


High-Fat Meal–Induced Changes in Markers of Inflammation and Angiogenesis in Healthy Adults Who Differ by Age and Physical Activity Level

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ABSTRACT

Background: Inflammation and angiogenesis are key facets of cardiovascular disease pathophysiology. Age and physical activity level can influence fasting systemic inflammation, but the impact of these factors on postprandial inflammation is unknown. In addition, markers of angiogenesis have never been tested in the context of a single high-fat meal (HFM).

Objective: The purpose of this study was to investigate the effects of an HFM on markers of inflammation and angiogenesis in individuals of different ages and physical activity levels.

Methods: Twenty-two healthy adults—8 younger active (YA) adults (4 men, 4 women; mean \pm SD age: 25 ± 5 y), 8 older active (OA) adults (4 men, 4 women; 67 ± 5 y), and 6 older inactive (OI) adults (3 men, 3 women; 68 ± 7 y)—consumed an HFM [63% fat (39% saturated fat, 14% monounsaturated fat, 10% polyunsaturated fat), 34% carbohydrate; 12 kcal/kg body mass; 927 ± 154 kcal]. Fourteen inflammatory and 9 angiogenic markers were measured at baseline and 3 and 6 h postmeal.

Results: Significant group effects were observed in interleukin (IL)-10 (YA > OA; $P = 0.02$), IL-23 (YA > OA; $P = 0.02$), tumor necrosis factor (TNF)- α (OA < OI; $P = 0.04$), and vascular endothelial growth factor (VEGF)-C (YA < OA; $P = 0.001$). IL-8, VEGF-A, VEGF-C, and heparin-binding epidermal growth factor–like growth factor significantly increased, whereas granulocyte-macrophage colony-stimulating factor, interferon- γ , IL-1 β , IL-5, IL-10, IL-12, IL-13, IL-17A, IL-23, TNF- α , leptin, angiopoietin-2, and follistatin significantly decreased after HFM consumption (P 's < 0.05). Notably, VEGF-A and VEGF-C were significantly higher at 3 h [mean difference: 22.5 pg/mL (VEGF-A); 73.5 pg/mL (VEGF-C)] and 6 h postmeal [mean difference: 26.9 pg/mL (VEGF-A); 81.2 pg/mL (VEGF-C)].

Conclusions: A novel finding of this study was the robust increase in VEGF after an HFM. There were also group differences in several inflammatory markers (IL-10 and IL-23 greater in YA than OA, and TNF- α lower in OA than OI) that suggest a potential influence of age and physical activity level. *Curr Dev Nutr* 2019;3:nzy098.

Introduction

Inflammation is an integral feature in the pathophysiology of cardiovascular disease (CVD) (1), which is the leading cause of death in the United States (2). As lipids are deposited in the arterial wall, an inflammatory response is propagated that involves the recruitment of various immune cells, and ultimately contributes to the process of atherosclerosis (1). Consequently, high levels of immune cells recognized to be players in the atherosclerotic cascade, also known as



Keywords: cytokine, interleukin, VEGF, postprandial, older adults, exercise

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Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HB-EGF, heparin-binding epidermal growth factor–like growth factor; HFM, high-fat meal; HGF, hepatocyte growth factor; MVPA, moderate- to vigorous-intensity physical activity; OA, older active; OI, older inactive; VEGF, vascular endothelial growth factor; YA, younger active.

systemic inflammation, are considered indicators of CVD risk (3). This rationale is supported by epidemiological evidence, in which circulating inflammatory markers, such as IL-6, C-reactive protein (CRP), and TNF- α , have been found to be positively associated with various forms of CVD (4–6).

One factor that can initiate or largely prevent the development of CVD, while also modifying inflammatory status, is dietary intake. The beneficial effects of a nutrient-dense diet are well-recognized, as are the detrimental effects of a Western diet (7). Moreover, recent research has revealed that a single high-fat meal (HFM), representative of a typical Western diet, can induce an inflammatory response (8). Specifically, postprandial increases have been observed in IL-6, TNF- α , and CRP (9–11). Because circulating concentrations of these markers have been found to be positively associated with CVD risk in the fasted state (4–6), acute fluxes in inflammatory markers such as IL-6, TNF- α , and CRP are noteworthy.

In addition to a nutrient-dense diet, physical activity and exercise can decrease risk of CVD, likely mediated in part through a reduction in inflammation (12). Several studies have demonstrated the anti-inflammatory effect of chronic exercise, particularly by revealing inverse associations between chronic physical activity level and several markers of inflammation, including CRP, IL-6, and TNF- α (12–14). Based on these findings, one could speculate that chronic physical activity may blunt the postprandial inflammatory response.

The majority of previous studies investigating postprandial inflammation have been conducted with younger or middle-aged individuals, and very few studies have focused on older adults. A recent study that investigated postprandial inflammation in healthy younger and older adults found no differences between groups in IL-6, TNF- α , or CRP after HFM intake (15). To our knowledge, no previous studies have investigated the impact of both age and chronic physical activity on the postprandial inflammatory response to an HFM.

Related to inflammation, angiogenesis has been suggested as a key facet of atherosclerosis, although this point has been highly contentious (16). Although angiogenic cytokines, such as vascular endothelial growth factor (VEGF), have been used as a therapeutic agent in preclinical studies to promote collateral blood vessel formation in ischemic tissue (17), neovascularization has also been shown to contribute to the expansion of atherosclerotic lesions (18) and to be an important factor in rupture-promoting plaque destabilization (19). Notably, angiogenic factors are tightly connected to vascular inflammation and there is evidence that inflammatory cells can drive angiogenesis in atherosclerotic plaque (20). Given their affiliation with inflammatory markers, it is reasonable to speculate that markers of angiogenesis may exhibit acute changes in response to HFM intake, as well as modification by factors such as age and physical activity level, although this has never, to our knowledge, been tested.

Therefore, the purpose of this experiment was to determine the effects of an HFM on markers of inflammation and angiogenesis in individuals of different ages and physical activity levels. We assessed the postprandial inflammatory and angiogenic responses in 3 groups: younger active (YA), older active (OA), and older inactive (OI) adults. We hypothesized that age and physical activity level would independently alter the postprandial inflammatory response, with the YA group displaying the least postprandial inflammatory response and the OI group exhibiting the greatest response. Given the exploratory

nature of assessing postprandial angiogenesis, we simply hypothesized that several angiogenic markers would increase post-HFM, while not expecting all markers to exhibit an increase.

Methods

Participants and physical activity level

The general methods from this study and the metabolic results have been published previously (21). Twenty-two individuals participated in the current study: 8 YA adults (age 18–35 y; 4 men, 4 women), 8 OA adults (age \geq 60 y; 4 men, 4 women), and 6 OI adults (3 men, 3 women). YA and OA participants regularly met physical activity guidelines [\geq 150 min/wk of moderate- to vigorous-intensity physical activity (MVPA)] (22). Inactive participants were not habitually involved in planned exercise ($<$ 30 min/wk) and reported participating in a mostly inactive lifestyle (i.e., not meeting physical activity guidelines). Participants had not meaningfully altered their physical activity habits in the past 5 y. OA and OI adults reported either having been habitually active or insufficiently active for most of their lives, respectively. There is no validated questionnaire to assess lifetime physical activity, therefore whether participants matched the physical activity inclusion criteria was determined by means of extensive interviewing with an investigator, as has been done previously (23). Differences in physical activity status between active and inactive participants were objectively determined using accelerometry (Actical Respironics). Accelerometers were worn on the nondominant wrist for 5–7 continuous days, including \geq 1 weekend day, and were initialized to record data in 30-s epochs. To ensure continuous wear of the accelerometer and no nonwear time, a securing band was utilized to secure the device to the nondominant wrist. There were no instances of accelerometer removal by participants over the course of the study. Participants did not have any known chronic disease, nor were they taking any lipid-lowering medications, as confirmed via interview and medical history questionnaire. This study was approved by the Institutional Review Board at Kansas State University (Protocol #8067). Before participation in the study, participants provided written consent to all research activities. Participants were compensated \$30 for their time involved in the study.

Initial assessment

Participants reported to the laboratory on 2 separate occasions: an initial assessment and a meal assessment. The initial assessment involved paperwork and anthropometric testing, performed by a trained research assistant. Height was measured via a portable stadiometer (Invictus Plastics) and weight was evaluated using a digital scale (Pelsar LLC). Height and weight were both measured twice. A third measurement was performed if the values differed by $>$ 0.5 cm or $>$ 0.5 kg, respectively. The 2 closest values were then averaged together and the mean was recorded. Body composition was assessed via a DXA scan (GE Lunar Prodigy). Finally, participants were given a 3-d food log and instructed to fill it out for the 3 d before their meal assessment.

Meal test protocol

The present study used an HFM consisting of chocolate pie (Marie Callender's Chocolate Satin Pie; Conagra Brands). The macronutrient

TABLE 1 Participant characteristics¹

	Younger Active (n = 8)	Older Active (n = 8)	Older Inactive (n = 6)	P value
Age, y	25.1 ± 4.8 ^a	66.5 ± 5.2 ^b	68.2 ± 7.4 ^b	<0.0001
Height, cm	174.2 ± 10.8	175.0 ± 8.8	168.3 ± 8.5	0.40
Body mass, kg	71.9 ± 10.4	81.6 ± 15.4	78.6 ± 11.3	0.32
BMI, kg/m ²	23.6 ± 2.0	26.6 ± 4.1	27.9 ± 4.7	0.11
Body fat, %	21.5 ± 8.9	30.0 ± 11.7	33.4 ± 8.9	0.09
Trunk fat, %	22.5 ± 9.2 ^a	32.6 ± 11.5 ^{ab}	37.7 ± 5.9 ^b	0.02
MVPA, min/d	159.4 ± 48.6 ^a	182.4 ± 77.1 ^a	62.3 ± 14.1 ^b	0.003
Test meal energy, kcal	863 ± 125	980 ± 185	943 ± 136	0.32
Protein, % of kcal	16.9 ± 5.1	13.8 ± 3.6	19.2 ± 4.2	0.11
Carbohydrate, % of kcal	46.5 ± 7.4	50.3 ± 4.6	46.5 ± 9.4	0.53
Total fat, % of kcal	34.4 ± 6.6	35.2 ± 5.8	34.1 ± 7.7	0.96
Saturated fat, % of kcal	11.3 ± 2.7	10.6 ± 2.2	11.9 ± 3.8	0.73
MUFA, % of kcal	5.6 ± 2.5	5.6 ± 1.6	6.4 ± 3.7	0.82
PUFA, % of kcal	3.4 ± 3.0	4.4 ± 2.3	2.6 ± 1.0	0.40
Fiber, % of recommended	71.2 ± 26.1	90.5 ± 26.8	54.1 ± 24.0	0.06

¹Values are mean ± SD. The P value column denotes main effects between groups assessed via 1-factor ANOVA. Within main effects (by row), column values without a common letter differ, determined by post hoc pairwise comparisons. See the Results section for post hoc pairwise comparison P values. MVPA, moderate- to vigorous-intensity physical activity.

distribution, as a percentage of calories, was 63% fat (39% saturated fat, 14% monounsaturated fat, 10% polyunsaturated fat), 34% carbohydrate, and 3% protein. Thus, saturated, monounsaturated, and polyunsaturated fat contributed 62%, 23%, and 15% of the fat in the HFM, respectively. Previous studies have indicated that meals rich in saturated fat tend to produce a greater inflammatory response than meals rich in monounsaturated or polyunsaturated fats (24, 25). The amount of test meal that each participant consumed was calculated in relation to their body mass (12 kcal/kg body mass; 0.84 g/kg fat, 1.02 g/kg carbohydrate, 0.09 g/kg protein). When accounting for participant body mass, the HFM contained a Mean ± SD of 927 ± 154 kcal across all of the participants. **Table 1** displays calories consumed in the test meal by group. The amount of pie consumed was generally characteristic of a standard serving at a restaurant or social event and was served in a homogeneous manner (with a similar distribution of each layer of pie across participants). To our knowledge, the present study is the first to utilize chocolate pie as an HFM challenge. However, the proportion of calories from fat and total caloric load are similar to previous studies investigating postprandial inflammation (e.g., 9).

Participants were instructed to avoid planned or structured exercise for 2 full days before their meal assessment. A 270-kcal snack was given to participants (Little Debbie Swiss Cake Roll; McKee Foods) that they were instructed to consume 10 h preceding their meal assessment (i.e., in the evening before their appointment). The intent of this small snack was to ensure that all participants were fasted for the same length of time and that the last food item consumed across participants was consistent.

Participants arrived at the laboratory on the morning of the meal assessment after a 10-h overnight fast. An indwelling safelet catheter was inserted into a forearm vein via 24-gauge needle (Exelint International) and kept patent with a continual infusion of 0.9% NaCl solution (~1 drip/s). A fasting blood draw was conducted on each participant once the catheter was in place. Blood samples were drawn into a 5-mL syringe (BD) and emptied into a 6-mL Vacutainer test tube (BD) coated with anticoagulant EDTA. After the baseline blood draw, participants consumed the test meal within 20 min. Water was available

for participants ad libitum with the meal and during the postmeal period. Water consumption during the assessment period was not regulated or measured. Participants remained in the laboratory for 6 h after the consumption of the test meal. The 6-h time period began after the last bite of the test meal.

Analyses of inflammation and angiogenesis markers

Blood draws were conducted to determine markers of inflammation and angiogenesis at baseline and 3 and 6 h post-HFM. Whole blood samples were centrifuged at 1800 × g at room temperature for 12 min and the plasma was pipetted into 0.6-mL containers (Fisher). Plasma was stored at −80°C until the study was complete (<1 y). After data collection, plasma samples were analyzed in duplicate via bead-based custom high-sensitivity T-cell Discovery inflammatory cytokine assay and a bead-based angiogenesis Discovery assay (Eve Technologies). The multiplex assays were conducted at Eve Technologies via the Bio-Plex 200 system (Bio-Rad Laboratories, Inc.) and a Milliplex high-sensitivity human cytokine kit and angiogenesis kit (Millipore) according to protocol specifications. The markers of inflammation, primarily cytokines, assessed in the present study (with their respective CV) were granulocyte-macrophage colony-stimulating factor (GM-CSF) (9.7%), IFN-γ (8.8%), IL-1β (12.7%), IL-2 (23.3%), IL-4 (16.5%), IL-5 (11.0%), IL-6 (13.9%), IL-8 (12.4%), IL-10 (10.1%), IL-12(p70) (13.6%), IL-13 (15.0%), IL-17A (8.6%), IL-23 (10.0%), and TNF-α (8.3%). The markers of angiogenesis assessed in the present study (with their respective CV) were angiopoietin-2 (3.2%), endoglin (5.7%), follistatin (7.6%), granulocyte colony-stimulating factor (G-CSF) (23.8%), heparin-binding epidermal growth factor-like growth factor (HB-EGF) (9.6%), hepatocyte growth factor (HGF) (13.6%), leptin (3.1%), VEGF-A (11.1%), and VEGF-C (2.9%). All samples were within the standard curve for inflammatory markers. With regard to markers of angiogenesis, 10 specific participant time points were extrapolated from the standard curve for VEGF-A and G-CSF. All other markers of angiogenesis were within the standard curve.

Statistical analyses

This study was originally powered to detect changes in TGs (21), not markers of inflammation and angiogenesis. In this context, we intended to recruit 8 individuals to each group (24 participants in total), which we accomplished with regard to the YA and OA groups. Owing to difficulties inherent in recruiting participants to the OI group who met study inclusion criteria, we were only able to recruit 6 individuals to the OI group. Although our study was not originally designed to detect postprandial changes in cytokines or growth factors, our total sample of 22 participants, with 6–8 participants in each group, is similar to other studies that have detected changes in postprandial inflammation (for a review, see: 8). In addition, our statistically significant findings detected in several markers of inflammation and angiogenesis suggest that our study was sufficiently powered to address our hypotheses. In consideration of sample size, Cohen's *d* values have been computed for detected group differences in order to provide information with regard to effect size.

Data were formally checked for normality using a Shapiro–Wilk normality test, as well as informally via inspection of central tendency. Participant characteristics and pre- and postmeal data for each marker passed normality testing and it was therefore appropriate to analyze them using parametric testing procedures. A 1-factor ANOVA was utilized to test for group differences in participant characteristics. Each marker of inflammation was analyzed using a 2-factor (group × time) repeated-measures ANOVA to determine significant interactions or significant main effects of group or time. If a significant main effect of time or group was detected, post hoc multiple comparisons were then conducted to test for group- or time-specific differences. In this instance, Tukey's adjustment for multiple comparisons was implemented in order to avoid type 1 error. All statistical analyses were conducted and figures were created using GraphPad Prism statistical software version 7.03 (GraphPad Software, Inc.).

Results

Participant characteristics

Participant characteristics are displayed in Table 1. The OA and OI groups were not different in age ($P = 0.60$). Height, body mass, BMI, body fat, or test meal energy were not different ($P > 0.05$) across groups. There were significant differences in trunk fat between groups ($P = 0.02$). Trunk fat was lower in the YA group than in the OI group ($P = 0.02$), but neither the YA and OA ($P = 0.09$) groups nor the OA and OI ($P = 0.33$) groups differed. MVPA was significantly different between groups ($P = 0.003$). The YA and OA groups obtained more MVPA relative to the OI group ($P = 0.01$ and $P = 0.003$, respectively), whereas the YA and OA groups were not different ($P = 0.44$). With regard to dietary composition of the participants, there were no group differences ($P > 0.05$) in protein, carbohydrate, fat, saturated fat, monounsaturated fat, or polyunsaturated fat (all presented as percentages of total calories). Fiber consumption, as a percentage of recommended intake, was also not different between groups ($P = 0.06$).

Markers of inflammation

Postprandial inflammation outcomes are reported in Figure 1. There was no interaction ($P = 0.29$) or group effect ($P = 0.76$) for IL-8,

but there was a time effect ($P = 0.01$). IL-8 significantly increased from baseline to 6 h post-HFM (mean difference: 0.8 pg/mL; 95% CI of difference: 0.2, 1.4 pg/mL; $P = 0.01$). There was also a time effect observed for IL-6 ($P = 0.01$), with no interaction ($P = 0.94$) or group effect ($P = 0.13$). IL-6 decreased from baseline to 3 h post-HFM (mean difference: 0.3 pg/mL; 95% CI of difference: 0.03, 0.53 pg/mL; $P = 0.02$) and then increased from 3 h to 6 h post-HFM (mean difference: 0.3 pg/mL; 95% CI of difference: 0.04, 0.54 pg/mL; $P = 0.02$), with no difference between baseline and 6 h postmeal ($P = 0.99$).

A significant time effect ($P = 0.03$) was detected for GM-CSF, but no interaction ($P = 0.10$) or group effect ($P = 0.16$). Post hoc comparison by time revealed that GM-CSF decreased from baseline to 3 h (mean difference: 17.3 pg/mL; 95% CI of difference: 1.5, 33.2 pg/mL; $P = 0.029$) and 6 h postmeal (mean difference: 15.9 pg/mL; 95% CI of difference: 0.1, 31.8 pg/mL; $P = 0.048$). There was a main effect of time for IFN- γ ($P = 0.01$), but there was no interaction ($P = 0.35$) or group effect ($P = 0.45$). IFN- γ decreased from baseline to 3 h postmeal (mean difference: 1.0 pg/mL; 95% CI of difference: 0.2, 1.9 pg/mL; $P = 0.019$). Similar to IFN- γ , there was no interaction or group effect for IL-1 β , but there was a significant time effect ($P = 0.03$). IL-1 β decreased from baseline to 3 h postmeal across groups (mean difference: 0.2 pg/mL; 95% CI of difference: 0.1, 0.4 pg/mL; $P = 0.01$). No interaction ($P = 0.35$) or group effect ($P = 0.31$) was observed for IL-5, but there was a time effect ($P < 0.0001$). IL-5 significantly decreased from baseline to 3 h postmeal (mean difference: 0.09 pg/mL; 95% CI of difference: 0.05, 0.13 pg/mL; $P < 0.0001$) and 6 h postmeal (mean difference: 0.1 pg/mL; 95% CI of difference: 0.06, 0.14 pg/mL; $P < 0.0001$). No interaction was detected for IL-10 ($P = 0.98$), but there was a time effect ($P = 0.006$) and a group effect ($P = 0.02$). Across groups, IL-10 decreased from baseline to 3 h (mean difference: 0.6 pg/mL; 95% CI of difference: 0.1, 1.2 pg/mL; $P = 0.01$) and 6 h postmeal (mean difference: 0.6 pg/mL; 95% CI of difference: 0.1, 1.1 pg/mL; $P = 0.02$). Across the postprandial period, IL-10 was greater in YA than in OA (mean difference: 3.7 pg/mL; 95% CI of difference: 0.5, 6.8 pg/mL; $P = 0.02$; Cohen's *d*: 10.7). A time effect was observed for IL-12 ($P = 0.01$), without a significant interaction ($P = 0.18$) or group effect ($P = 0.12$). IL-12 significantly decreased from baseline to 3 h (mean difference: 0.32 pg/mL; 95% CI of difference: 0.04, 0.59 pg/mL; $P = 0.02$) and 6 h post-HFM (mean difference: 0.33 pg/mL; 95% CI of difference: 0.06, 0.61 pg/mL; $P = 0.01$). A significant interaction ($P = 0.02$) was observed for IL-13, as well as a time effect ($P = 0.01$), but no group effect ($P = 0.94$). In post hoc analyses, IL-13 decreased in YA from baseline to 3 h post-HFM (mean difference: 0.7 pg/mL; 95% CI of difference: 0.3, 1.2 pg/mL; $P = 0.002$). No other specific changes or differences were observed in IL-13. Similar to other markers, there was no interaction ($P = 0.44$) or group effect ($P = 0.32$) for IL-17A, but there was a significant effect of time ($P = 0.03$). IL-17A significantly decreased from baseline to 3 h postmeal (mean difference: 1.1 pg/mL; 95% CI of difference: 0.1, 2.2 pg/mL; $P = 0.03$). Time ($P = 0.01$) and group ($P = 0.02$) effects were detected in IL-23, but no interaction ($P = 0.07$). Within main effects of time, IL-23 decreased from baseline to 3 h (mean difference: 76.9 pg/mL; 95% CI of difference: 18.0, 135.9 pg/mL; $P = 0.008$) across groups. Within main effects of group, YA was greater than OA (mean difference: 490.2 pg/mL; 95% CI of difference: 67.2, 913.3 pg/mL; $P = 0.02$; Cohen's *d*: 8.1). A time effect ($P < 0.0001$) and group effect ($P = 0.04$) were detected for TNF- α , but no interaction ($P = 0.14$). TNF- α decreased

from baseline to 3 h (mean difference: 0.8 pg/mL; 95% CI of difference: 0.5, 1.2 pg/mL; $P < 0.0001$) and 6 h postmeal (mean difference: 0.8 pg/mL; 95% CI of difference: 0.5, 1.2 pg/mL; $P < 0.0001$). Within main effects of group, OI was greater than OA (mean difference: 2.9 pg/mL; 95% CI of difference: 0.3, 5.4 pg/mL; $P = 0.03$; Cohen's d : 7.3).

No significant interactions or time or group effects were observed for IL-2 or IL-4.

Markers of angiogenesis

Markers of angiogenesis at baseline and in the postprandial period are displayed in **Figure 2**. A significant time effect was determined for

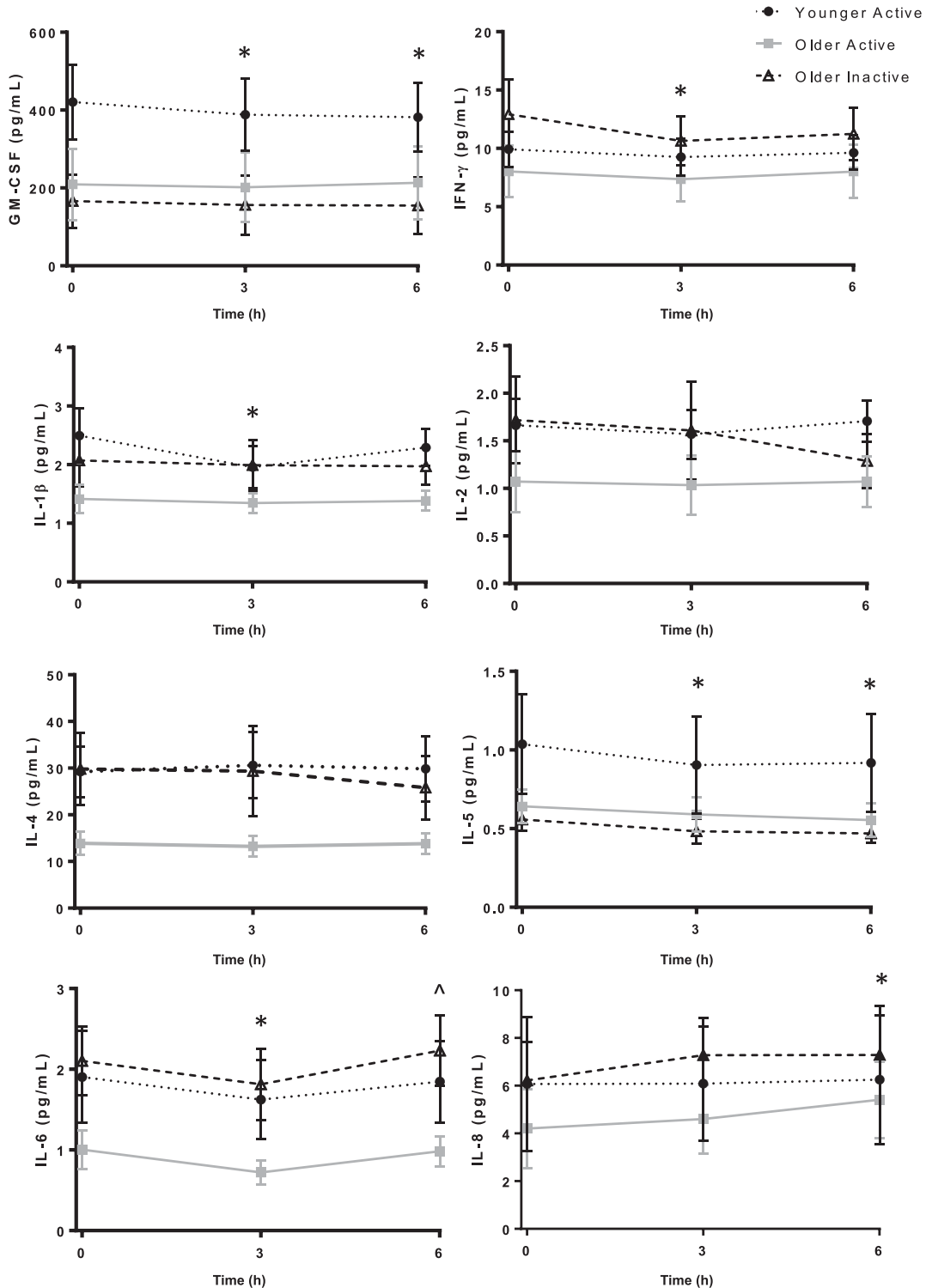


FIGURE 1 (Continued)

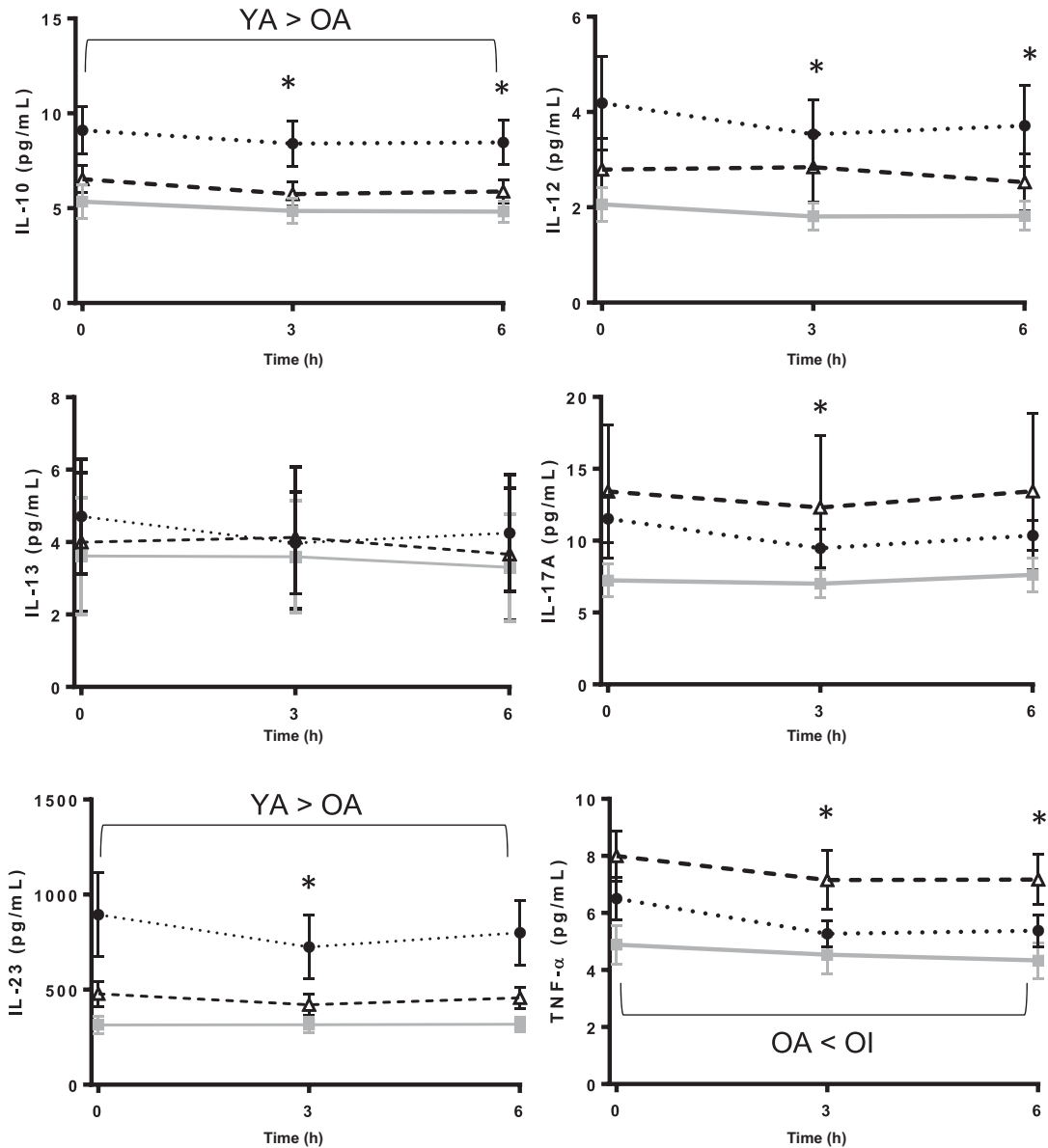


FIGURE 1 Markers of inflammation at baseline and postmeal. Concentrations of inflammatory markers in plasma from baseline/fasting (time 0) to 3 h and 6 h post-HFM. Data were analyzed with a 2-factor (group × time) repeated-measures ANOVA with post hoc pairwise comparisons. Data are mean ± SEM. *Significantly different from baseline (main effect of time). ^Significantly different from 3 h postmeal, but not baseline (main effect of time). GM-CSF, granulocyte-macrophage colony-stimulating factor; HFM, high-fat meal; OA, older active; OI, older inactive; YA, younger active.

VEGF-A ($P = 0.0006$), but not an interaction ($P = 0.39$) or group effect ($P = 0.56$). VEGF-A significantly increased from baseline to 3 h (mean difference: 22.5 pg/mL; 95% CI of difference: 6.6, 38.5 pg/mL; $P = 0.004$) and 6 h postmeal (mean difference: 26.9 pg/mL; 95% CI of difference: 11.0, 42.9 pg/mL; $P = 0.0006$). A significant time effect ($P < 0.0001$) and group effect ($P = 0.001$) were observed in VEGF-C, but no interaction ($P = 0.14$). Similar to VEGF-A, VEGF-C increased from baseline to 3 h (mean difference: 73.5 pg/mL; 95% CI of difference: 36.5, 110.5 pg/mL; $P < 0.0001$) and 6 h post-HFM (mean difference: 81.2 pg/mL; 95% CI of difference: 44.2, 118.2 pg/mL; $P < 0.0001$). Within group effects, OA was significantly greater than YA (mean difference: 133.8 pg/mL;

95% CI of difference: 58.5, 209.0 pg/mL; $P = 0.0007$; Cohen's d : 2.4). There was not an interaction ($P = 0.58$) or group effect ($P = 0.13$) for HB-EGF, but there was a time effect ($P = 0.04$). HB-EGF significantly increased from baseline to 3 h postmeal (mean difference: 6.1 pg/mL; 95% CI of difference: 0.5, 11.8 pg/mL; $P = 0.03$).

For leptin, there was a significant time effect ($P = 0.001$), but no interaction ($P = 0.74$) or group effect ($P = 0.47$). Across groups, leptin decreased from baseline to 3 h (mean difference: 3135.7 pg/mL; 95% CI of difference: 1237.5, 5033.9 pg/mL; $P = 0.0007$) and 6 h postmeal (mean difference: 2297.2 pg/mL; 95% CI of difference: 399.0, 4195.4 pg/mL; $P = 0.01$). A significant time effect ($P = 0.002$) was

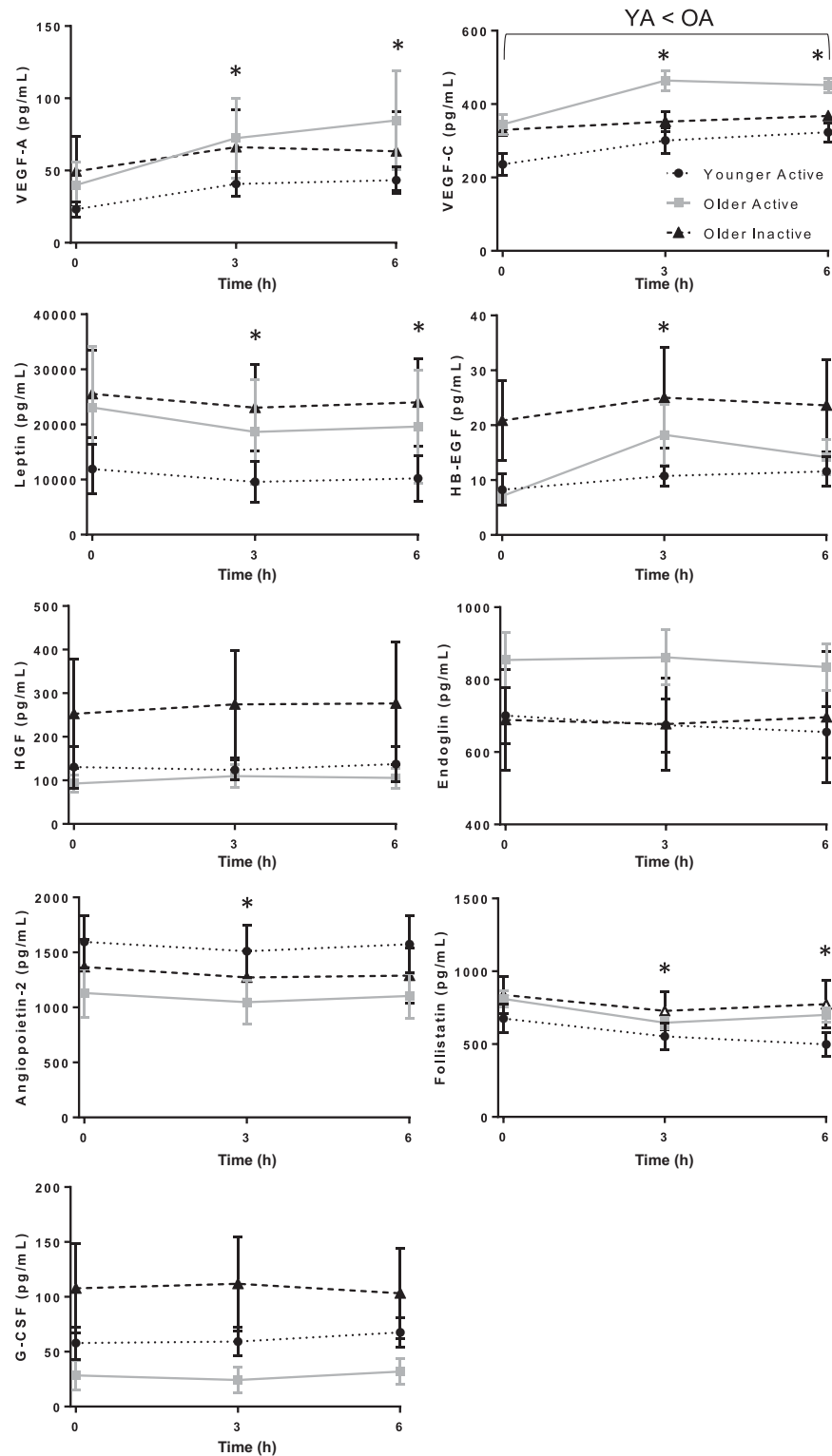


FIGURE 2 Markers of angiogenesis at baseline and postmeal. Concentrations of angiogenic markers in plasma from baseline/fasting (time 0) to 3 h and 6 h post-HFM. Data were analyzed with a 2-factor (group \times time) repeated-measures ANOVA with post hoc pairwise comparisons. Data are mean \pm SEM. *Significantly different from baseline (main effect of time). G-CSF, granulocyte colony-stimulating factor; HB-EGF, heparin-binding epidermal growth factor–like growth factor; HFM, high-fat meal; HGF, hepatocyte growth factor; OA, older active; VEGF, vascular endothelial growth factor; YA, younger active.

detected for angiotensin-2, but no interaction ($P = 0.85$) or group effect ($P = 0.35$). Angiotensin-2 decreased from baseline to 3 h post-HFM (mean difference: 87.1 pg/mL; 95% CI of difference: 33.1, 141.2 pg/mL; $P = 0.001$). Similarly, no interaction ($P = 0.27$) or group effect ($P = 0.28$) was detected for follistatin, but there was a significant time effect ($P < 0.0001$). Follistatin significantly decreased from baseline to 3 h (mean difference: 133.9 pg/mL; 95% CI of difference: 71.5, 196.2 pg/mL; $P < 0.0001$) and 6 h postmeal (mean difference: 121.5 pg/mL; 95% CI of difference: 59.1, 183.8 pg/mL; $P < 0.0001$).

A significant interaction was observed for G-CSF ($P = 0.01$), but no time ($P = 0.37$) or group effects ($P = 0.13$). The only post hoc pairwise comparison that was significantly different was that YA increased from baseline to 6 h post-HFM (mean difference: 9.6 pg/mL; 95% CI of difference: 0.8, 18.5 pg/mL; $P = 0.03$). There were no significant effects or interactions observed for HGF or endoglin.

Discussion

The purpose of this study was to evaluate the effects of an HFM on markers of inflammation and angiogenesis in YA, OA, and OI individuals. We hypothesized that the YA group would exhibit the lowest postprandial inflammatory response and the OI group would exhibit the greatest response. Although noteworthy group differences were detected in the postprandial period for IL-10, IL-23, and TNF- α , the remaining inflammatory markers did not reveal differences across our 3 groups. This finding agrees with a recent study that also generally did not observe differences between younger and older adults in the postprandial inflammatory response (15). We also hypothesized that some, but not all, markers of angiogenesis would increase after HFM intake. This hypothesis was supported, as VEGF-A, VEGF-C, and HB-EGF increased in the postprandial period, although the other angiogenic markers did not.

Findings for markers of inflammation

IL-10 was greater in the YA group than in the OA group throughout the postprandial period. IL-10 is traditionally considered to be an anti-inflammatory cytokine (26). IL-10 has been found to inhibit the production of an array of classical proinflammatory cytokines, including IL-1 β , IL-8, TNF- α , and GM-CSF (27). When IL-10 is added to LPS-stimulated human mononuclear cells and neutrophils, it suppresses cytokine synthesis, inhibiting transcription of their respective genes (28). Therefore, greater IL-10 in the YA group suggests a more optimal inflammatory state. Exercise training has been shown to increase IL-10 in middle-aged individuals (29) and IL-10 has been found to be higher in active older men than in less active older men (30). Thus, it is not clear why there was no difference between the YA and OI or OA and OI groups, although this may be partially due to the smaller sample size in the OI group. In our opinion, our finding of greater IL-10 in YA than in OA merits further investigation.

TNF- α is produced primarily by macrophages of the innate immune system and is a key component of the immune response. Although TNF- α plays many physiologically important roles, it is classically considered to be proinflammatory, due to its function as a key player in LPS-induced septic shock and other inflammatory pathologies, such as rheumatoid arthritis and inflammatory bowel diseases (31).

In the postprandial context, it is one of the most frequently studied proinflammatory cytokines and has been shown in some instances to be responsive to an HFM (9, 11, 32–34). However, a recent systematic review of inflammatory markers in the postprandial period found that ~70% of studies found no change in TNF- α after HFM intake (35). In fact, whereas 5 of the 28 studies investigating postprandial TNF- α reported an increase (9, 11, 33, 36, 37), 3 studies reported a postprandial decrease (38–40). In the present study, TNF- α decreased at both 3 h and 6 h postmeal across groups. Therefore, our findings are not necessarily in disagreement with previous studies, despite the traditional notion that TNF- α increases post-HFM. TNF- α can decrease with exercise training (41) and its receptors have been found to be significantly lower in physically active individuals (14). Our findings partially agree with the notion that regular exercise modifies TNF- α concentrations, because TNF- α was higher in the OI group than in the OA group. Of note, although there were no significant group differences in fiber consumption, there was a trend toward greater fiber consumption in OA than in OI. It could be speculated that differences between groups in TNF- α may be due in part to higher fiber intake in OA, a possibility that cannot be completely excluded. However, a post hoc analysis did not reveal a significant correlation between fiber consumption and the TNF- α response in the present sample. Interestingly, we did not detect any differences between the YA and OI groups. This finding could be explained in part by the smaller sample size in the OI group. In addition, despite evidence that TNF- α is higher in older individuals (42), there were no differences between younger and older adults in the present study with regard to TNF- α .

Our findings regarding IL-23 revealed a statistically significant group effect. Specifically, the YA group had higher IL-23 concentrations than the OA group. Less is known regarding IL-23 relative to certain other, more prominent cytokines, but it has been found to be a key driver of intestinal inflammation via direct activity on T cells that promotes Th17 cell proliferation (43). Evidence has also linked IL-23 to autoimmune inflammation in the brain (44). Although it appears that the proinflammatory effects of IL-23 are more prominent in mucosal tissue, rather than promoting systemic inflammation (45), studies have found associations between circulating IL-23 concentrations and obesity (46), amyotrophic lateral sclerosis (47), and ankylosing spondylitis (48). Interestingly, a recent case-control study reported a significant positive association between IL-23 and disease progression of carotid atherosclerosis (49). In this context, our finding of elevated IL-23 in YA relative to OA at baseline and throughout the postprandial period is potentially meaningful, but counter to what would be expected. However, future research should further investigate the influence of age on IL-23 in the postprandial period.

IL-6 has been found to be significantly associated with several characteristics of metabolic syndrome including blood pressure, fasting insulin, and insulin sensitivity (50). IL-6 concentrations have also been associated with mortality and coronary artery disease (4, 51). IL-6 is the most commonly assessed and best-understood cytokine in the postprandial period, having been found to increase in >70% of previous studies (35). Our findings generally disagree with these studies; although we found a significant time effect, IL-6 decreased at 3 h postmeal, and returned to baseline concentrations at 6 h. However, the present study is not the first to observe a postprandial decrease in IL-6 (52). IL-6 tends to be higher in older adults (53) and physically

inactive individuals (54). However, in agreement with the only previous investigation known to assess postprandial IL-6 in younger compared with older adults (15), we did not observe any IL-6 group differences in the present study.

Although IL-6 is commonly assessed as a proinflammatory cytokine, it also has beneficial health effects as an anti-inflammatory myokine in the context of exercise (55). IL-6 is secreted from skeletal muscle during exercise, so most of the existing physical activity literature has investigated changes in IL-6 after an acute bout of exercise (e.g., 56), although several chronic adaptations have been documented as well. Dixon et al. (57) found that regularly active middle-aged men had lower fasting IL-6 than their inactive counterparts with no differences in postprandial IL-6 between physical activity levels. However, the subjects were only asked to refrain from exercise for 24 h before the HFM. In our study, no differences in fasting IL-6 were reported between groups, which could be because subjects were asked to refrain from exercise for 48 h.

Findings for markers of angiogenesis

VEGF-A and VEGF-C increased significantly after HFM consumption across groups, and were elevated at 3 and 6 h postmeal. To our knowledge, the present study is the first to demonstrate a significant increase in markers of angiogenesis in response to intake of a single meal. VEGF-A and VEGF-C are key angiogenic markers, functioning as mitogens for vascular endothelial cells, with their primary difference being the specific VEGF receptors they trigger (58). Some have suggested a therapeutic role of VEGF in promoting the development of collateral blood vessels in ischemic conditions (17). VEGF is upregulated by hypoxia and ischemia *in vitro* and *in vivo*, respectively, suggesting that VEGF acts as an important mediator in ischemia-induced myocardial neovascularization (59). However, clinical trials utilizing VEGF as a therapeutic agent have produced mixed results (60, 61). From a pathological perspective, VEGF is upregulated in many tumor types (62) and there is extensive evidence that VEGF inhibition blunts growth in a variety of tumor cell lines (63). VEGF is also upregulated in the context of atherosclerosis (18) and is thought to promote plaque growth and instability by stimulating intraplaque neovascularization and consequent hemorrhage (16). Thus, the significant increase in VEGF after HFM intake observed in the present study is potentially meaningful. Because VEGF can be driven by inflammatory cells (20), it is possible that elevated VEGF is a downstream result of increased inflammation in the postprandial period. However, in the present study, VEGF-A and VEGF-C exhibited more robust postmeal responses than the measured inflammatory markers. Overall, the postprandial increase in VEGF across groups observed in this study is an interesting and novel finding, and potentially speaks to another facet of the acute adverse response induced by an HFM, although more research is certainly needed to better understand the role and reason for this postprandial change.

In addition to VEGF, there was also a significant increase in HB-EGF, a growth factor produced primarily by monocytes that is upregulated in cancer (64). We observed that HB-EGF was significantly elevated across groups 3 h after the HFM compared with baseline. Similarly to VEGF, given its role in cancer, the postprandial increase in HB-EGF is likely not a favorable outcome, but our limited data preclude excessive

speculation regarding its specific functioning in the acute hours after HFM consumption.

The majority of angiogenesis markers either did not change or actually decreased in the postprandial period. Specifically, leptin, angiopoietin-2, and follistatin each significantly decreased in response to HFM consumption, although these decrements were not very large in magnitude. Endoglin, HGF, and G-CSF did not significantly change in the postprandial period. Each of these markers, like VEGF and HB-EGF, promotes the growth and development of new blood vessels. The observation that some markers of angiogenesis increased, whereas others did not change or even decreased, is similar to the heterogeneous responses seen in inflammatory cytokines from this study and others (8). Ideally, our preliminary findings can now be used to inform more targeted studies in meal-induced changes in angiogenic markers.

Strengths and limitations

The current study features several strengths that should be considered. First, the test meal was largely representative of a typical nutrient-poor Western meal. When compared with previously used test meals (>1500 kcal), the test meal used in the present study resembled a realistic serving that would likely be offered in a social setting (~930 kcal), although it was still large enough to feasibly induce an inflammatory response. The present study did observe a significant decrease in several circulating markers after meal intake, which may have been partially due to the consideration that the HFM, although proportionally high in fat (63% of kcal) and saturated fat (62% of fat kcal), was lower in overall calories than the meals used in some other studies (e.g., 65). However, it should be noted that a postprandial decrease in inflammatory markers has been observed before (52, 66). Another strength of the current study was the large array of inflammatory and angiogenic markers assessed, making possible a more comprehensive view of the systemic response to HFM consumption. However, the present study does have several noteworthy limitations. First, this investigation did not feature a group that was younger and inactive. Our main research purpose was to assess the independent effects of aging and physical activity level on the postmeal response. Thus, we were most intent on comparing YA with OA and OA with OI, and therefore did not consider a younger inactive group to be critical for accomplishing our research purpose. Instead, we decided to direct our resources toward the YA, OA, and OI groups. Second, although we did measure outcomes for 6 h, the frequency was only every 3 h. Therefore, it is possible that we simply did not detect the peak responses of some cytokines, as they may have peaked at time points we did not measure. Little is known regarding the consistent time-course of postprandial changes in markers of inflammation, but a systematic review has suggested that certain commonly assessed markers (IL-6, TNF- α , and CRP) tend to peak between 4 and 6 h after an HFM (35). More work is needed to better determine the time frame in which individual markers respond. In light of this, it is very possible that our assessment of markers at only baseline and 3 and 6 h postmeal influenced our results. Third, the sample size for this study was not originally determined to detect changes in cytokines or growth factors post-HFM, but rather lipid changes. The sample size of the present study was similar to other postprandial studies that have detected changes in postprandial inflammation (for reviews, see: 8, 35). Nevertheless, it is possible that we simply did not have sufficient power, especially with regard to the OI

group, to detect changes in response to the meal or differences between groups in all of the markers assessed. Related to this point, some of the inflammatory and angiogenic markers presented relatively high CVs, weakening the ability to detect significant changes or differences. When compared with single-plex ELISA, multiplex platforms, as presently utilized, have a limited ability to detect low-grade inflammatory changes in the plasma of healthy humans (67). This weakness could explain in part our null findings for several markers. However, compared with several other multiplex systems, the presently used Bio-Plex has been observed to be a suitable system for key inflammatory biomarkers (68). Next, although participants were not eligible to participate if they were taking lipid-lowering medications, we did not further restrict or assess medication use. Given the high prevalence of medication use in older adults (69), it is possible that participants may have been taking other medications. Lastly, the present study did not feature any form of negative control meal (e.g., water). Thus, it is possible that some of the time-based changes observed could have simply been due to circadian oscillations.

Conclusions and future decisions

We observed interesting differences in IL-10, IL-23, and TNF- α in groups that differed by either age or physical activity level, and numerous inflammatory markers exhibited time-based differences in the postprandial period. Whereas IL-8 increased in response to the HFM, the majority of inflammatory markers decreased in the postprandial period. With regard to markers of angiogenesis, a key novel finding was that VEGF-A, VEGF-C, and HB-EGF significantly increased after HFM consumption, with a group-based difference observed in VEGF-C. However, the postprandial responses of markers of inflammation and angiogenesis can be very heterogeneous and marker-specific. We observed that several markers did not change; moreover, some differences and changes that were statistically significant were not necessarily robust in magnitude.

We recommend future studies further investigate the impact of aging and activity level on postprandial inflammation, potentially focusing on IL-10, TNF- α , and IL-23. Future investigations should also assess the impact of aging and activity on the postprandial changes of other immune markers that are not plasma-borne, as these have been suggested to more consistently increase after HFM intake (8). There are complex inflammatory processes involved with both acute and chronic exercise that may interact with meal consumption, which may explain several of the inflammatory responses observed in the present study. Although the purpose of this study was not to identify precise mechanisms, this should be a focus in future investigations. Lastly, it would be worthwhile for additional studies to investigate HFM-induced changes in markers of angiogenesis, aiming to better understand the role and explanation for this potential postprandial change. Given the relevance of inflammation and angiogenesis to numerous pathologies, efforts to understand how these phenomena are modified by consumption of a single meal could potentially be clinically meaningful.

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and CMS: analyzed the data and wrote the paper; SRE: had primary responsibility for final content; and all authors: edited the paper and read and approved the final manuscript.

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