aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GT)], creatinine, uric acid, and fasting glucose were analyzed in serum by SynLab (Munich, Germany). Leptin, chemerin, high-sensitivity C-reactive protein (hsCRP), regulated on activation normal, T cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), high-molecular-weight adiponectin (HMW adiponectin), soluble cluster of differentiation (sCD14) and lipopolysaccharide-binding protein (LBP) (all: R&D), insulin (Dako), and zonulin (Immundiagnostik AG) were assayed in plasma using commercially available ELISAs. Non-esterified fatty acid (NEFA) concentrations were determined in plasma using an enzymatic colorimetric method (Acyl-CoA synthetase-Acyl-CoA oxidase-method, Wako Chemicals GmbH). Insulin resistance was estimated according to the Homeostatic Model Assessment–Insulin Resistance (HOMA-IR) (22). Fecal calprotectin was measured by ELISA [CALPROLAB^{TM} Calprotectin ELISA (HRP), FROST Diagnostika GmbH] in feces.

Hyperinsulinemic euglycemic clamp. Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp (HEC) technique. Initially, glucose infusion (20%, Braun) was started at 4 mg/kg body weight · min, followed by an insulin bolus [60 mU/(m^2 surface area · min)] for 5 min to suppress endogenous glucose production. After the priming dose, insulin was infused at a constant rate [40 mU/(m^2 surface area · min)] for ≥150 min. Glucose infusion was adjusted on the basis of continuous analyses of blood glucose concentrations (HemoCue Glucose 201$^+$, plasma-calibrated; HemoCue AB) at intervals of 5 min during the clamp. Steady state was defined as stable blood glucose

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Abbreviations used: ALT, alanine transaminase; AST, aspartate transaminase; EN%, energy percent; HEC, hyperinsulinemic-euglycemic clamp; HFD, high-fat diet; HMW adiponectin, high-molecular weight adiponectin; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide-binding protein; MCP-1, monocyte chemoattractant protein-1; M_r, molecular mass; NEFA, nonesterified fatty acid; OTU, operational taxonomic unit; PEG, polyethylene glycol; RANTES, regulated on activation normal, T cell expressed and secreted; RMR, resting metabolic rate, γ-GT, γ-glutamyltransferase.

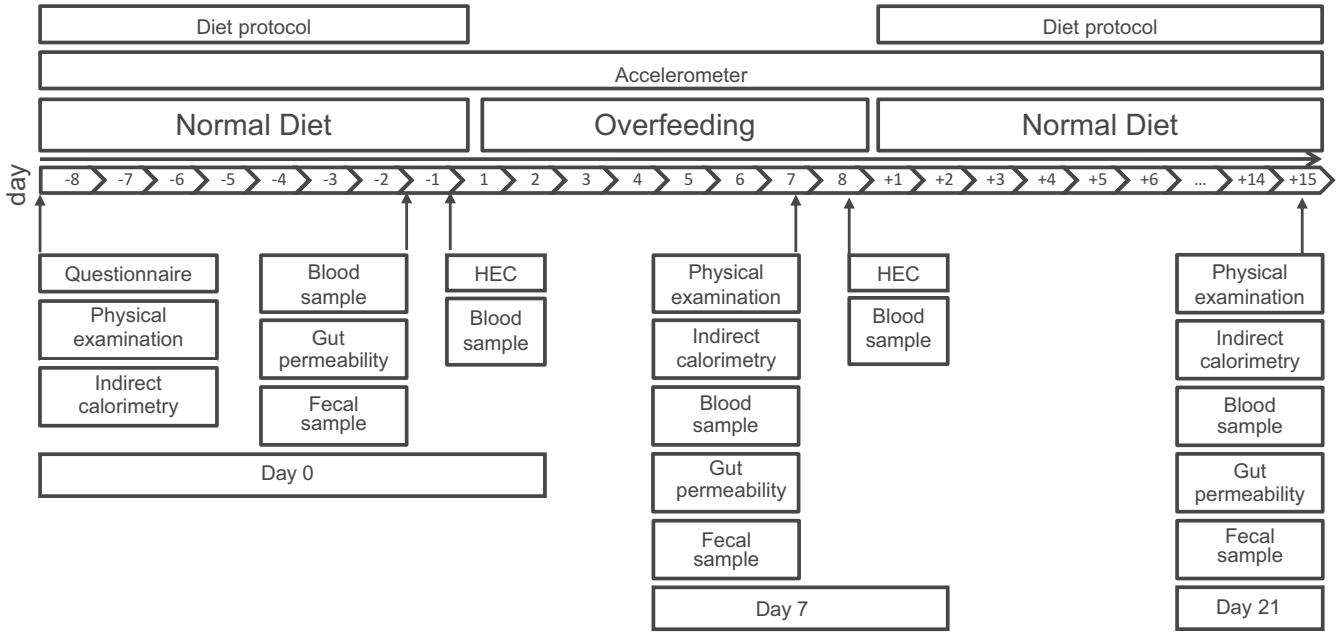

FIGURE 1 Study design. Overview of the timeline and the different examinations performed. Visit days are indicated with arrows and corresponding analyses performed are listed below. HEC, hyperinsulinemic euglycemic clamp.

concentrations of 4.4 ± 2.8 mmol/L after a clamp time of 150–180 min. The glucose infusion rate during the final 15 min of the clamp was used to calculate insulin sensitivity. The results of the clamps were analyzed in a blinded manner. The M value is defined as the average glucose infusion rate over a period of time from the start of the insulin infusion. For calculation of the M value we used the following formula: M value (mmoL/min) = glucose infusion rate (mL/h) · 200/60 · 0.0055.

Measurement of gut permeability. Gut barrier function was assessed by means of 2 non-invasive tests: *1*) sugar and polyethylene glycol (PEG) absorption tests (performed in parallel); *2*) measurement of the gut permeability marker zonulin in plasma via ELISA (23).

The sugar absorption test was performed as described by Norman et al. (24). Participants received a sugar test solution following an overnight fast and after collecting a baseline urine sample. The 100 mL sugar test solution contained mannitol (5 g), lactulose (10 g), and sucrose (20 g) and 6 tablets of sucralose (333.3 mg/tablet). The subjects were instructed to collect their whole urine at time-defined intervals (0–5 h, 5–26 h). Urine was sampled in containers with sodium acid (0.002 g) as a preservative and stored at –20°C until analysis. The sugars were quantified by HPLC with pulsed electrochemical detection (chromatography module: 250; Dionex) (24). Data are expressed as percentage of ingested sugars, referred to as percentage of urine recovery.

Participants received 100 mL of a PEG test solution containing 1 mg of molecular mass (M_r) 400 (PEG_6–PEG_{13}; mass range: 285–678 Da) and 200 mg of M_r 1500 (PEG_{20}–PEG_{45}; mass range: 899–2000 Da) (Merck). Five hours after ingestion of the sugar solution, the PEG test solution was drunk and urine was sampled during the following 21 h. PEGs were analyzed by LC-MS as described by Lichtenegger and Rychlik (25). Data are presented as percentage of ingested PEGs that was discovered in the urine, referred to as percentage of urine recovery.

Collection of fecal samples. Fecal samples were collected directly into sterile plastic containers (1000 mL; VWR International). Participants were asked to bring feces within 1 h to the human study center, where 10 aliquots (100–200 mg) were collected into screw cap tubes and immediately stored at –80°C.

High-throughput 16S rRNA gene amplicon sequencing. Samples were processed as described previously (26). Briefly, microbial cells were lysed by bead beating and heat treatment and the metagenomic DNA was purified using gDNA columns (Macherey-Nagel). Concentrations and purity were tested using the NanoDrop® system (Thermo Scientific). The V4 region of 16S rRNA genes was amplified (25 cycles) from 24 ng DNA using primers 519F (5′ CAG CMG CCG CGG TAA TWC) and 785R (5′ GAC TAC HVG GGT ATC TAA TCC) (27). After purification (AMPure XP system, Beckmann Coulter Biomedical GmbH) and pooling in an equimolar amount, the 16S rRNA gene amplicon libraries were sequenced in paired-end modus (PE175) using a MiSeq system (Illumina, Inc.) following the manufacturer's instructions.

Sequence analysis. Raw read files were processed based on the UPARSE approach (28) using IMNGS (29). Sequences were tested for the presence of chimeras using UCHIME (30) and operational taxonomic units (OTUs) were clustered at a threshold of 97% sequence similarity. To avoid analysis of spurious OTUs, only those with a relative abundance >0.5% total sequences in ≥1 sample were kept. SILVA (SILVA Incremental Aligner, version 1.2.11) (31) and RDP classifier (set 15; 80% confidence) were used to assign taxonomic classification to the OTUs' representative sequences (32). Specific OTUs with differential abundances between groups were further identified using EzTaxon. A species-level identification was reported for these OTUs under the condition that the 16S rRNA gene sequence of only one cultured strain in the database returned a similarity >97%. If several hits above this conservative species-level threshold were obtained, or if the closest relative was >95%, the OTU's identity was reported as unknown species within the corresponding genus. Phylogenetic relations were examined using the generalized UniFrac procedure (33). Shannon-effective counts were determined to estimate diversity within samples (α diversity) as described by Jost et al. (34).

Statistical analysis. Data were analyzed in the R programming environment. Anthropometric and metabolic data are presented as mean ± SD. P values <0.05 were regarded as statistically significant. A Shapiro-Wilk test was performed to test for normality. According to the distribution a paired t test or Wilcoxon-signed rank test was applied to assess mean differences before and after intervention. Comparing parameters of all 3 time points, ANOVA repeated measurements and Tukey post-hoc tests were used for normally distributed parameters. For nonparametric data a Friedman rank test with a paired Wilcoxon signed rank sum test was used to test significance between all 3 time points.

Rhea (version 1.0.1-5) was used for analysis of fecal microbiota profiles (35). The effect of HFD on OTUs and taxonomic counts was tested using the Friedman rank test for the analysis of a nonparametric randomized block design. Missing values were handled by using the Skillings-Mack test. A Wilcoxon signed rank sum test for matched pairs was applied for pairwise comparisons. The Benjamini-Hochberg method was used for adjustment after multiple testing. For β-diversity analysis, generalized UniFrac distances were calculated using the package GUniFrac (33, 36).

Results

Effect of overfeeding on anthropometric and metabolic parameters. The baseline characteristics of the participants are presented in Table 1. The 24 participants were young normal-weight healthy men with a mean age of 23.0 ± 2.8 y. The analysis of dietary protocols revealed an average energy intake of 2731 ± 708 kcal/d at baseline before the high-fat high-caloric intervention (day 0). A macronutrient composition of 48 EN% from fat, 18 EN% from protein, and 34 EN% from carbohydrates was targeted. Before and after the overfeeding intervention, participants recorded their daily dietary intake for 7 d (Table 2). Seven days before the intervention (day 0) the macronutrient composition of 38 EN% fat, 15 EN% protein and 47 EN% carbohydrates was approximating the recommendations of the German Nutrition Society for young men (37). The calculated caloric intake for the overfeeding period intervention (day 7) was 3926 ± 340 kcal/d on average. During the overfeeding period (day 7), fat intake increased by 100 ± 31 g/d, including an increased intake of SFAs by 66 ± 15 g/d. During the 2 wk following the intervention (day 21), mean energy intake returned to 2589 ± 628 kcal/d. There was no change in physical activity throughout the study; participants remained at a light intensity level (data not shown).

TABLE 1 Anthropometric and metabolic characteristics of 24 men at baseline (day 0), after 7 d of HFD (day 7) and after 2-wk follow-up (day 21)[1]

	Day 0	Day 7	Day 21
Weight, kg	76.6 ± 10.3[b]	77.6 ± 10.23[a]	77.3 ± 10.4[ab]
BMI, kg/m^2	23.0 ± 2.1[b]	23.3 ± 2.1[a]	23.2 ± 2.1[ab]
Waist circumference, cm	83.5 ± 5.9	84.2 ± 5.9	83.7 ± 6.1
Hip circumference, cm	87.8 ± 5.5	88.4 ± 5.1	87.8 ± 4.9
Lean mass, kg	66.9 ± 8.0	67.3 ± 7.8	67.1 ± 8.0
Fat mass, kg	9.8 ± 3.9[b]	10.3 ± 3.8[a]	10.2 ± 3.9[ab]
RMR, kcal/d	1959 ± 233	2011 ± 290	1963 ± 291
Serum total cholesterol, mmol/L	4.3 ± 0.7[b]	4.8 ± 0.8[a]	4.3 ± 0.9[b]
Serum HDL cholesterol, mmol/L	1.4 ± 0.3[b]	1.6 ± 0.3 [a]	1.4 ± 0.3[b]
Serum LDL cholesterol, mmol/L	2.5 ± 0.7[b]	2.8 ± 0.8[a]	2.5 ± 0.8[b]
Serum triglycerides, mmol/L	1.0 ± 0.3[a]	0.7 ± 0.3[b]	0.9 ± 0.5[ab]
LDL-C/total cholesterol	0.56 ± 0.08[b]	0.58 ± 0.08[a]	0.57 ± 0.08[ab]
HDL-C/total cholesterol	0.33 ± 0.08[b]	0.34 ± 0.08[a]	0.34 ± 0.0[b]
LDL-C/HDL-C	1.9 ± 0.7	1.8 ± 0.7	1.9 ± 0.7
Plasma NEFA, mmol/L	0.4 ± 0.2[a]	0.2 ± 0.1[b]	0.3 ± 0.3[a]
Fasting plasma blood glucose, mmol/L	4.5 ± 0.4[a]	4.4 ± 0.3[b]	4.5 ± 0.2[a]
Fasting plasma insulin concentrations, pmol/L	29.2 ± 6.2	30.5 ± 8.5	28.4 ± 4.9
HOMA-IR	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.2
Plasma hsCRP, mg/L	0.3 ± 0.4	0.3 ± 0.2	0.46 ± 0.5
Plasma LBP, μg/L	6.5 ± 2.9	5.9 ± 1.7	5.8 ± 2.1
Plasma sCD14, μg/L	1.3 ± 0.2[a]	1.1 ± 0.2[b]	1.2 ± 0.2[a]
LBP:sCD14 ratio	4.9 ± 0.2	5.6 ± 0.3	4.8 ± 0.2
Plasma calprotectin, μg/L	460 ± 412[ab]	601 ± 548[a]	381 ± 479[b]
Plasma HMW adiponectin, μg/L	3.4 ± 2.1[b]	4.5 ± 2.3[a]	3.5 ± 2.4[b]
Plasma leptin, μg/L	1.3 ± 0.7	1.5 ± 0.9	1.4 ± 0.8
Plasma RANTES, μg/L	26.9 ± 14.9[a]	17.3 ± 7.7[b]	16.3 ± 8.7[ab]
Plasma MCP-1, ng/L	80.8 ± 14.3[ab]	79.6 ± 14.9[b]	87.9 ± 16.4[a]
Plasma chemerin, μg/L	37.7 ± 10.6	37.0 ± 8.6	35.9 ± 7.9

[1] Values are means ± SDs, $n = 24$. Labeled means in a row without a common superscript letter differ, $P < 0.05$. HDL-C, HDL cholesterol; HFD, high-fat diet; HMW adiponectin, high-molecular-weight adiponectin; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide-binding protein; LDL-C, LDL cholesterol; MCP-1, monocyte chemoattractant; NEFA, nonesterified fatty acid; RANTES, regulated on activation normal, T cell expressed and secreted; RMR, resting metabolic rate; sCD14, soluble cluster of differentiation.

TABLE 2 Macronutrient intake of 24 men at baseline (day 0), after 7 d of HFD (day 7) and after 2-wk follow-up (day 21)[1]

	Day 0	Day 7	Day 21
Energy intake, kcal/d	2731 ± 708[b]	3926 ± 340[a]	2589 ± 628[b]
Fat intake, g/d	108 ± 35[b]	209 ± 18[a]	101 ± 33[b]
SFAs, g/d	46 ± 16[b]	111 ± 6[a]	43 ± 15[b]
Protein intake, g/d	100 ± 25[b]	176 ± 15[a]	95 ± 26[b]
Carbohydrate intake, g/d	303 ± 110[b]	333 ± 29[a]	280 ± 73[b]

[1] Values are means ± SDs, $n = 24$. Labeled means in a row without a common superscript letter differ, $P < 0.05$. HFD, high-fat diet.

The 24 participants gained 938 ± 616 g during the 7 d of HFD (day 7, Table 1). This significant body weight gain was associated with a significant increase in body fat mass ($P = 0.03$). During the 2 wk after overfeeding (day 21), body weight and fat mass did not change significantly. Waist circumference, hip circumference, lean body mass, and resting metabolic rate did not differ between the 3 study periods (Table 1).

The short-term overfeeding (day 7) resulted in significantly elevated serum total cholesterol, serum LDL cholesterol, serum HDL cholesterol concentrations, LDL cholesterol/total cholesterol, and both LDL cholesterol:total cholesterol and HDL cholesterol:total cholesterol ratios (each $P < 0.01$). These parameters returned to baseline levels 2 wk after discontinuation of the HFD (day 21). In contrast, fasting serum triglyceride and plasma NEFA concentrations significantly declined during overfeeding (day 7) and increased again under the normal diet (day 21, Table 1).

The HFD did not trigger significant changes in inflammatory markers such as plasma hsCRP and plasma LBP. In addition to plasma LBP, plasma sCD14 was measured and the LBP:sCD14 ratio increased after overfeeding (day 7) significantly ($P = 0.05$). Plasma sCD14 significantly decreased ($P < 0.01$) after overfeeding (day 7) and significantly increased ($P < 0.01$) during the 2 wk after overfeeding (day 21). Furthermore, fecal calprotectin was not affected by the dietary intervention (day 7), but declined significantly during the subsequent 14 d (day 21, $P = 0.02$). The proinflammatory adipokine leptin measured in plasma also did not change. Plasma HMW adiponectin rose significantly during the HFD (day 7, $P < 0.001$) and decreased again during the following 2 wk after intervention (day 21, $P = 0.001$). RANTES measured in plasma showed a significant decrease during the intervention (day 7, $P < 0.001$), but remained unchanged during the subsequent 2 wk of the HFD (day 21). Plasma MCP-1 did not vary during the HFD (day 7), but rose significantly in the third study phase (day 21, $P = 0.008$) (Table 1).

Effect of the HFD on insulin sensitivity. Fasting plasma glucose concentrations significantly decreased during the 7 d of HFD (day 7, $P = 0.03$) and were stable during the subsequent

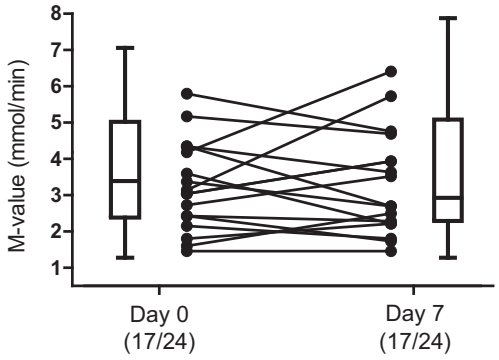

FIGURE 2 Measurement of insulin sensitivity. M value as a marker for insulin sensitivity at baseline (day 0) and after 7 d of high-fat diet (day 7) illustrated by boxplots and corresponding individual changes (connected lines). Only men reaching steady state (17/24) were included in the analysis. Numbers in brackets below the *x* axis indicate prevalence (number of analyzed samples/total number of samples).

2 wk (day 21, Table 1). Fasting plasma insulin concentrations and HOMA-IR remained constant throughout the study. The mean M value measured by the HEC was 3.62 ± 1.65 mmoL/min before overfeeding (day 0) and remained unchanged after overfeeding (day 7) (3.75 ± 1.92 mmoL/min; $P = 0.72$) (Figure 2). The glucose disposal rate did not change significantly before or after the HFD (198 ± 89.6 and 205 ± 105 mL/h, respectively; $P = 0.73$). There was also no effect of the intervention on insulin sensitivity when M values were normalized for body weight and fat-free mass.

Effect of the HFD on gut permeability. Table 3 summarizes the results obtained using different approaches for measurement of gut permeability. The mean urine appearance of the 4 sugars did not change during the 7 d of HFD (day 7). Regarding the PEG approach, no consistent change of gut permeability was observed. The gut permeability marker plasma zonulin remained unchanged throughout the study period.

The effect of HFD on gut microbiota composition. After quality- and chimera-check, a total of ~860,000 sequences (12,468 ± 2262/sample) clustering in 276 OTUs were analyzed. The short-term HFD (day 7) did not affect α-diversity (Figure 3A). β-Diversity analysis revealed marked interindividual differences and no distinct and significant clustering according to time points (Figure 3B). In spite of these interindividual differences, explorative analysis of taxonomic groups across all individuals revealed a decreased relative abundance of sequences classified within the family *Bacteroidaceae*, and an increase in those classified within the order *Betaproteobacteria* associated with overfeeding (Figure 3C). Of note, 6 individuals were characterized by drastic shifts (>40% dissimilarity) in gut microbial diversity after the high-fat feeding (day 7, Figure 3D). Explorative analysis of taxa for these individuals revealed lower relative abundance of sequences within the family *Bacteroidaceae* (data not shown) and higher relative abundances of 3 molecular species: *Blautia wexlerae* (OTU 2), *Coprococcus comes* (OTU 21), and *Alistipes* sp. (OTU 93) (Figure 3E). In summary, the data suggest that a short-term HFD did not trigger consistent and substantial rearrangements of fecal bacterial populations in this cohort of healthy male subjects.

TABLE 3 Gut permeability parameters of 24 men at baseline (day 0), after 7 d of HFD (day 7) and after 2-wk follow-up (day 21)[1]

	Urine recovery, %		
	Day 0	Day 7	Day 21
Sucrose	0.14 ± 0.05	0.09 ± 0.05	0.11 ± 1.02
Mannitol	14 ± 5	15 ± 5	15 ± 4
Lactulose	0.23 ± 0.09	0.24 ± 0.09	0.23 ± 0.08
Sucralose	0.46 ± 0.47	0.39 ± 0.6	0.75 ± 1.02
L:M ratio	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
PEG_9	14 ± 7[a]	11 ± 7[b]	12 ± 7[ab]
PEG_{11}	13 ± 9	9 ± 7	10 ± 8
PEG_{13}	3 ± 2	2 ± 2	3 ± 2
PEG_{25}	0.30 ± 0.17[a]	0.21 ± 0.12[b]	0.26 ± 0.19[ab]
PEG_{30}	0.19 ± 0.12	0.14 ± 0.09	0.15 ± 0.12
PEG_{35}	0.20 ± 0.18	0.14 ± 0.12	0.12 ± 0.12
PEG_{40}	0.21 ± 0.17	0.16 ± 0.13	0.13 ± 0.13
Plasma zonulin, μg/	49 ± 14	46 ± 12	45 ± 14

[1] Values are means ± SDs, $n = 24$. Labeled means in a row without a common superscript letter differ, $P < 0.05$. Metabolites were measured in plasma or urine, depending on the analytic method. HFD, high-fat diet; L:M ratio, lactulose:mannitol ratio; PEG, polyethylene glycol.

Discussion

Multiple studies in rodents have shown that HFDs are associated with an increase in gut permeability, possibly contributing to the low-grade inflammation status observed in metabolic diseases (7, 38). However, it is not yet conclusively known if this holds true for humans. In the present study in young healthy men, 7 d of an HFD resulted in an increase of body weight of ~940 g on average as expected from the additional energy intake of 1000 kcal/d. However, there was no significant change in insulin sensitivity after the intervention, even after normalizing for body weight and fat-free mass. Gut barrier function, as measured by different approaches, also did not significantly change following the HFD.

Only a few human studies have looked at the effect of short-term high-fat overfeeding on insulin sensitivity as measured by HEC. Brøns et al. (39) investigated the impact of an energy surplus of 50% above the regular diet for 5 d. Their HFD (60% fat, 32.5% carbohydrate, and 7.5% protein) resulted in increased fasting glucose and insulin concentrations due to an increased hepatic glucose production, but there was not significant evidence of an effect on insulin-mediated glucose uptake or the M value. Likewise, Adochio et al. (40) assessed the effect of a high-fat, high-calorie diet mainly as dairy, nuts, and oils (50% fat, 30% carbohydrate) on insulin sensitivity and did not observe a significant change in whole-body insulin sensitivity expressed as glucose disposal rate and M value. Furthermore, Chen et al. (41) studied the impact of 3 d of an HFD (+1250 kcal/d, 45% fat) on insulin sensitivity and did not observe significant alterations in insulin sensitivity. The results of these 3 studies are thus in line with our findings, but are in contrast to other human overfeeding studies using surrogate markers of insulin sensitivity such as HOMA-IR (42, 43). Hence, based on HEC as the gold standard for assessment of whole-body insulin sensitivity, there is some consistency that short-term overfeeding ≤7 d does not result in impaired insulin sensitivity in healthy, normal-weight subjects. Even greater duration of overfeeding based on cheese, butter, and almonds (56 d, +760 kcal/d) did not result in a significant decrease in insulin sensitivity measured by the HEC (44).

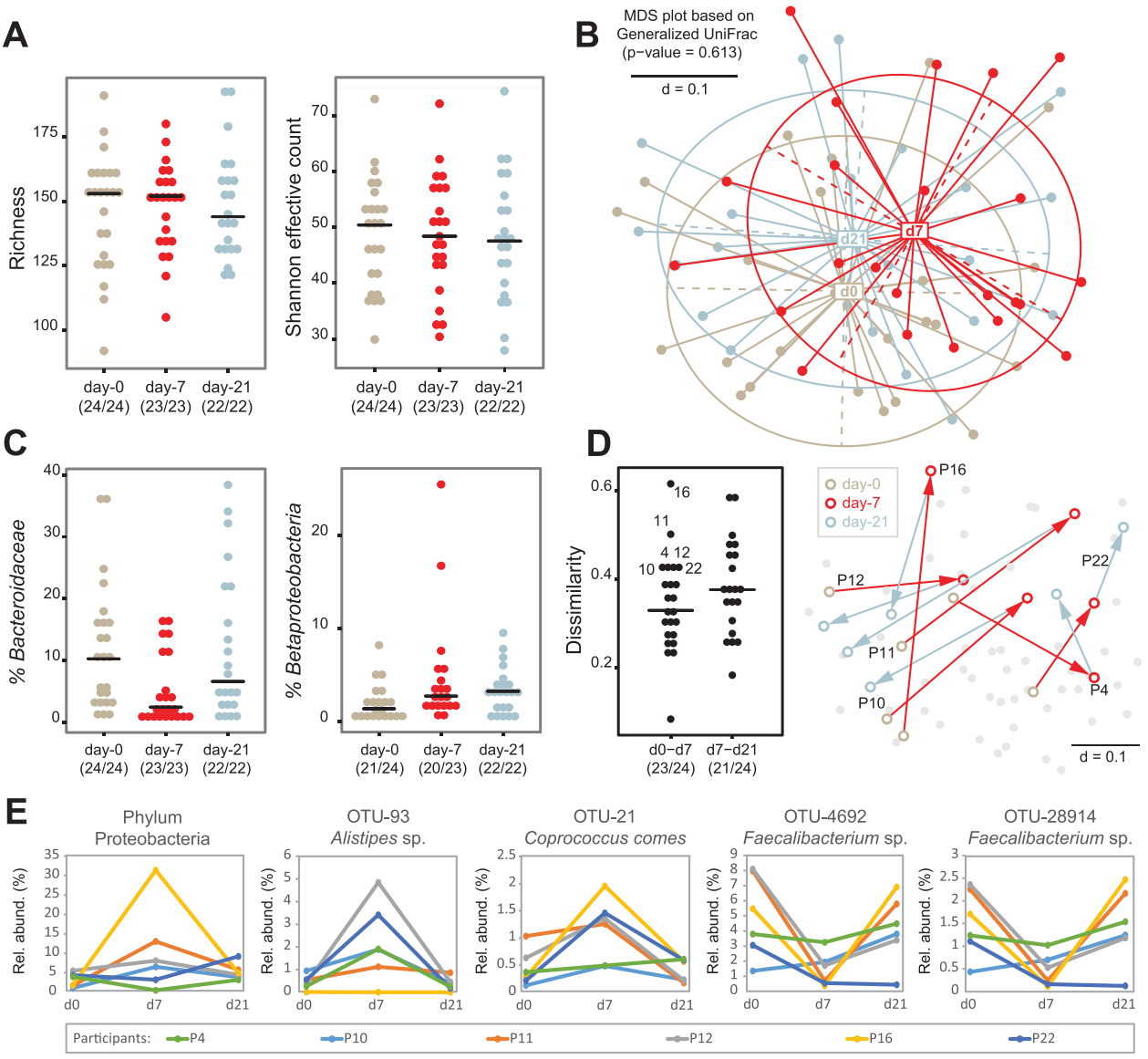

FIGURE 3 Fecal microbiota analysis of 24 men at baseline (day 0), after 7 d of HFD (day 7) and after 2-wk follow-up (day 21). (A) Diversity within samples (α-diversity). Numbers in brackets below the *x* axis indicate prevalence (number of analyzed samples/total number of samples). (B) Multidimensional scaling plot of phylogenetic distances (β-diversity). (C) Bacterial taxonomic groups altered by the intervention ($P < 0.05$). (D) Overtime analysis of phylogenetic profiles revealed 6 individuals with marked shifts after overfeeding. (E) Individuals in (D) were characterized by significant changes in the relative abundances of specific taxa. d, day; HFD, high-fat diet; OTU, operational taxonomic unit; P, person; Rel. abund., relative abundance.

In the current study, whipping cream was used as a high-fat product rich in SFAs for supplementation. It is noteworthy in this context that the food matrix may play an important role for the functional consequences of specific foods (45). Rosqvist et al. (46) demonstrated that milk fat globule membranes present in whipping cream modulate plasma lipoproteins so that the lipoprotein profile was not impaired. Vors et al. (47) investigated dietary lipids incorporated in food products with different physiochemical structures and showed that the fat structure in the meal could modify postprandial lipid metabolism including the size of chylomicrons and β-oxidation. Thus, we cannot exclude the possibility that the food matrix of the whipping cream diminished the impact of SFAs regarding insulin sensitivity and other outcomes of our study. The decrease in fasting serum triglycerides and plasma NEFAs during HFD consumption may also be explained by the specific fat matrix provided, potentially leading to increased β-oxidation (47).

Likewise, and again in contrast to animal data, the inflammation markers hsCRP, leptin, and MCP-1 measured in plasma did not change significantly over the 7 d of HFD. This finding is in line with another overfeeding study including healthy, young, lean participants (41). Fecal calprotectin, as a marker of intestinal inflammation, slightly increased after the HFD and declined after the 2-wk follow-up period, suggesting a modest increase of the inflammatory status in the gastrointestinal tract. Plasma HMW adiponectin increased during overfeeding and returned to baseline during the following 2 wk. A similar finding was reported by Brøns et al. (39) after 5 d of an HFD (60% fat, 50% overfeeding). Other studies have also detected elevated plasma adiponectin concentrations after overfeeding for 3 d (48) and

7 d (49). The effect on plasma adiponectin may be explained by an anti-inflammatory effect of dairy products (50).

We measured plasma sCD14 and plasma LBP concentrations before, immediately after, and 14 d after overfeeding. Plasma sCD14 concentrations were significantly decreased after overfeeding, thereby confirming another study by Laugerette et al. (15). The circulating plasma LBP was used as a biomarker of intestinal bacterial translocation, since LPS has only a short half-life and is hard to detect (51). Recent studies showed a positive correlation between caloric intake and plasma LPS concentrations (9, 14). In our study, there was no increase in plasma LBP concentrations, suggesting that short-term overfeeding does not affect bacterial translocation (52). This is also supported by our measurements of paracellular gut permeability markers, including PEG 1500 (except PEG_{25}), lactulose, and plasma zonulin, which remained unchanged following 7 d of high-fat overfeeding. The urinary excretion of PEG_{25} was significantly decreased after short-term overfeeding, in contrast to the other homologues PEG_{30}, PEG_{35}, and PEG_{40}. PEG is a nontoxic, nondegradable substance that is absorbed in the gut lumen (53), and entirely excreted by the kidneys (54). In addition to PEG 1500, PEG 400 was measured at the same time points. PEG 400 significantly decreased following the intervention. One explanation could be that the baseline results of PEG 400 were contaminated by several bodycare products (e.g., body lotion, face cream) known to contain PEG 400. In summary, the data from the PEG absorption test do not provide evidence for impaired gut permeability after 7 d of an HFD.

PEG-based permeability only provides information regarding the small intestine. We extended the assessment of gut permeability from the gastroduodenal tract to the colon by using a combined sugar absorption test. The 4-sugar absorption test reflects both paracellular and transcellular pathways and revealed no significant changes regarding paracellular and transcellular absorption following short-term overfeeding.

To date, the impact of an HFD on gut permeability in humans is poorly understood and few data are available. In contrast, animal studies have shown that an HFD increases intestinal permeability (8, 55). These unphysiological diets consisted of 60% (8) and ≤75% fat of total energy intake (55). Others were not able to reproduce these results (56). In mice, it is also interesting to note that the housing conditions may play an important role in the link between HFD and gut barrier function (57).

Finally, fecal microbiota profiles were measured before, immediately after, and 2 wk following overfeeding. There was no uniform response to the defined dietary intervention, but the shifts in the phylogenetic make-up of fecal bacteria were substantial in some individuals. Richness and Shannon effective counts were not affected by overfeeding and only 2 taxonomic groups (*Bacteroidaceae* and *Betaproteobacteria*) showed altered relative abundance of sequences across all individuals. Previous studies reported shifts in human gut microbiota even after short-term dietary challenges of 1–2 wk (58, 59). However, in these studies the dietary interventions were more drastic than in the present study: exclusively plant- or animal-based diets or drastic shifts in fiber and fat content (e.g. from 14 to 55 g/d and from 16% to 52%, respectively).

The main strength of our study is the high degree of standardization. The overfeeding experiment took place in a specialized study unit and participants had to eat their meals under supervision. In addition, potential changes in gut permeability were studied using a variety of methods. The HEC technique, the gold standard for the assessment of insulin sensitivity, was used. Nevertheless, a potential limitation is the short-term overfeeding period of 7 d, which cannot exclude changes in the outcomes after longer high-fat overfeeding. Moreover, PUFA, and in particular n–3 fatty acid, intakes were not assessed and these can affect inflammation.

In conclusion, 7 d of high-fat overfeeding did not result in impaired insulin sensitivity in young, healthy men. In addition, the HFD had no major effect on gut permeability and no consistent impact on fecal microbiota across individuals. However, the large variation in individual responses to a dietary intervention should be considered in future studies.

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