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CONSUMING A BALANCED HIGH FAT DIET FOR 16 WEEKS IMPROVES BODY COMPOSITION, INFLAMMATION AND VASCULAR FUNCTION PARAMETERS IN OBESE PREMENOPAUSAL WOMEN

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Abstract

Objective—Inflammation, insulin resistance and vascular dysfunction characterize obesity and predict development of cardiovascular disease (CVD). Although women experience CVD events at an older age, vascular dysfunction is evident 10 years prior to coronary artery disease. Questions remain whether replacing SFA entirely with MUFA or PUFA is the optimal approach for cardiometabolic benefits. This study tested the hypotheses that: a) body composition, inflammation and vascular function would improve with a high fat diet (HFD) when type of fat is balanced as 1/3 SFA, 1/3 MUFA and 1/3 PUFA; and b) body composition, inflammation and vascular function would improve more when balanced HFD is supplemented with 18C fatty acids, in proportion to the degree of 18C unsaturation.

Methods—Obese premenopausal women were stabilized on balanced HFD and randomized to consume 9 g/d of encapsulated stearate (18:0), oleate (18:1), linoleate (18:2) or placebo.

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Author Contributions

Drs. H.J. Silver, K.D. Niswender, J.A. Muldowney III, D.E. Vaughan and H. Kocalis were involved in the study concept. Drs. H.J. Silver and K.D. Niswender designed the study. Dr. H.J. Silver was responsible for study implementation and supervision. C.D. Keil was responsible for database management, data entry and quality control. Drs. H. Kang and H.J. Silver were responsible for statistical analysis. Drs. H.J. Silver, J.A. Muldowney III, S. Fazio and K.D. Niswender provided intellectual content, drafted the manuscript and manuscript revisions.

Disclosure Statement

The authors have no conflicts with the present study to report.

Results—Significant improvements occurred in fat oxidation rate ($\uparrow 6\%$), body composition (%fat: $\downarrow 2.5 \pm 2.1\%$; %lean: $\uparrow 2.5 \pm 2.1\%$), inflammation ($\downarrow$ IL-1 α , IL-1 β , IL-12, IL-17, IFN γ , TNF α , TNF β) and vascular function ($\downarrow$ BP, $\downarrow$ PAI-1, $\uparrow$ tPA activity). When compared to HFD +placebo, HFD+stearate had the greatest effect on reducing IFN γ ($\downarrow 74\%$) and HFD+linoleate had the greatest effect on reducing PAI-1 ($\downarrow 31\%$).

Conclusions—Balancing the type of dietary fat consumed (SFA/MUFA/PUFA) is a feasible strategy to positively affect markers of CVD risk. Moreover, reductions in inflammatory molecules involved in vascular function might be enhanced when intake of certain 18C fatty acids is supplemented. Long term effects need to be determined for this approach.

Keywords

Obesity; cardiovascular; inflammation; endothelial; fatty acid

1. Introduction

Inflammation, insulin resistance and vascular dysfunction are characteristics of obesity that predict development of cardiovascular disease (CVD) [1]. Dietary fatty acids, particularly long chain saturated fatty acids (SFA), promote obesity related inflammation and vascular dysfunction by regulating production of adipocyte derived chemokines and cytokines [2, 3] and macrophage accumulation into vascular walls. During weight stability, diets high in SFA can impair endothelial function, a marker of coronary vascular function that predicts atherosclerosis [4]. Conversely, weight loss may improve endothelial function [5, 6], whether caloric restriction encompasses low or high fat intake [7, 8]. Rather than focusing solely on amount of fat, recent strategies target replacing SFA with monounsaturated (MUFA) or polyunsaturated (PUFA) fat.

Although high MUFA or PUFA intake decreases total and LDL-cholesterol, and apolipoprotein B levels [9, 10], a high MUFA diet has not wholly improved vascular function or reduced coronary artery atherosclerosis [11]. Alternatively, high PUFA intake may improve endothelial function [12] and reduce coronary disease risk [9], although the type of PUFA could impact effects [13]. Additionally, not all SFA can be considered unfavorable as metabolic effects differ, partly due to carbon chain length [14]. While the 16-carbon SFA palmitate raises total, LDL- and HDL-cholesterol levels and robustly promotes inflammation and vascular dysfunction [15, 16], the 18-carbon SFA stearate has neutral effects [17]. Hence, a high fat diet where the proportion of total fat from SFA, MUFA and PUFA is balanced may potentiate the greatest CVD risk reduction.

Sex differences have been recognized in vascular function, apparently due to the smaller size and greater stiffness of the female heart and vasculature as well as distinct hormonal milieu [18]. Although women experience cardiovascular events later than men, vascular dysfunction has been detected 10 years prior to actual CVD [19]. The aim of the present study was to test the hypothesis that body composition, inflammation and vascular function in obese premenopausal women would improve with a high fat diet (HFD) when the type of fat is balanced as 1/3 SFA, 1/3 MUFA and 1/3 PUFA. We further hypothesized that, while consuming the balanced HFD, improvements in body composition, inflammation and

vascular function would be greater when supplemented with 18C fatty acids - in proportion to degree of 18C fatty acid unsaturation. To test these hypotheses, subjects were stabilized on balanced HFD during a two-week standardization period and then randomized to 9 g/d of encapsulated pure stearate (18:0), oleate (18:1), linoleate (18:2) or placebo while continuing HFD.

2. Methods

2.1. Subjects

Of 731 respondents, 245 met inclusion criteria of being female, age 21–50 years, premenopausal, and Class I/II obesity (BMI 30–39.9 kg/m²) with weight stability ($\pm$ 2.2kg) over three months prior (Figure). Exclusion criteria were tobacco use; alcohol or substance abuse; diabetes, cardiovascular, thyroid, kidney or liver disease; inflammatory condition; hypertension (systolic 150/diastolic 95mmHg); hyperlipidemia (serum triglycerides 200mg/dL or LDL-cholesterol 130mg/dL); pregnancy or lactation. Additional exclusions were evidence of disordered eating by Eating Attitudes Test score $\geq$ 20 [20] and/or Three Factor Eating Questionnaire score $<$ 14 [21]; appetite reducing medications, daily aspirin, Coumadin or ACE inhibitors, or dietary supplements other than multivitamin/mineral; and consuming $>$ 4 cups regular coffee or black tea daily.

Upon consent, demographic information was collected and a fasting venous sample was obtained for complete blood count, basic metabolic panel, liver enzymes and lipid profile to rule out serious illness and hyperlipidemia. Physical measures were obtained in triplicate for seated blood pressure, height ($\pm$ 0.1cm), weight ($\pm$ 0.1kg), and waist and hip circumferences ($\pm$ 0.1cm), and BMI was calculated for eligibility.

Subjects were interviewed for diet, weight and gastrointestinal health history and the Modified Baecke Physical Activity Questionnaire (PAQ) [22]. Three 24-hour diet recalls were performed by the Vanderbilt Nutrition and Diet Assessment Core using standardized probes (NDSR 2011, Nutrition Coordinating Center, Minn., MN), portion estimation utensils, and multiple-pass methodology [23]. Of 245 qualifying respondents, 230 were enrolled and scheduled for baseline testing at the Vanderbilt Clinical Research Center (VCRC). The Vanderbilt University Institutional Review Board approved the study protocol which was registered in the U.S. National Institutes of Health ClinicalTrials.gov registry (NCT00696228).

2.2. Diet and supplement protocol

This study was a randomized double-blind placebo controlled trial to compare balanced high fat diet (HFD) to HFD supplemented with stearate (HFD+S), oleate (HFD+O) or linoleate (HFD+L). One hundred and forty-four women began the two-week HFD stabilization period by following daily menus calculated in NDSR to provide a macronutrient composition of 50% fat, 30% carbohydrate and 20% protein. Total fat was balanced as 1/3 SFA, 1/3 MUFA and 1/3 PUFA, and total carbohydrate as 1/2 simple and 1/2 complex (Table 1). To further standardize fat intakes, all sources of fats including oils, spreads, nuts and seeds were provided weekly in pre-portioned containers. Daily caloric intake was derived from

measured resting energy expenditure (REE) multiplied by a modest 1.2 activity factor (as PAQ scores denoted sedentary behavior [24]).

Upon completing the HFD stabilization period, subjects were randomized to stearate (HFD+S), oleate (HFD+O), linoleate (HFD+L) or placebo (HFD+P) supplementation while continuing balanced HFD for 14 weeks. Each pure (98.5%) food grade 18C fatty acid was purchased from one lot and compounded into 1gram capsules for a dose of 9g/d. Placebo capsules contained 1g of food grade silicon dioxide powder. Supplements were odorless, tasteless and colorless. The compounding pharmacist performed monthly quality control according to GMP standards. The menus for subjects assigned to HFD+P were adjusted to compensate for the additional energy intake (81kcal/day) in subjects randomized to fatty acid supplements. Adherence was promoted by the study dietitian during weekly clinic visits and compliance was assessed by reviewing daily checklists disclosing the amount of HFD menu items consumed, random 24-hr recalls, capsule counts [25] and plasma lipid composition [26].

2.3. Metabolic testing

Subjects were scheduled for follow-up testing at weeks 2, 9 and 16. They were instructed to avoid alcohol, excess caffeine intake and non-routine physical activities on the day before each VCRC visit, and fast from 9:00pm until arrival at 7:00am. After weight and vital signs were obtained, REE and substrate oxidation rates were measured in thermoneutral conditions using a metabolic cart system (ParvoMedics TrueOne 2400®, Sandy, UT). Data was collected for 15 minutes at steady state with average change in minute VO_2 10% and respiratory quotient (RQ) 5%. REE was calculated via the Weir equation [27] and substrate oxidation was adjusted for urinary urea nitrogen.

Whole and regional body composition was acquired using dual energy x-ray absorptiometry (DEXA) on a Lunar iDXA™ (GE Healthcare) phantom calibrated before each use.

Coefficients of variation from repeated measures of 12 randomly selected subjects were 0.58% for fat mass, 0.42% for lean mass, 1.35% for trunk fat and 1.02% for trunk lean [28].

2.4. Hemodynamic testing

Arterial elasticity was assessed by applanating a calibrated tonometer (HD/PulseWave™ CR-2000, Hypertension Diagnostics Inc., Eagen, MN) at the radial bony prominence of the left wrist and placing a sphygmomanometer cuff around the right arm at the brachial artery [29]. A hand/wrist stabilizer optimized position and minimized movement. Waveforms were calibrated to brachial systolic and diastolic cuff pressures and collected as an average of 30-second recordings with a pressure sensitivity of 10%. The primary arterial compliance parameters were small and large artery elasticity index (SAEI/LAEI ml/mm Hg x 100).

Flow mediated dilatation (FMD) was performed using ultrasound (Philips iE33 Medical, Bothell, WA) with a broadband linear array transducer on the brachial artery above the right elbow and a sphygmomanometer cuff below the elbow. Two-dimensional images were acquired for artery diameter at the end of diastole at rest (1-minute) and 30, 60, 90 and 120 seconds after reactive hyperemia induced by cuff inflation to 110 mm Hg above systole for 5 minutes. Peak diameter was the largest diameter during the 2-minute post-deflation segment.

Baseline diameter was the average of three diameters at the 1-minute rest period. FMD was calculated as maximum percent change in diameter after hyperemia compared to baseline. Since estrogen fluctuations alter vascular reactivity, scheduling accommodated subjects' menses.

2.5. Biochemical analyses

Serum assays were performed using standard methods at the Vanderbilt Department of Pathology Clinical Laboratory for high sensitivity CRP (hsCRP), lipoprotein profile (triglyceride, total, LDL- and HDL-cholesterol), liver function, glucose and insulin. Plasma cytokines were measured in duplicate (intra-assay CV 11%) using Multiplex Kit #MPXHCYTO-60K (EMD Millipore, St. Charles, MO) on a Luminex100® at the Vanderbilt Diabetes Hormone Core. The Core also quantified plasma nonesterified fatty acid (FFA), triglyceride (TG) and phospholipid (PL) content by gas chromatography using a 7890A system (Agilent Technologies). For each lipid class, fatty acid content is presented as percent total fatty acids. Plasma FFA, TG and PL 16-carbon desaturation indices were calculated as 16:1/16:0 and 18-carbon desaturation indices as 18:1/18:0 [30]. For plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen antigen (tPA) levels, plasma was collected in acidified sodium citrate (0.105mol/l, Biopool, Umea, Sweden) and measured by commercially available 2-site ELISA using chromogenic substrates at the Vaughan laboratory at Northwestern University's Feinberg Cardiovascular Research Institute. PAI-1 and tPA levels are expressed in units of mass (ng/ml) and tPA activity in units of activity (IU/ml).

2.6. Statistical analyses

Analyses were performed using R Statistical Software version 2.15.1 (<http://www.r-project.org/>) to explain longitudinal changes in body composition, inflammation and vascular function variables due to the effects of balanced HFD with 18-carbon fatty acid supplementation (HFD+S, HFD+O or HFD+L) *versus* balanced HFD with placebo (HFD+P). Based on physiological relevance, eight parameters (systolic BP [31], diastolic BP [31], SAEI [32], LAEI [32], FMD percent change [33], tPA [34], PAI-1 [35], tPA activity [34]) characterized vascular function. The sample size was based on a power calculation using change in tPA level. The study design provided 83% power to detect a clinically relevant difference in tPA of 10 ng/min/100mL from baseline levels with α of 0.05.

Visual inspection of data by Q-Q plots depicted mild deviation from normality for some variables so nonparametric approaches were employed in univariate analyses. The Wilcoxon rank-sum test for interval variables and McNemar's test for nominal variables were used to detect differences between completers and dropouts at weeks 2, 9 and 16. The Kruskal-Wallis test was used to assess differences across the four groups in baseline characteristics and attrition rates. Wilcoxon signed-rank tests were used to determine the significance of within-group changes in outcome variables from baseline to week 2, and thus, the need to control for these changes in linear regression modeling. Wilcoxon signed-rank tests were also used to assess within-group changes at week 16.

Linear mixed-effect models with an autoregressive order one (AR1) correlation structure of the repeated measures were used to assess mean time effect response profiles (main effect of time) and the difference in mean response across the four groups (main effect of supplement type). The same models were also used to assess statistical differences in overall response profiles across groups (time X supplement interaction). The validity of AR1 correlation structure was examined against an unstructured correlation pattern. Since log transformations of the variables revealed as being skewed in the Q-Q plots did not improve normality, unchanged values were used in fitting regression models. Changes in outcome variables during the two-week HFD stabilization period and total weight loss were included as covariates. Statistical significance was set at $P < 0.05$.

3. RESULTS

3.1. Subjects

The women were middle-aged (36.7 ± 6.8 years), obese (BMI 34.8 ± 2.7 kg/m²), normotensive, normoglycemic and normolipidemic. At baseline, there were no significant differences among the four groups by age, race, BMI, anthropometrics, biochemical indicators of metabolic disease or inflammation (Table 2). Further, no differences were detected in baseline characteristics between completers and non-completers and no differences in attrition rate by group ($P = 0.23$). The balanced HFD, placebo and fatty acid supplements were well tolerated; there were no significant differences among groups based on a composite gastrointestinal tolerance score obtained weekly. Compliance was excellent [36] with 90% of placebo and supplement capsules consumed daily and no significant differences among groups.

3.2. Balanced HFD During the Stabilization Period (Study Weeks 0 – 2)

To test the effects of 18C fatty acid supplements under standardized dietary conditions, subjects completed a two-week HFD stabilization period. Total fat intake increased (38.1 ± 7.4 to 48.5 ± 2.4 %kcal, $P < 0.001$), the proportion of SFA and MUFA intake decreased and PUFA intake increased (Table 3). Concomitantly, the proportion of SFA in plasma FFA and TG decreased; MUFA in plasma FFA and PL increased; and PUFA in plasma TG and PL increased. Simultaneously, energy intake decreased ~10% (1905.8 ± 448.3 kcal/d to 1715.3 ± 217.2 kcal/d, $P < 0.001$) resulting in weight loss of 2.6 ± 1.3 kg ($P < 0.001$). Weight loss and changes in outcome variables during HFD stabilization were included as covariates in analyses comparing balanced HFD with 18C fatty acid supplementation to balanced HFD with placebo.

3.3. Balanced HFD (HFD+P) versus HFD + 18C Fatty Acid Supplementation (Study Weeks 3 – 16)

Dietary and Plasma Lipids—Analysis of daily HFD checklists and 24-hour diet recalls showed that increased total fat at week 2 (48.5 ± 2.4 %kcal) was maintained throughout the study in all groups (Supplement Table 1). When compared to HFD+P, there were no main effects of time or supplement type on fat, carbohydrate or protein intake. Plasma lipid composition revealed compliance with diet and supplement prescriptions.

HFD+P—With HFD+P, dietary SFA and the proportion of SFA in plasma FFA, TG and PL did not change after the stabilization period. The proportion of PUFA in plasma FFA decreased and trended downward in plasma TG ($P = 0.07$), but did not change in plasma PL. There was a main effect of time for increased 18C desaturation index in plasma FFA and TG ($P = 0.002$) which was associated with increased dietary oleate and total MUFA ($r = 0.26$, $P = 0.01$) and the proportion of MUFA in plasma FFA ($r = 0.60$, $P < 0.001$) and TG ($r = 0.49$, $P < 0.001$). There was also a main effect of time for increased proportion of MUFA in plasma PL ($P < 0.001$) and increased plasma PL 18C desaturation index was associated with the proportion of MUFA in TG ($r = 0.41$, $P < 0.001$) and PL ($r = 0.56$, $P < 0.001$).

HFD+S—As expected, fat intake from total SFA increased with HFD+S ($4.6 \pm 7.0\%$, $P = 0.009$), but no changes occurred in the proportion of SFA, MUFA or PUFA in plasma FFA. There was decreased proportion of oleate and total MUFA with increased linoleate and total PUFA in plasma TG and PL. Additionally, there was a main effect for a 1.4 unit/wk increase in plasma TG 18C desaturation index as well as a time x supplement interaction ($P = 0.03$) when comparing HFD+S to HFD+P.

HFD+O—With HFD+O, fat intake from SFA decreased while MUFA intake increased ($5.5 \pm 5.6\%$, $P < 0.001$). In plasma FFA, the proportion of palmitate and total SFA decreased, oleate and total MUFA increased, and FFA 18C desaturation index increased. There was a main effect for a 2.72%/wk decrease in the proportion of PUFA in plasma FFA. In plasma TG, the proportion of palmitate and total SFA decreased with increased oleate, total MUFA, linoleate and total PUFA. In plasma PL, the proportion of stearate, oleate and total MUFA decreased with increased PUFA.

HFD+L—Fat intake from total PUFA increased with HFD+L ($9.6 \pm 7.5\%$, $P < 0.001$). The proportion of oleate, total MUFA and linoleate increased in plasma FFA with decreased palmitate and total SFA. In plasma TG, the proportion of linoleate increased, but total PUFA did not change. No significant changes in plasma PL were detected and no main effects of HFD+L were observed in plasma FFA, TG or PL when compared to HFD+P.

Fat Oxidation—Indicating that subjects had the capacity (i.e., metabolic flexibility [37]) to respond positively to balanced HFD, fat oxidation rate (% total O_2 consumption) increased ~6% overall ($47.6 \pm 9.9\%$ to $53.3 \pm 10.0\%$, $P = 0.001$). While there was no main effect of supplement type on fat oxidation rate when compared to HFD+P, increased fat oxidation rate was associated with reduced palmitate intake ($r = -.28$, $P = 0.009$).

Body Weight and Composition—No further significant reductions occurred in energy intake after week 2 and REE reduced only 4% overall (1604.3 ± 183.6 kcal/d to 1535.8 ± 165.2 kcal/d, $P < 0.001$), thus, the rate of weight loss declined 77% after the stabilization period (1.3 ± 0.7 kg/wk to 0.3 ± 0.2 kg/wk). Yet, there was a main effect of time on weight overall ($P < 0.001$) and BMI decreased from 34.6 ± 2.7 kg/m² to 32.3 ± 2.9 kg/m² ($P < 0.001$). The reductions in weight and BMI were similar among groups and there were no main effects of supplement type on weight or BMI when compared to HFD+P.

Despite only modest weight loss, significant improvement occurred in body composition with decreased proportion of fat ($-2.5 \pm 2.1\%$) to lean mass ($+2.5 \pm 2.1\%$), $P_s < 0.001$ (Figure 2). When compared to HFD+P, there were no main effects of supplement type on the relative change in fat to lean mass. However, there were main effects of time on %fat in all regions (ranging from -2.5 to -4.8% less total body, trunk, android and gynoid fat, $P_s < 0.001$). When compared to HFD+P, there were main effects for 2.6% higher gynoid fat and 2.1% higher total body fat with HFD+S and 3.2% lower android fat with HFD+O.

Inflammation—Although elevated at baseline (6.0 ± 6.8 mg/dl), hsCRP levels did not decrease. Yet, significant reductions in serum cytokines occurred in all groups (Supplement Figure 1). With HFD+P, the levels of IL-1 β , IL-12, IL-17, TNF β and IFN γ decreased. With HFD+S, the levels of IL-1 α , IL-17 and IFN γ decreased. Compared to HFD+P, there was a main effect for lower IFN γ (-14.8 pg/ml, $P = 0.01$) and a time x supplement interaction indicating -1.4 pg/ml/wk ($P = 0.02$) with HFD+S intake. With HFD+O, the levels of IL-1 α , IL-12, IL-17 and IFN γ decreased. Yet, when compared to HFD+P, there were main effects for higher IL-1 β (3.9 pg/ml, $P = 0.03$), IL-6 (6.9 pg/ml, $P = 0.04$), IL-10 (6.8 pg/ml, $P = 0.07$), IL-12 (29.1 pg/ml, $P = 0.03$) and TNF α (6.9 pg/ml, $P = 0.005$) with HFD+O. With HFD+L, the levels of IL-1 α , IL-10, IL-12 and IFN γ decreased, but no main effects on cytokines were observed when comparing HFD+L to HFD+P.

Vascular Function

Blood Pressure: Despite normal SBP and DBP at baseline, considerable reductions occurred in all groups, averaging -7.0 ± 11.1 mm Hg for SBP and -3.9 ± 7.7 mm HG for DBP ($P_s < 0.001$). Compared to HFD+P, there were no main effects of supplement type on BP. The reductions in BP were associated with improved body composition (SBP: %body fat, $r = 0.26$, $P = 0.02$ and %trunk fat, $r = 0.33$, $P = 0.003$; DBP: %body fat, $r = 0.30$, $P = 0.009$ and %trunk fat, $r = 0.37$, $P = 0.001$).

PAI-1, tPA Antigen, tPA Activity: With HFD+P, there was a trend for decreased PAI-1 levels (-5.6 ± 17.7 ng/ml, $P = 0.08$), but more significant decreases occurred with HFD+S ($P = 0.008$), HFD+O ($P = 0.04$) and HFD+L ($P = 0.009$). When compared to HFD+P, there was a strong main effect with HFD+L for an 11.1 ng/ml greater decrease in PAI-1 ($P = 0.01$). Overall, reduced PAI-1 was associated with decreased tPA antigen ($r = 0.29$, $P = 0.007$) and increased tPA activity ($r = -0.39$, $P < 0.001$). While tPA antigen decreased with HFD+P, HFD+O and HFD+L, there were no main effects of supplement type on tPA antigen when compared to HFD+P. Remarkably, tPA activity increased (Figure 4) 1.6-fold with HFD+P ($P < 0.001$), 1.25-fold with HFD+S ($P = 0.001$), 1.5-fold with HFD+O ($P = 0.006$) and 0.6-fold with HFD+L ($P = 0.003$).

SAEI, LAEI, FMD: Although univariate analyses indicated positive associations between cytokines and SAEI (IL-12: $r = -0.27$, $P = 0.03$; INF γ : $r = -0.26$, $P = 0.06$) and LAEI (INF γ : $r = -0.26$, $P = 0.03$; IL-17: $r = -0.26$, $P = 0.03$), neither SAEI, LAEI or FMD percent changed significantly with HFD+P, HFD+O or HFD+L. Intriguingly, FMD declined with HFD+S (from $9.0 \pm 7.4\%$ to $7.4 \pm 5.2\%$, $P = 0.02$). When compared to HFD+P, there was a

main effect of HFD+S for reduced FMD percent change ($P = 0.03$) and a negative time x supplement interaction ($P = 0.04$).

4.0 Discussion

Given the challenge of managing body weight, it is important to determine whether cardiometabolic health can be improved using dietary approaches without major weight loss. We observed improvements in body composition, inflammatory and vascular function markers in obese premenopausal women that were independent of weight loss. These improvements occurred with increased fat intake ($\sim 49\%$ of energy) and total fat balanced as 1/3 SFA, 1/3 MUFA and 1/3 PUFA. Dietary fat changes were reflected in plasma lipid profiles as well as 16C and 18C desaturation indices. Contrarily, fat intake in the typical American diet comprises $\sim 38\%$ SFA, 41% MUFA and 21% PUFA [38]. Accordingly, a higher SFA and lower PUFA plasma lipid profile is associated with the metabolic syndrome and CVD risk [39].

4.1 Fat oxidation

With balanced HFD, fatty acid oxidation rate increased significantly ($\sim 6\%$). Although unsaturated fats should be oxidized more efficiently than saturated [40], increased fat oxidation occurred in all groups regardless of degree of 18C fatty acid supplement unsaturation. This finding suggests intact metabolic adaptation to balanced HFD in contrast to reports of impaired long chain fatty acid oxidation in obese women [41]. Importantly, enhanced oxidation should decrease vulnerability to inflammation and insulin resistance [42]. Data from very low carbohydrate interventions suggest increased fat oxidation would be driven more from reducing carbohydrate than increasing fat intake. However, these studies severely restricted carbohydrate to $\sim 12\%$ of energy leading to reliance on ketone bodies for energy [43], whereas carbohydrate in the balanced HFD was 30% of energy.

4.2 Body composition

While prior interventions suggest obese individuals may lose more lean mass on a high fat diet [44], others reveal sex differences in response to modified fat intake. Whereas men appear to lose more lean mass with high fat intake, women seem to lose more with low fat diet [44, 45]. That this cohort had increased lean to fat mass on balanced HFD supports this concept. Importantly, retention of lean mass protects against chronic inflammation, insulin resistance and metabolic dysfunction [46]. It also promotes maintenance of resting energy expenditure [47]. We observed a reduction in REE of only 4%, further substantiating preserved metabolic efficiency as this amount represents biological day-to-day variation rather than physiologic decline [48].

4.3 Inflammation

Despite large inter-individual variability in CRP and cytokine concentrations at baseline, we observed substantial reductions in cytokine levels that are likely related to modified fat intake along with reduced adiposity. A more typical high fat diet would promote chronic low-grade inflammation, mediated in part by adipose tissue macrophages. Although we did

not measure tissue levels, it is notable that balanced HFD+S showed a large reduction in IFN γ , a driver of macrophage activity and endothelial cell inflammation [49]. In contrast, palmitate induces CRP synthesis and promotes inflammation in endothelial cells [50], highlighting biological differences in SFA by carbon chain length. It is curious that balanced HFD+O showed less reduction in some cytokines (IL-1 α and TNF β) compared to HFD+P since oleate rescues palmitate-induced inflammation by affecting interleukin secretion [51] and chemokine expression [52]. These seeming discrepancies with oleate intake may reflect the unique pathophysiological roles of different categories of cytokines [53].

4.4 Vascular function

Subjects experienced an 8 mmHg reduction in SBP and a 6 mmHg reduction in DBP despite being normotensive at baseline. Improved BP was most associated with reduced adiposity. Given the large BP response to balanced HFD, the minimal changes in SAEI, LAEI and FMD were surprising, although other macronutrient-based interventions have reported similar non-response [54] unless coupled with exercise [55]. Brachial artery FMD assesses capacity to dilate in response to increased flow, regulated by endothelial nitric oxide release. Lack of improved FMD suggests that BP reduction was independent of nitric oxide vasodilation.

Unlike FMD, tPA release and plasma PAI-1 are not mediated by nitric oxide [56]. While tPA antigen levels changed only moderately, tPA activity increased strikingly, up 60 to 160%. Improved tPA activity appears to be mediated by reduced PAI-1. As PAI-1 is regulated by adipose tissue derived inflammatory cytokines, as well as the renin-angiotensin aldosterone system [57], it is conceivable that reduced adiposity and cytokine concentrations contributed. Although all supplemented groups had reductions in pro-inflammatory cytokines and PAI-1, it is intriguing that HFD+L showed the largest improvement when compared to HFD+P. While unsaturated fats have induced PAI-1 release from endothelial cells, our findings raise the possibility that degree of unsaturation may be influential. Importantly, increased fibrinolysis, via increased tPA activity and reduced PAI-1, suggests reduced diabetes and CVD risk [58].

4.5 Strengths and limitations

Subjects were carefully phenotyped to assure they were premenopausal, normotensive, normoglycemic and normolipidemic, enabling detection of effects on inflammatory and vascular parameters prior to clinically evident CVD. Further, subjects were stabilized on balanced HFD for two weeks to allow determination of acute biological responses, included as covariates in modeling comparisons to HFD with 18C supplementation. Meticulous calculation of HFD menus along with providing all dietary fats/oils to subjects and the use of encapsulated pure fatty acids ensured strong experimental control. Lastly, adherence to HFD and compliance with supplement prescription was excellent as revealed by daily menu checklists, random 24-hour recalls, pill counts and changes in plasma lipids.

The primary limitation is that results are only generalizable to obese metabolically healthy women. Beyond heterogeneity, another concern when studying free-living individuals is drop-out. Although higher than laboratory-based experiments, retention was similar to prior

dietary fat interventions [59]. Closing interviews revealed satisfaction with the practicality of balanced HFD but unavoidable stressful life circumstances interfered with retention. Finally, the study was designed to investigate risk biomarkers not actual CVD risk reduction.

5.0 Conclusions

Focusing on type rather than amount of fat is appealing because dietary fat improves consistency, texture and palatability of foods [60]. Our findings suggest that balancing dietary fat composition (ratio of SFA/MUFA/PUFA) is a feasible strategy for increasing fat oxidation and decreasing percent body fat in obese premenopausal women. Although molecular mechanisms remain unresolved, preclinical data indicates that mitochondrial dysfunction, oxidant stress and inflammation at the cell and tissue level can be influenced by altering the type of fat consumed, based on fatty acid carbon chain length and/or degree of unsaturation [15]. Improved mitochondrial function would permit greater fat oxidation, decreased accumulation of lipotoxic intermediates, and improved adipocyte function. By doing so, a balanced HFD may potentiate improvement in inflammation and vascular function. Indeed, reduced adiposity, pro-inflammatory cytokines, and PAI-1 levels would be expected to attenuate CVD risk. Moreover, increasing intake of some long chain fatty acids may enhance inflammatory and vascular benefits. Although long term effects have not yet been determined, the practicality of the balanced HFD approach offers a readily adoptable dietary strategy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AR1	autoregressive order one
BP	blood pressure
BMI	body mass index
CVD	cardiovascular disease
DEXA	dual energy x-ray absorptiometry (DEXA)

FFA	free fatty acid
FMD	flow mediated dilatation
HDL	high density lipoprotein
HFD	high fat diet
HFD+P	high fat diet + placebo
HFD+S	high fat diet + stearate
HFD+O	high fat diet + oleate
HFD+L	high fat diet + linoleate
HOMA-IR	homeostatic model assessment of insulin resistance
IL	interleukin
PAQ	modified Baecke physical activity questionnaire
LDL	low density lipoprotein
PL	phospholipid
PAI-1	plasminogen activator inhibitor-1
tPA	tissue plasminogen antigen
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
RQ	respiratory quotient
REE	resting energy expenditure
SAEI/LAEI	small and large artery elasticity index
TG	triglyceride
VCRC	Vanderbilt clinical research center

References

1. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999; 340(2):115–126. [PubMed: 9887164]
2. Li X, Gonzalez O, Shen X, et al. Endothelial acyl-CoA synthetase 1 is not required for inflammatory and apoptotic effects of a saturated fatty acid-rich environment. *Arterioscler Thromb Vasc Biol*. 2013; 33(2):232–240. [PubMed: 23241406]
3. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science*. 1993; 259(5091):87–91. [PubMed: 7678183]
4. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004; 109(23 Suppl 1):III27–III32. [PubMed: 15198963]

5. Raitakari M, Ilvonen T, Ahotupa M, et al. Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. *Arterioscler Thromb Vasc Biol*. 2004; 24(1):124–128. [PubMed: 14656740]
6. Haspicova M, Milek D, Siklova-Vitkova M, et al. Post-prandial endothelial dysfunction is ameliorated following weight loss in obese premenopausal women. *Medical science monitor : international medical journal of experimental and clinical research*. 2011; 17(11):CR634–CR639. [PubMed: 22037742]
7. Clifton PM, Keogh JB, Foster PR, et al. Effect of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. *Int J Obes (Lond)*. 2005; 29(12):1445–1451. [PubMed: 16172619]
8. Mohler ER 3rd, Sibley AA, Stein R, et al. Endothelial function and weight loss: Comparison of low-carbohydrate and low-fat diets. *Obesity (Silver Spring)*. 2012; 21(3):504–509. [PubMed: 23404949]
9. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS medicine*. 2010; 7(3):e1000252. [PubMed: 20351774]
10. Berglund L, Lefevre M, Ginsberg HN, et al. Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. *Am J Clin Nutr*. 2007; 86(6):1611–1620. [PubMed: 18065577]
11. Sanders TA, Lewis FJ, Goff LM, et al. SFAs do not impair endothelial function and arterial stiffness. *Am J Clin Nutr*. 2013; 98(3):677–683. [PubMed: 23964054]
12. Thies F, Miles EA, Nebe-von-Caron G, et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids*. 2001; 36(11):1183–1193. [PubMed: 11795850]
13. Baum SJ, Kris-Etherton PM, Willett WC, et al. Fatty acids in cardiovascular health and disease: a comprehensive update. *Journal of clinical lipidology*. 2012; 6(3):216–234. [PubMed: 22658146]
14. Hughes TA, Heimberg M, Wang X, et al. Comparative lipoprotein metabolism of myristate, palmitate, and stearate in normolipidemic men. *Metabolism*. 1996; 45(9):1108–1118. [PubMed: 8781298]
15. Yuzefovych LV, Solodushko VA, Wilson GL, et al. Protection from palmitate-induced mitochondrial DNA damage prevents from mitochondrial oxidative stress, mitochondrial dysfunction, apoptosis, and impaired insulin signaling in rat L6 skeletal muscle cells. *Endocrinology*. 2012; 153(1):92–100. [PubMed: 22128025]
16. Kim F, Tysseling KA, Rice J, et al. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler Thromb Vasc Biol*. 2005; 25(5):989–994. [PubMed: 15731493]
17. Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. *Am J Clin Nutr*. 2010; 91(1):46–63. [PubMed: 19939984]
18. Shaw LJ, Bugiardini R, Merz CN. Women and ischemic heart disease: evolving knowledge. *J Am Coll Cardiol*. 2009; 54(17):1561–1575. [PubMed: 19833255]
19. Bugiardini R, Manfrini O, Pizzi C, et al. Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation*. 2004; 109(21):2518–2523. [PubMed: 15136498]
20. Garner DM, Olmsted MP, Bohr Y, et al. The eating attitudes test: psychometric features and clinical correlates. *Psychological medicine*. 1982; 12(4):871–878. [PubMed: 6961471]
21. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*. 1985; 29(1):71–83. [PubMed: 3981480]
22. Voorrips LE, Ravelli CJ, Dongelmans PCA, et al. Modified Baecke Questionnaire for Older Adults. 1991:S117–S121.
23. Conway JM, Ingwersen LA, Vinyard BT, et al. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. *Am J Clin Nutr*. 2003; 77(5):1171–1178. [PubMed: 12716668]

24. Institute of Medicine. Dietary Reference Intakes For Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids Food and Nutrition Board. Washington, DC: National Academy Press; 2002.
25. Huang HY, Maguire MG, Miller ER 3rd, et al. Impact of pill organizers and blister packs on adherence to pill taking in two vitamin supplementation trials. *American journal of epidemiology*. 2000; 152(8):780–787. [PubMed: 11052557]
26. Raatz SK, Bibus D, Thomas W, et al. Total fat intake modifies plasma fatty acid composition in humans. *J Nutr*. 2001; 131(2):231–234. [PubMed: 11160538]
27. de V, Weir J. New method for calculating metabolic rate with special reference to protein metabolism. *J Physiology*. 1949; 109:1–9.
28. Silver HJ, Niswender KD, Kullberg J, et al. Comparison of gross body fat-water magnetic resonance imaging at 3 Tesla to dual-energy X-ray absorptiometry in obese women. *Obesity (Silver Spring)*. 2013; 21(4):765–774. [PubMed: 23712980]
29. Nelson MR, Stepanek J, Cevette M, et al. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? *Mayo Clin Proc*. 2010; 85(5):460–472. [PubMed: 20435839]
30. Vessby B, Gustafsson IB, Tengblad S, et al. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. *Br J Nutr*. 2013:1–9.
31. Taddei S, Salvetti A. Endothelial dysfunction in hypertension. *Advances in experimental medicine and biology*. 1997; 432:235–246. [PubMed: 9433530]
32. Cohn JN, Quyyumi AA, Hollenberg NK, et al. Surrogate markers for cardiovascular disease: functional markers. *Circulation*. 2004; 109(25 Suppl 1):IV31–IV46. [PubMed: 15226249]
33. Ghiadoni L, Versari D, Giannarelli C, et al. Non-invasive diagnostic tools for investigating endothelial dysfunction. *Current pharmaceutical design*. 2008; 14(35):3715–3722. [PubMed: 19128224]
34. Muldowney JA 3rd, Vaughan DE. Tissue-type plasminogen activator release: new frontiers in endothelial function. *J Am Coll Cardiol*. 2002; 40(5):967–969. [PubMed: 12225724]
35. Mertens I, Verrijken A, Michiels JJ, et al. Among inflammation and coagulation markers, PAI-1 is a true component of the metabolic syndrome. *Int J Obes (Lond)*. 2006; 30(8):1308–1314. [PubMed: 16389265]
36. Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. *Clinical therapeutics*. 2001; 23(8):1296–1310. [PubMed: 11558866]
37. Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. *The Proceedings of the Nutrition Society*. 2004; 63(2):363–368. [PubMed: 15294056]
38. Vadiveloo M, Scott M, Quatromoni P, et al. Trends in dietary fat and high-fat food intakes from 1991 to 2008 in the Framingham Heart Study participants. *Br J Nutr*. 2013:1–11.
39. Mayneris-Perxachs J, Guerdinain M, Castellote AI, et al. Plasma fatty acid composition, estimated desaturase activities, and their relation with the metabolic syndrome in a population at high risk of cardiovascular disease. *Clinical nutrition*. 2013
40. Jones PJ, Pencharz PB, Clandinin MT. Whole body oxidation of dietary fatty acids: implications for energy utilization. *Am J Clin Nutr*. 1985; 42(5):769–777. [PubMed: 3933323]
41. Binnert C, Pachiaudi C, Beylot M, et al. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. *Am J Clin Nutr*. 1998; 67(4):595–601. [PubMed: 9537605]
42. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab*. 2008; 295(5):E1009–1017. [PubMed: 18765680]
43. Forsythe CE, Phinney SD, Fernandez ML, et al. Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids*. 2008; 43(1):65–77. [PubMed: 18046594]
44. Meckling KA, O'Sullivan C, Saari D. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *J Clin Endocrinol Metab*. 2004; 89(6):2717–2723. [PubMed: 15181047]

45. Volek JS, Sharman MJ, Love DM, et al. Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism*. 2002; 51(7):864–870. [PubMed: 12077732]
46. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey. *J Clin Endocrinol Metab*. 2011; 96(9):2898–2903. [PubMed: 21778224]
47. Illner K, Brinkmann G, Heller M, et al. Metabolically active components of fat free mass and resting energy expenditure in nonobese adults. *Am J Physiol Endocrinol Metab*. 2000; 278(2):E308–315. [PubMed: 10662716]
48. Henry CJ, Lightowler HJ, Marchini J. Intra-individual variation in resting metabolic rate during the menstrual cycle. *Br J Nutr*. 2003; 89(6):811–817. [PubMed: 12828798]
49. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. *Clin Sci (Lond)*. 2005; 108(3):205–213. [PubMed: 15540988]
50. Mugabo Y, Mukaneza Y, Renier G. Palmitate induces C-reactive protein expression in human aortic endothelial cells. Relevance to fatty acid-induced endothelial dysfunction. *Metabolism*. 2011; 60(5):640–648. [PubMed: 20727556]
51. Coll T, Eyre E, Rodriguez-Calvo R, et al. Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells. *J Biol Chem*. 2008; 283(17):11107–11116. [PubMed: 18281277]
52. Soumura M, Kume S, Isshiki K, et al. Oleate and eicosapentaenoic acid attenuate palmitate-induced inflammation and apoptosis in renal proximal tubular cell. *Biochemical and biophysical research communications*. 2010; 402(2):265–271. [PubMed: 20937260]
53. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol*. 2009; 78(6):539–552. [PubMed: 19413999]
54. Ruth MR, Port AM, Shah M, et al. Consuming a hypocaloric high fat low carbohydrate diet for 12 weeks lowers C-reactive protein, and raises serum adiponectin and high density lipoprotein-cholesterol in obese subjects. *Metabolism*. 2013; 62(12):1779–1787. [PubMed: 24075505]
55. Seligman BG, Polanczyk CA, Santos AS, et al. Intensive practical lifestyle intervention improves endothelial function in metabolic syndrome independent of weight loss: a randomized controlled trial. *Metabolism*. 2011; 60(12):1736–1740. [PubMed: 21700302]
56. Muldowney JAS, Painter CA, Sanders-Bush E, et al. Acute tissue-type plasminogen activator release in human microvascular endothelial cells: The roles of G alpha(q), PLC-beta, IP3 and 5,6-epoxyeicosatrienoic acid. *Thrombosis and haemostasis*. 2007; 97(2):263–271. [PubMed: 17264956]
57. Vaughan DE, Lazos SA, Tong K. Angiotensin II regulates the expression of plasminogen activator inhibitor-1 in cultured endothelial cells. A potential link between the renin-angiotensin system and thrombosis. *J Clin Invest*. 1995; 95(3):995–1001. [PubMed: 7884001]
58. Festa A, D'Agostino R Jr, Tracy RP, et al. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*. 2002; 51(4):1131–1137. [PubMed: 11916936]
59. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*. 2003; 348(21):2082–2090. [PubMed: 12761365]
60. Montmayeur, J-P.; Le Coutre, J. Boca Raton: CRC Press/Taylor & Francis; 2010. Fat detection : taste, texture, and post ingestive effects.

FIGURE 1.
Schematic of study subject recruitment and retention flow

Table 1

Composition of the Balanced High Fat Diet (HFD)

	Planned	Consumed ³
Energy (kcal)	1600–2400 ^{1,2}	1905.8 ± 448.3
Fat (%kcal)	50	48.9 ± 0.3
Carbohydrate (%kcal)	30	30.2 ± 0.2
Protein (%kcal)	20	21.3 ± 0.5
Total SFA (%Fat)	33.3	30.6 ± 0.3
Total MUFA (%Fat)	33.3	35.1 ± 0.1
Total PUFA (%Fat)	33.3	34.2 ± 0.1
Starch (%CHO)	50	52.5 ± 0.9
Sugar (%CHO)	50	48.0 ± 0.5

¹ Menus were created using NDSR for caloric intakes ranging from 1600 to 2400 kcal/day.

² Actual energy (kcal) goals were individualized based on measured resting energy expenditure x 1.2 physical activity factor.

³ Data are expressed as means ± SD.

Table 2

Baseline Characteristics of 144 Obese Women by 18C Fatty Acid Supplement Group¹

	HFD + Placebo n = 41	HFD + Stearate n = 34	HFD + Oleate n = 36	HFD + Linoleate n = 33	P Value ²
Race					0.62
Caucasian	30 (73.2%)	22 (64.7%)	28 (77.8%)	21 (63.6%)	
African American	11 (26.8%)	12 (35.3%)	8 (22.2%)	12 (36.4%)	
Age (y)	37.0 ± 7.3	36.4 ± 7.5	37.3 ± 6.4	36.1 ± 6.1	0.82
Height (cm)	163.4 ± 5.1	163.7 ± 6.5	163.3 ± 7.0	164.0 ± 7.4	0.96
Weight (kg)	93.6 ± 8.8	95.4 ± 12.6	91.5 ± 10.3	93.1 ± 12.0	0.51
BMI (kg/m ²)	35.0 ± 2.5	35.5 ± 2.7	34.7 ± 2.8	34.5 ± 2.7	0.25
Waist Circumference (cm)	103.7 ± 8.0	102.5 ± 8.4	101.6 ± 6.5	101.5 ± 8.3	0.61
Total Body Fat (%)	46.7 ± 3.2	47.7 ± 3.4	47.5 ± 3.0	46.9 ± 3.9	0.54
Energy Intake (kcal)	1928 ± 515	1874 ± 409	1973 ± 476	1838 ± 369	0.69
Respiratory Quotient (kcal/l)	0.82 ± 0.05	0.82 ± 0.06	0.82 ± 0.05	0.82 ± 0.05	0.96
Glucose (mg/dl)	90.6 ± 12.5	93.8 ± 12.3	92.8 ± 11.7	92.6 ± 12.9	0.72
Insulin (μU/ml)	12.0 ± 7.0	10.3 ± 5.9	11.1 ± 9.5	10.5 ± 8.3	0.76
HOMA-IR (units)	2.8 ± 2.1	2.5 ± 1.7	2.7 ± 2.5	2.5 ± 2.4	0.90
Total Cholesterol (mg/dl)	169.3 ± 33.1	165.3 ± 32.9	174.3 ± 25.6	162.7 ± 21.7	0.38
LDL Cholesterol (mg/dl)	103.3 ± 26.5	101.6 ± 28.9	105.1 ± 25.5	99.1 ± 19.3	0.79
HDL Cholesterol (mg/dl)	47.5 ± 12.8	47.7 ± 11.9	48.3 ± 11.9	48.2 ± 13.5	0.99
Triglycerides (mg/dl)	96.4 ± 51.3	79.1 ± 36.7	91.0 ± 58.0	88.1 ± 52.0	0.52
hsCRP (mg/dl)	6.3 ± 7.9	6.6 ± 9.0	5.4 ± 3.9	5.8 ± 5.6	0.89
Systolic BP (mm Hg)	120.7 ± 12.9	121.8 ± 11.2	123.6 ± 10.8	120.2 ± 9.8	0.61
Diastolic BP (mm Hg)	69.2 ± 8.7	71.8 ± 8.6	70.2 ± 8.5	68.8 ± 8.1	0.50

¹Data are expressed as means ± SD.²The Kruskal-Wallis test was used to detect differences among the four groups at baseline.

Table 3

Dietary and Plasma Lipid Changes During HFD Stabilization Period (Week 0 – 2)[†]

	HFD + Placebo			HFD + Stearate			HFD + Oleate			HFD + Linoleate		
	Baseline	Change at Week 2 [‡]	Baseline	Change at Week 2 [‡]	Baseline	Change at Week 2 [‡]	Baseline	Change at Week 2 [‡]	Baseline	Change at Week 2 [‡]	Baseline	Change at Week 2 [‡]
Dietary												
Energy Intake (kcal)	1928 ± 515	-135 ± 488	1874 ± 409	-89 ± 467	1973 ± 476	-297 ± 503 [‡]	1838 ± 369		1838 ± 369	-176 ± 343 [*]		
Fat (% Energy)	38.7 ± 8.5	10.6 ± 9.6 [‡]	37.0 ± 7.1	11.6 ± 7.1 [‡]	37.8 ± 6.8	10.7 ± 6.9 [‡]	39.7 ± 6.9		39.7 ± 6.9	8.4 ± 6.3 [‡]		
Carbohydrate (% Energy)	43.9 ± 10.0	-15.5 ± 10.6 [‡]	46.3 ± 8.4	-16.8 ± 8.4 [‡]	45.2 ± 8.2	-16.0 ± 8.0 [‡]	43.1 ± 8.7		43.1 ± 8.7	-12.9 ± 8.1 [‡]		
Protein (% Energy)	16.9 ± 4.7	5.0 ± 4.5 [‡]	16.5 ± 4.9	5.0 ± 4.9 [‡]	16.1 ± 3.1	5.5 ± 3.3 [‡]	16.8 ± 4.2		16.8 ± 4.2	4.7 ± 4.3 [‡]		
Total SFA (% Fat)	33.3 ± 5.7	-2.1 ± 5.8	34.6 ± 6.2	-2.8 ± 5.9 [‡]	33.5 ± 5.7	-2.2 ± 5.8 [*]	33.4 ± 7.5		33.4 ± 7.5	-3.4 ± 7.9 [*]		
Palmitic C16:0	18.0 ± 2.5	-1.8 ± 2.8 [‡]	18.0 ± 2.3	-1.7 ± 2.6 [‡]	17.7 ± 2.4	-1.7 ± 2.6 [‡]	17.2 ± 3.2		17.2 ± 3.2	-1.9 ± 3.3 [‡]		
Stearic C18:0	8.7 ± 1.6	-2.5 ± 1.7 [‡]	8.7 ± 1.5	-2.4 ± 1.5 [‡]	8.5 ± 1.7	-2.2 ± 1.6 [‡]	8.5 ± 1.6		8.5 ± 1.6	-2.4 ± 1.6 [‡]		
Total MUFA (% Fat)	36.8 ± 4.1	-2.0 ± 3.8 [*]	35.5 ± 4.3	-0.3 ± 4.3	36.5 ± 5.4	-1.0 ± 5.3	36.6 ± 5.3		36.6 ± 5.3	-1.1 ± 5.6		
Palmitoleic C16:1	1.6 ± 0.6	-0.2 ± 0.7	1.6 ± 0.5	-0.2 ± 0.6	1.6 ± 0.5	-0.2 ± 0.5	1.5 ± 0.7		1.5 ± 0.7	-0.2 ± 0.8		
Oleic C18:1	34.4 ± 3.7	-2.0 ± 3.7 [‡]	33.2 ± 4.3	-0.2 ± 4.5	34.2 ± 5.4	-1.0 ± 5.4	34.4 ± 5.1		34.4 ± 5.1	-1.0 ± 5.5		
Total PUFA (% Fat)	21.2 ± 6.9	5.3 ± 7.5 [‡]	21.7 ± 6.3	3.8 ± 6.2 [‡]	21.9 ± 6.2	3.8 ± 5.6 [‡]	22.0 ± 7.4		22.0 ± 7.4	5.0 ± 7.4 [‡]		
Linoleic C18:2	18.8 ± 6.4	4.9 ± 7.0 [‡]	19.3 ± 5.6	3.5 ± 5.5 [‡]	19.5 ± 5.9	3.6 ± 5.3 [‡]	19.6 ± 7.1		19.6 ± 7.1	4.8 ± 7.0 [‡]		
Plasma												
Total SFA (% TG)	34.8 ± 4.3	-2.0 ± 4.6 [*]	33.4 ± 3.9	-1.3 ± 3.9	34.7 ± 3.7	-2.5 ± 4.4 [‡]	35.0 ± 4.4		35.0 ± 4.4	-2.9 ± 4.4 [‡]		
Palmitic C16:0	27.5 ± 3.3	-0.7 ± 3.6	27.0 ± 3.0	-0.3 ± 2.8	27.7 ± 2.9	-1.4 ± 2.6 [‡]	27.7 ± 3.5		27.7 ± 3.5	-1.1 ± 3.6		
Stearic C18:0	5.0 ± 1.4	-0.6 ± 1.1 [‡]	4.7 ± 1.2	-0.7 ± 1.2 [‡]	4.9 ± 1.1	-0.2 ± 2.2 [*]	5.3 ± 1.6		5.3 ± 1.6	-0.9 ± 1.2 [‡]		
Total MUFA (% TG)	38.1 ± 2.7	0.2 ± 3.7	39.4 ± 3.3	-0.3 ± 3.6	39.1 ± 3.1	-0.3 ± 3.7	38.2 ± 2.9		38.2 ± 2.9	0.7 ± 3.5		
Palmitoleic C16:1	2.9 ± 1.1	-0.2 ± 0.9	2.8 ± 0.9	-0.1 ± 0.9	3.1 ± 0.8	-0.3 ± 0.8 [*]	3.0 ± 1.0		3.0 ± 1.0	-0.5 ± 0.8 [‡]		
Oleic C18:1	35.0 ± 3.0	0.4 ± 3.7	36.1 ± 3.5	-0.2 ± 3.5	35.4 ± 3.0	0.1 ± 3.4	34.7 ± 2.7		34.7 ± 2.7	1.2 ± 3.2 [*]		
Total PUFA (%TG)	27.1 ± 4.6	1.7 ± 4.6	27.3 ± 5.2	1.6 ± 4.5	26.2 ± 4.4	2.8 ± 4.8 [‡]	26.8 ± 5.3		26.8 ± 5.3	2.2 ± 4.9 [*]		
Linoleic C18:2	22.8 ± 4.3	2.6 ± 3.6 [‡]	23.3 ± 4.7	1.8 ± 4.3 [*]	21.9 ± 3.9	3.1 ± 4.2 [‡]	22.3 ± 5.1		22.3 ± 5.1	2.8 ± 4.5 [‡]		
Total SFA (%FFA)	38.6 ± 6.2	-2.6 ± 7.3	38.7 ± 5.7	-2.5 ± 6.1 [*]	40.4 ± 7.1	-4.2 ± 7.6 [‡]	40.3 ± 5.4		40.3 ± 5.4	-4.3 ± 4.5 [‡]		
Palmitic C16:0	26.0 ± 2.7	-0.5 ± 2.8	25.4 ± 2.3	0.2 ± 2.8	26.2 ± 3.0	-1.0 ± 2.9	26.2 ± 2.6		26.2 ± 2.6	-1.0 ± 2.1 [*]		

	HFD + Placebo			HFD + Stearate			HFD + Oleate			HFD + Linoleate		
	Baseline	Change at Week 2 ²	Baseline	Baseline	Change at Week 2 ²	Baseline	Baseline	Change at Week 2 ²	Baseline	Baseline	Change at Week 2 ²	Baseline
Stearic C18:0	10.7 ± 4.5	-2.2 ± 5.1 [*]	11.6 ± 4.2	11.6 ± 4.2	-2.9 ± 3.8 [‡]	12.3 ± 4.7	12.3 ± 4.7	-3.0 ± 5.1 [‡]	12.2 ± 3.7	12.2 ± 3.7	-3.3 ± 3.3 [‡]	12.2 ± 3.7
Total MUFA (% FFA)	37.2 ± 4.5	2.0 ± 5.6	37.6 ± 4.3	37.6 ± 4.3	2.7 ± 4.4 [‡]	37.3 ± 5.4	37.3 ± 5.4	3.5 ± 5.4 [‡]	36.9 ± 4.1	36.9 ± 4.1	3.9 ± 4.5 [‡]	36.9 ± 4.1
Palmitoleic C16:1	3.1 ± 1.1	0.5 ± 1.1 [*]	3.0 ± 1.1	3.0 ± 1.1	0.6 ± 1.1 [‡]	3.1 ± 0.9	3.1 ± 0.9	0.5 ± 0.9 [‡]	3.0 ± 1.0	3.0 ± 1.0	0.5 ± 1.0 [‡]	3.0 ± 1.0
Oleic C18:1	34.0 ± 4.0	1.3 ± 4.5	34.2 ± 3.7	34.2 ± 3.7	2.1 ± 3.8 [‡]	33.7 ± 5.0	33.7 ± 5.0	3.0 ± 4.6 [‡]	33.4 ± 3.8	33.4 ± 3.8	3.5 ± 4.2 [‡]	33.4 ± 3.8
Total PUFA (% FFA)	24.2 ± 4.0	0.6 ± 3.8	23.7 ± 4.0	23.7 ± 4.0	-0.1 ± 3.0	22.3 ± 3.3	22.3 ± 3.3	0.6 ± 3.5	22.8 ± 3.9	22.8 ± 3.9	0.4 ± 2.4	22.8 ± 3.9
Linoleic C18:2	21.5 ± 3.5	0.6 ± 3.3	20.9 ± 3.4	20.9 ± 3.4	0.2 ± 2.7	19.6 ± 2.7	19.6 ± 2.7	0.7 ± 3.0 [*]	20.0 ± 3.3	20.0 ± 3.3	0.8 ± 1.9 [*]	20.0 ± 3.3
Total SFA (% PL)	41.3 ± 1.9	0.4 ± 2.5	41.9 ± 2.6	41.9 ± 2.6	-0.1 ± 2.5	42.1 ± 2.6	42.1 ± 2.6	0.2 ± 2.1	41.5 ± 3.5	41.5 ± 3.5	0.1 ± 2.8	41.5 ± 3.5
Palmitic C16:0	26.7 ± 2.1	1.5 ± 1.7 [‡]	27.2 ± 2.4	27.2 ± 2.4	1.0 ± 1.8 [‡]	27.4 ± 2.2	27.4 ± 2.2	1.2 ± 1.6 [‡]	26.6 ± 2.3	26.6 ± 2.3	1.5 ± 2.2 [‡]	26.6 ± 2.3
Stearic C18:0	14.3 ± 1.7	-1.1 ± 2.3 [*]	14.3 ± 1.7	14.3 ± 1.7	-1.1 ± 2.1 [‡]	14.3 ± 1.9	14.3 ± 1.9	-0.9 ± 1.5 [‡]	14.5 ± 2.1	14.5 ± 2.1	-1.3 ± 2.5 [‡]	14.5 ± 2.1
Total MUFA (% PL)	8.9 ± 1.1	-0.6 ± 0.9 [‡]	9.5 ± 1.1	9.5 ± 1.1	-1.1 ± 1.1 [‡]	10.2 ± 2.7	10.2 ± 2.7	-1.5 ± 1.1 [‡]	9.7 ± 2.3	9.7 ± 2.3	-1.5 ± 2.1 [‡]	9.7 ± 2.3
Palmitoleic C16:1	0.5 ± 0.2	-0.1 ± 0.2 [*]	0.5 ± 0.2	0.5 ± 0.2	-0.1 ± 0.2 [*]	0.6 ± 0.3	0.6 ± 0.3	-0.1 ± 0.2 [‡]	0.6 ± 0.3	0.6 ± 0.3	-0.1 ± 0.5 [‡]	0.6 ± 0.3
Oleic C18:1	8.3 ± 0.9	-0.5 ± 0.9 [‡]	8.7 ± 0.9	8.7 ± 0.9	-1.0 ± 1.1 [‡]	9.3 ± 2.5	9.3 ± 2.5	-1.4 ± 1.0 [‡]	8.8 ± 2.0	8.8 ± 2.0	-1.4 ± 1.9 [‡]	8.8 ± 2.0
Total PUFA (% PL)	49.7 ± 2.6	0.2 ± 2.9	48.6 ± 2.9	48.6 ± 2.9	1.2 ± 2.7 [*]	47.6 ± 3.7	47.6 ± 3.7	1.3 ± 2.7 [*]	48.8 ± 4.6	48.8 ± 4.6	1.5 ± 3.6 [*]	48.8 ± 4.6
Linoleic C18:2	26.8 ± 2.8	-1.0 ± 3.6 [*]	26.8 ± 2.6	26.8 ± 2.6	-0.6 ± 2.6	26.0 ± 2.8	26.0 ± 2.8	-0.8 ± 2.4 [*]	26.6 ± 4.2	26.6 ± 4.2	-1.4 ± 2.9 [*]	26.6 ± 4.2

¹ Data are expressed as mean ± SD.

² Within-group changes assessed using Wilcoxon signed rank tests:

^{*} $P < 0.05$,

[‡] $P < 0.01$,

[‡] $P < 0.001$.

Table 4

Responses in Body Composition, Inflammation and Vascular Function to Balanced HFD with 18C Fatty Acid Supplementation[†]

	HFD + Placebo		HFD + Stearate		HFD + Oleate		HFD + Linoleate	
	Baseline	Change at Week 16 [‡]	Baseline	Change at Week 16 [‡]	Baseline	Change at Week 16 [‡]	Baseline	Change at Week 16 [‡]
Body Composition								
Total Fat Mass (kg)	40.9 ± 5.2	-5.1 ± 3.2 [‡]	45.3 ± 8.2	-4.8 ± 3.7 [‡]	42.4 ± 5.7	-4.6 ± 2.9 [‡]	40.6 ± 7.1	-4.9 ± 3.2 [‡]
Total Lean Mass (kg)	47.6 ± 4.3	-1.1 ± 1.8 [†]	47.9 ± 4.6	-1.2 ± 1.1 [‡]	46.2 ± 5.2	-1.0 ± 1.6 [†]	45.4 ± 6.7	-0.8 ± 1.7
Body fat (%)	46.2 ± 3.1	-2.8 ± 2.3 [‡]	48.3 ± 5.2	-2.2 ± 2.2 [‡]	47.8 ± 2.8	-2.4 ± 1.7 [‡]	47.1 ± 4.0	-2.9 ± 2.3 [‡]
Lean (%)	53.8 ± 3.1	2.8 ± 2.3 [‡]	51.6 ± 3.2	2.3 ± 2.2 [‡]	52.2 ± 2.8	2.4 ± 1.7 [‡]	52.9 ± 3.9	2.9 ± 2.3 [‡]
Trunk fat (%)	49.3 ± 4.3	-3.6 ± 3.4 [‡]	51.0 ± 4.4	-3.1 ± 2.5 [‡]	51.1 ± 3.5	-2.5 ± 2.1 [‡]	48.7 ± 4.4	-3.3 ± 3.3 [‡]
Android fat (%)	56.8 ± 3.9	-4.5 ± 5.1 [‡]	57.5 ± 4.7	-3.4 ± 3.4 [‡]	58.2 ± 3.7	-2.5 ± 2.2 [‡]	55.8 ± 4.4	-3.8 ± 3.6 [‡]
Gynoid fat (%)	53.9 ± 3.3	-2.9 ± 2.8 [‡]	56.5 ± 2.9	-2.2 ± 2.5	56.0 ± 3.8	-2.4 ± 1.8 [‡]	55.7 ± 4.8	-2.7 ± 1.8 [‡]
Inflammation								
IL-1α (pg/ml)	15.0 ± 24.9	-3.4 ± 13.3	10.3 ± 18.7	-9.1 ± 20.3 [*]	18.6 ± 30.2	-7.0 ± 16.0 [*]	16.0 ± 34.7	-4.6 ± 10.7 [*]
IL-1β (pg/ml)	2.5 ± 4.9	-0.7 ± 3.9 [†]	3.7 ± 8.0	-2.7 ± 9.3	6.7 ± 11.1	-4.1 ± 10.7	2.4 ± 4.2	-1.4 ± 4.5
IL-6 (pg/ml)	4.2 ± 6.9	-1.9 ± 5.0	5.9 ± 9.0	-4.2 ± 8.4	10.9 ± 22.4	-5.6 ± 21.4	4.9 ± 11.0	-0.8 ± 2.9
IL-10 (pg/ml)	4.9 ± 9.1	-1.1 ± 8.0 [*]	5.4 ± 8.9	-3.1 ± 9.4	12.3 ± 22.1	-5.6 ± 12.4	6.8 ± 8.3	-2.9 ± 5.6 [*]
IL-12 (pg/ml)	17.3 ± 38.3	-1.9 ± 27.9 [*]	20.5 ± 46.2	-12.6 ± 48.5	48.8 ± 76.3	-31.8 ± 51.6 [†]	14.4 ± 24.4	-8.3 ± 20.4 [*]
IL-17 (pg/ml)	6.7 ± 8.2	-3.2 ± 5.4 [†]	12.7 ± 17.8	-8.0 ± 14.1 [†]	10.9 ± 20.3	-8.0 ± 16.0 [‡]	10.3 ± 17.1	-4.4 ± 11.3
IFN-γ (pg/ml)	11.5 ± 19.3	-6.5 ± 17.9 [†]	24.7 ± 50.5	-23.8 ± 49.0 [†]	11.2 ± 15.0	-8.9 ± 12.4 [‡]	7.8 ± 11.0	-3.9 ± 8.1 [†]
TNFα (pg/ml)	6.8 ± 3.0	0.2 ± 5.8	8.3 ± 6.4	-3.5 ± 7.7	12.0 ± 15.9	-6.6 ± 14.6	6.9 ± 4.5	-2.0 ± 4.4
TNFβ (pg/ml)	5.4 ± 10.0	-2.7 ± 8.2 [†]	6.7 ± 13.9	-5.2 ± 15.4	10.4 ± 19.4	-6.6 ± 18.4	3.7 ± 8.3	-0.2 ± 13.1
Vascular Function								
Systolic BP (mm Hg)	120.7 ± 13.0	-3.9 ± 9.1	121.8 ± 11.2	-7.2 ± 8.9 [†]	123.6 ± 10.8	-9.0 ± 13.9 [†]	120.2 ± 9.9	-7.3 ± 10.8 [†]
Diastolic BP (mm Hg)	69.2 ± 8.7	-2.2 ± 7.3	71.8 ± 8.6	-4.6 ± 6.5 [†]	70.2 ± 8.5	-4.4 ± 9.5 [†]	68.8 ± 8.1	-4.1 ± 7.0 [*]
PAI-1 Antigen (ng/ml)	26.7 ± 17.2	-5.6 ± 17.7	22.0 ± 11.1	-7.6 ± 10.6 [†]	25.3 ± 15.9	-8.3 ± 18.7 [*]	22.9 ± 13.4	-7.0 ± 10.2 [†]
tPA Antigen (ng/ml)	12.8 ± 5.9	-5.6 ± 4.9 [‡]	9.6 ± 5.6	-2.6 ± 5.6	12.1 ± 6.1	-4.2 ± 5.5 [†]	12.1 ± 5.4	-4.8 ± 5.2 [‡]
tPA Activity (IU/ml)	0.16 ± 0.25	0.25 ± 0.21 [‡]	0.16 ± 0.32	0.22 ± 0.25 [‡]	0.13 ± 0.32	0.18 ± 0.39 [†]	0.17 ± 0.33	0.15 ± 0.32 [†]
SAEI	7.9 ± 2.7	-0.2 ± 2.7	7.3 ± 3.4	0.3 ± 3.9	7.9 ± 2.7	1.1 ± 4.2	7.6 ± 2.4	0.5 ± 2.7

	HFD + Placebo		HFD + Stearate		HFD + Oleate		HFD + Linoleate	
	Baseline	Change at Week 16 ²	Baseline	Change at Week 16 ²	Baseline	Change at Week 16 ²	Baseline	Change at Week 16 ²
L/AEI	16.7 ± 4.4	-0.2 ± 2.6	16.3 ± 4.5	0.9 ± 5.0	15.8 ± 4.2	0.8 ± 4.5	16.4 ± 3.7	0.1 ± 4.1
FMD (% change)	8.6 ± 6.2	1.7 ± 9.3	9.0 ± 7.4	-3.6 ± 6.9 [*]	8.2 ± 6.8	-1.8 ± 7.9	7.8 ± 5.9	0.0 ± 7.7

¹ Data are expressed as means ± SD.

² Within-group changes assessed using Wilcoxon signed rank tests:

^{*} $P < 0.05$,
[†] $P < 0.01$,
[‡] $P < 0.001$.

Table 5

Results from Linear Mixed Effects Models for Main Effects of Time and Treatment on Inflammation and Vascular function¹

	For time effect (per week)	For treatment effect ²			For time x treatment effect			
		HFD+Stearate	HFD+Oleate	HFD+Linoleate	HFD+Stearate	HFD+Oleate	HFD+Linoleate	
Inflammation	IL-1α (pg/ml)	(-0.2 ± 0.2) 0.36	(-2.9 ± 7.5) 0.70	(7.6 ± 7.3) 0.29	(-3.4 ± 7.6) 0.65	(-0.3 ± 0.4) 0.38	(-0.2 ± 0.3) 0.59	(-0.1 ± 0.4) 0.84
	IL-1β (pg/ml)	(-0.1 ± 0.1) 0.66	(0.7 ± 1.9) 0.71	(3.9 ± 1.9) 0.04*	(-0.5 ± 1.9) 0.79	(-0.1 ± 0.1) 0.29	(-0.2 ± 0.1) 0.08	(-0.1 ± 0.1) 0.65
	IL-6 (pg/ml)	(-0.1 ± 0.1) 0.37	(2.9 ± 3.5) 0.39	(6.9 ± 3.4) 0.04*	(-1.6 ± 3.5) 0.64	(-0.1 ± 0.2) 0.54	(-0.2 ± 0.2) 0.19	(0.1 ± 0.2) 0.74
	IL-10 (pg/ml)	(-0.1 ± 0.1) 0.54	(-0.1 ± 3.9) 0.97	(6.8 ± 3.8) 0.07	(-0.3 ± 3.9) 0.93	(-0.1 ± 0.2) 0.41	(-0.3 ± 0.1) 0.06	(-0.1 ± 0.2) 0.42
	IL-12 (pg/ml)	(-0.1 ± 0.4) 0.83	(0.4 ± 13.9) 0.97	(29.1 ± 13.6) 0.04*	(-6.8 ± 14.1) 0.63	(-0.7 ± 0.6) 0.28	(-1.8 ± 0.6) 0.002 [†]	(-0.4 ± 0.6) 0.48
	IL-17 (pg/ml)	(-0.2 ± 0.1) 0.17	(5.9 ± 4.3) 0.17	(2.6 ± 4.2) 0.55	(-0.6 ± 4.4) 0.89	(-0.3 ± 0.2) 0.10	(-0.3 ± 0.2) 0.12	(-0.1 ± 0.2) 0.66
	IFNγ (pg/ml)	(-0.4 ± 0.3) 0.13	(-14.8 ± 5.7) 0.01*	(1.4 ± 5.6) 0.81	(-3.6 ± 5.8) 0.54	(-1.0 ± 0.4) 0.02*	(-0.1 ± 0.4) 0.75	(-0.2 ± 0.4) 0.65
	TNFα (pg/ml)	(0.1 ± 0.1) 0.97	(2.8 ± 2.5) 0.25	(6.9 ± 2.4) 0.005 [†]	(0.5 ± 2.5) 0.85	(-0.2 ± 0.2) 0.28	(-0.4 ± 0.2) 0.02*	(-0.1 ± 0.2) 0.43
	TNFβ (pg/ml)	(-0.2 ± 0.2) 0.28	(0.9 ± 3.1) 0.77	(4.4 ± 3.1) 0.15	(-2.6 ± 3.2) 0.41	(-0.1 ± 0.2) 0.53	(-0.2 ± 0.2) 0.31	(0.1 ± 0.2) 0.54
Vascular Function	Systolic BP (mm Hg)	(-0.1 ± 0.1) 0.60	(0.6 ± 2.5) 0.81	(0.6 ± 2.3) 0.81	(-0.4 ± 2.5) 0.88	(0.1 ± 0.2) 0.89	(-0.2 ± 0.2) 0.25	(-0.1 ± 0.2) 0.97
	Diastolic BP (mm Hg)	(-0.1 ± 0.1) 0.89	(-0.1 ± 1.9) 0.98	(0.5 ± 1.8) 0.77	(-0.3 ± 1.9) 0.88	(0.1 ± 0.2) 0.61	(-0.1 ± 0.1) 0.66	(-0.1 ± 0.1) 0.94
	PAI-1 Antigen (ng/ml)	(-0.5 ± 0.2) 0.04*	(-7.0 ± 4.6) 0.13	(-5.7 ± 4.7) 0.24	(-11.1 ± 4.6) 0.01*	(0.1 ± 0.4) 0.81	(0.6 ± 0.4) 0.12	(0.1 ± 0.4) 0.74
	tPA Antigen (ng/ml)	(-0.2 ± 0.9) 0.08	(0.9 ± 1.8) 0.62	(2.0 ± 1.7) 0.25	(-1.4 ± 1.8) 0.43	(-0.3 ± 0.1) 0.81	(0.1 ± 0.1) 0.89	(0.1 ± 0.1) 0.63
	tPA Activity (IU/ml)	(0.1 ± 0.1) 0.34	(0.1 ± 0.1) 0.75	(0.1 ± 0.1) 0.73	(0.1 ± 0.1) 0.27	(-0.1 ± 0.1) 0.17	(-0.1 ± 0.1) 0.04*	(-0.1 ± 0.1) 0.33
	SAEI	(0.1 ± 0.1) 0.59	(0.6 ± 1.1) 0.61	(-0.2 ± 1.0) 0.86	(1.1 ± 1.1) 0.32	(-0.1 ± 0.1) 0.50	(0.1 ± 0.1) 0.19	(-0.1 ± 0.1) 0.63
	LAEI	(-0.1 ± 0.1) 0.33	(-1.7 ± 1.5) 0.29	(-1.5 ± 1.4) 0.30	(-1.6 ± 1.5) 0.31	(0.1 ± 0.1) 0.24	(0.1 ± 0.1) 0.19	(0.1 ± 0.1) 0.72

	For time effect (per week)	For treatment effect ²		For time x treatment effect		
		HFD+Stearate	HFD+Oleate	HFD+Stearate	HFD+Oleate	HFD+Linoleate
FMD (% change)	(0.2 ± 0.1)	(2.8 ± 1.8)	(1.8 ± 1.8)	(-0.3 ± 0.2)	(-0.3 ± 0.2)	(-0.1 ± 0.2)
	0.04 *	0.13	0.31	0.04 *	0.04 *	0.44

Data are expressed as (Value $\pm$ SE)/P Value. Models included total weight loss and change in variable during HFD stabilization period (Baseline to Week 2) as covariates.

²Treatment (HFD + 18C fatty acid supplement) compared to HFD + Placebo. Significant P-values flagged;

^{*} $P<0.05$,[†] $P < 0.01$,[†] $P < 0.001$