ORIGINAL RESEARCH

Protein, Carbohydrate, and Fat Metabolism

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# A Pilot Study of Associations Between Visceral Fat, IL-6, and Urinary $F_2$-Isoprostanes in Older Adults Exposed to a Diet Intervention

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## ABSTRACT

**Background:** Short-term markers of successful visceral adipose tissue (VAT) loss are needed. Urinary $F_2$-isoprostanes might serve as a marker for intensified lipid metabolism, whereas circulating IL-6 might stimulate fat oxidation and enhance mobilization of VAT.

**Objectives:** This pilot study was designed to explore the hypotheses that *1*) reduction in VAT is associated with increase in IL-6, and *2*) that increases in urinary $F_2$-isoprostanes are associated with increases in IL-6 and reduction in VAT.

**Methods:** Eighteen participants (aged 60–75 y, BMI 30–40 kg/m$^2$) were randomly assigned to either a very-low-carbohydrate diet (VLCD; <10:25:>65% energy from carbohydrate:protein:fat) or a low-fat diet (LFD; 55:25:20%) for 8 wk. Changes in fat distribution were assessed by MRI. Four urinary $F_2$-isoprostane isomers were quantified in 24-h urine collection using LC-MS/MS analyses. Changes in 4 $F_2$-isoprostane isomers were summarized using factor analysis ($\Delta$-$F_2$-isoprostane factor). Statistical significance was set at $P < 0.1$.

**Results:** Within the VLCD group, change in VAT was inversely associated with change in IL-6 ($r = -0.778$, $P = 0.069$) and $\Delta$-$F_2$-isoprostane factor ($r = -0.690$, $P = 0.086$), demonstrating that participants who maintained higher concentrations of $F_2$-isoprostane factor across the intervention showed greater decreases in VAT. A positive relation between $\Delta$-$F_2$-isoprostane factor and change in IL-6 was observed ($r = 0.642$, $P = 0.062$). In the LFD group, no significant associations between changes in VAT, $F_2$-isoprostane factor, or IL-6 were observed.

**Conclusions:** Results from this exploratory study in older adults with obesity suggest that, in the context of a VLCD, IL-6 could be involved in VAT mobilization, and urinary $F_2$-isoprostanes could reflect intensified oxidation of mobilized fatty acids. Trial registration: This study is registered at clinicaltrials.gov as NCT02760641. *Curr Dev Nutr* 2021;5:nzab082.

**Keywords:** visceral adipose tissue, interleukin-6, $F_2$-isoprostanes, older adults, obesity



Manuscript received March 31, 2021. Initial review completed May 12, 2021. Revision accepted May 14, 2021. Published online May 21, 2021.
Research reported in this publication was supported by the Egg Nutrition Center, and by the National Institute of Diabetes and Digestive and Kidney Diseases (grant numbers P30DK56336, P60DK079626). SEH was supported by a T32 Predoctoral Award from the UAB Predoctoral Training Program in Obesity Related Research (T32HL105349). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Author disclosures: The authors report no conflicts of interest.
Supplemental Figure 1 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: CHO, carbohydrate; $F_2$isoP1, iPF(2$\alpha$)-III; $F_2$isoP2, 2,3-dinor-iPF(2$\alpha$)-III; $F_2$isoP3, PF(2$\alpha$)-VI; $F_2$isoP4, 8,12-iso-iPF(2$\alpha$)-VI; hsCRP, high-sensitivity C-reactive protein; LFD, low-fat diet; UAB, University of Alabama at Birmingham; VAT, visceral adipose tissue; VLCD, very-low-carbohydrate diet.

## Introduction

In older adults, age-related redistribution of adipose tissue results in accumulation of visceral adipose tissue (VAT), which contributes to elevated risk of cardiometabolic disease, such as type 2 diabetes and cardiovascular disease (1–11). Accumulation of VAT is commonly associated with insulin resistance, whereas depletion of this adipose tissue depot reduces metabolic disease risk. Therefore, there is a need for interventions targeting VAT loss in older adults with obesity. The development of effective interventions for obesity would be aided by using serum- or urine-derived analytes associated with VAT loss, which could be used as a noninvasive and cost-effective marker for loss of this metabolically harmful fat depot.

One such marker is interleukin-6 (IL-6), which has been implicated in the loss of VAT. Although traditionally considered a proinflammatory cytokine, emerging evidence suggests that IL-6 has a more complex role in metabolic regulation and, specifically, fat oxidation (12). In animal models, IL-6 receptor expression is increased in VAT compared with subcutaneous adipose tissue (13), and exercise-induced loss of VAT requires IL-6 receptor signaling (14). Further, IL-6 has been shown to mediate improvement of insulin sensitivity (15, 16), and IL-6 knockout mice develop mature-onset obesity, which is partially reversed by IL-6 administration (17). In humans, IL-6 has been shown to mediate VAT loss (14), and IL-6 infusion stimulates fatty acid release and oxidation (18, 19). The positive regulation of exercise-induced IL-6 secretion on fat oxidation in mice is amplified by addition of a

carbohydrate-reduced diet (20), which in humans promotes selective loss of VAT (21–23). Thus, the carbohydrate-restricted diet can stimulate metabolic processes that facilitate both VAT mobilization and fat oxidation. Associations among IL-6 and VAT loss in humans exposed to a carbohydrate-restricted diet have not been reported.

Other potential markers are urinary $F_2$-isoprostanes, which have recently been proposed as markers of intense fat oxidation. As the nonenzymatic products of free radical–initiated peroxidation of arachidonic acid, urinary $F_2$-isoprostanes are traditionally considered the gold standard measurement of oxidative damage in vivo in humans (24–29). Cross-sectional evidence linking oxidative damage to disease risk shows elevated $F_2$-isoprostanes in individuals with high visceral fat accumulation and insulin resistance (30). However, prospective studies suggest that $F_2$-isoprostanes can also reflect other biochemical processes. For example, elevated $F_2$-isoprostanes are predictive of lower weight gain in middle age (31) and in older adults (32), and inversely related to incident type 2 diabetes (33). These observations support evidence that $F_2$-isoprostanes are more broadly reflective of mitochondrial oxidative metabolism (25, 34–36).

A very-low-carbohydrate diet (VLCD) could provide a unique model to explore the relations among diet-induced VAT loss, changes in IL-6, and changes in urinary $F_2$-isoprostanes within older adults with obesity, because we have previously demonstrated greater VAT loss in response to a VLCD than a low-fat diet (LFD) in this population (21). Therefore, the objective of this secondary analysis was to explore the hypotheses that *1*) reduction in VAT is associated with increase in IL-6, and *2*) that increases in urinary $F_2$-isoprostanes are associated with reduction in VAT and increases in IL-6.

## Methods

### Participants

Forty men and women were recruited. Specific inclusion and exclusion criteria have been described elsewhere (21). Briefly, inclusion criteria included BMI 30–40 kg/m$^2$, 60–75 y of age, and sedentary ($\leq$2 h/wk of moderate intentional exercise). Exclusion criteria included individuals with diabetes, unwillingness to eat the study diets, use of any medication known to affect metabolism, recent weight change ($\pm$4.5 kg in the last 12 mo), poorly controlled blood pressure (systolic blood pressure >159 mmHg or diastolic blood pressure >95 mmHg), renal failure, major liver dysfunction (elevation of liver transaminases >3× normal in past 2 y; or current/recent smoker (within 6 mo). Participants were informed of the experimental design, and oral and written consent was obtained. Participants were compensated for study visits. The study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham (UAB). The trial is registered at clinicaltrials.gov (NCT02760641). Eighteen participants who completed 24-h urine collection at baseline and after interventions were included in this analysis.

### Study design

In a 2-arm, parallel design, participants were randomly allocated to receive either a weight-maintaining VLCD or an LFD intervention for 8 wk (21). Screening for eligibility took place at the UAB Webb Nutrition Sciences building. Testing took place in the core facilities of UAB's Center for Clinical and Translational Science, Nutrition Obesity Research Center, and Diabetes Research Center. MRI analysis, hyperinsulinemic-euglycemic clamp, urine collection, and fasting blood draws were performed at baseline and after completion of the diet intervention.

### Diets

Specific details have been described elsewhere (21). Briefly, participants were counseled during weekly individual meetings with a registered dietitian to consume either a eucaloric VLCD (<20:25:>55% energy from carbohydrate:protein:fat) or an LFD (55:25:20%) according to diet prescription. The number of carbohydrate (CHO), protein, and fat servings counseled was determined based on group assignment and total energy requirements as measured by indirect calorimetry (Vmax ENCORE 29N Systems; SensorMedics Corporation) with an activity factor of 1.35 for women and 1.5 for men. The average daily total dietary fiber intake was 8.30 g in the VLCD and 20.86 g in the LFD group, based on 3-d food records (2 weekdays and 1 weekend day) completed at the study midpoint (21). Participants in both arms were provided with food lists, sample menus, and recipes throughout the intervention period, and breakfast foods compatible with their diet prescription during weekly individual meetings. Breakfast foods were purchased from the local grocery store. VLCD participants received 3 eggs/d (~216 kcal, 18.9 g protein, 14.3 g fat, and 1.2 g CHO) and LFD participants received breakfast bars (~180 kcal, 4 g protein, 10 g fat, 22 g CHO) each week. $\beta$-Hydroxybutyrate and respiratory quotient were taken as measures of dietary compliance and to support differences in diet composition (21).

### Fat distribution

VAT was determined by MRI. 3D volumetric T1-weighted magnetization-prepared rapid acquisition gradient echo using a 1.5-T Philips Achieva system was used to collect transaxial abdominal images (21). Contrast between adipose and nonadipose tissues was enhanced by selecting echo time, repetition time, and pulse flip angles. SliceOmatic image analysis software (version 4.3; Tomovision) was used to quantify the volume (cubic centimeters) of the tissues of interest. VAT was analyzed using the abdomen images from the L1 to the L5 vertebrae.

### Laboratory analyses

Analyses were conducted in the Core Laboratory of the Nutrition Obesity Research Center and Diabetes Research Center except where noted. Circulating measures were assayed by immunoassay in fasted morning sera before and after the intervention. Glucose was measured using a SIRRUS analyzer (Stanbio Laboratories). Insulin was measured using a TOSOH immunoassay analyzer (TOSOH AIA-600 II analyzer; TOSOH Bioscience); intra-assay CV of 1.5% and interassay CV of 4.4%. High-sensitivity C-reactive protein (hsCRP) was assessed by turbidometric methods by using a SIRRUS analyzer (Stanbio Laboratory), with reagents obtained from Pointe Scientific. Minimum detectable concentration was 0.05 mg/L. Mean intra-assay CV was 7.49%, and mean interassay CV was 2.13%. TNF-$\alpha$ and IL-6 were assessed by using electrochemiluminescence (Meso Scale Discovery). Minimum detectable concentrations for each assay were 0.507 pg/mL and 0.25 pg/mL, respectively. Mean intra-assay CVs were 7.61% and 6.68%, respectively. Mean interassay CVs were 5.47% and 9.72%, respectively. Four isomers of $F_2$-isoprostanes—iPF(2$\alpha$)-III ($F_2$isoP1), 2,3-dinor-iPF(2$\alpha$)-III ($F_2$isoP2),

**TABLE 1** Baseline characteristics of study participants by diet[1]

| Variable | LFD ($n$ = 8) | VLCD ($n$ = 10) |
|---|---|---|
| Race, $n$ (European American/African American) | 7/1 | 8/2 |
| Sex (% female) | 62.5 | 60.0 |
| Age, y | 68.75 ± 2.92 | 67.80 ± 5.43 |
| BMI, kg/m$^2$ | 38.76 ± 13.35 | 34.40 ± 3.12 |
| Weight, kg | 104.60 ± 42.80 | 97.63 ± 16.22 |
| Fat mass, kg | 39.69 ± 9.12 | 42.44 ± 6.05 |
| Fasting glucose, mg/dL | 99.95 ± 7.30 | 111.03 ± 16.30 |
| Fasting insulin, $\mu$U/mL | 17.06 ± 8.99 | 12.15 ± 4.60 |
| HOMA-IR | 4.23 ± 2.20 | 3.34 ± 1.38 |

[1]Data are mean ± SD, unless otherwise indicated. LFD, low-fat diet; VLCD, very-low-carbohydrate diet.

iPF(2$\alpha$)-VI ($F_2$isoP3), and 8,12-iso-iPF(2$\alpha$)-VI ($F_2$isoP4)—were quantified at Duke University in 24-h urine samples (stored at −70°C) by LC with tandem MS detection and corrected by urinary creatinine to account for differences in urine dilution as previously described (26).

### Statistical analysis

Data were analyzed using SPSS version 25.0 (IBM Corp.). Statistical tests were 2-sided, with an $\alpha$ level of 0.10 denoting significance due to the small sample size and exploratory nature of these analyses. Statistical assumptions were tested using the Levene test for equality of variance, and the Kolmogorov–Smirnov and Shapiro–Wilk tests for normal distribution.

Principal components analysis was used to create a combined variable to account for the large degree of correlation between the Δ scores of isoprostane isomers $F_2$isoP1, $F_2$isoP2, $F_2$isoP3, and $F_2$isoP4. The Δ-$F_2$-isoprostane isomers were normalized (mean = 0, SD = 1) and loaded onto a single factor, called Δ-$F_2$-isoprostane factor.

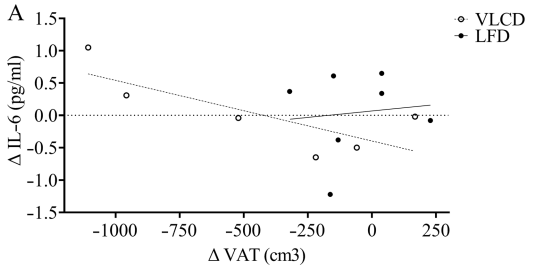
In both groups considered individually and combined, Pearson correlations were used to evaluate relations between the Δ-$F_2$-isoprostane factor and changes in each individual $F_2$-isoprostane isomer with changes in related variables of interest, and to evaluate the relation between the change in IL-6 and the change in VAT.

## Results

A total of 34 participants completed the study, with 19 on the VLCD and 15 on the LFD. Six European American females aged 67–72 y discontinued the intervention for reasons unrelated to the study. The main results for this study population have been published elsewhere (21). Briefly, although participants were counseled weight-maintaining diets, both groups experienced some weight loss, and weight loss was greater in the VLCD than the LFD group (21). Unique to this report are 18 participants who, in addition to the diet intervention, also completed 24-h urine collection for $F_2$-isoprostane analysis at baseline and after 8 wk, with 10 in the VLCD group and 8 in the LFD group. As shown in **Table 1**, the participants were primarily European American females with an average age of 67.80 y in the VLCD group and 68.75 y in the LFD group. There were no significant differences in BMI, weight, total fat mass, fasting glucose, fasting insulin, or HOMA-IR between groups at baseline.

All changes in $F_2$-isoprostane isomers were linearly related (representative plot in **Supplemental Figure 1**) and were absent of multicollinearity and singularity. The standardized changes in $F_2$-isoprostane isomers were considered factorable with all correlations >0.31, Bartlett test of sphericity <0.001, and Kaiser–Meyer–Olkin measure = 0.678. The eigenvalue for the best linear combination of the changes in $F_2$-isoprostanes was 2.731, indicating that the Δ-$F_2$-isoprostane factor explained 68.28% of the information contained in the change in $F_2$-isoprostane markers. No other factor had an eigenvalue >1.

Our findings were diet specific. The relation between change in VAT and change in IL-6 is shown in **Figure 1**. In the VLCD but not the LFD group, VAT loss was associated with an increase in IL-6 ($r$ = −0.778, $P$ = 0.069). Simple correlations of Δ-$F_2$-isoprostane factor with changes in fat distribution and IL-6 are shown in **Figure 2**, and simple correlations of individual $F_2$-isoprostane isomers with changes in fat distribution and inflammatory markers are shown in **Supplemental Table 1**. Within the VLCD group, Δ-$F_2$-isoprostane factor was inversely associ-

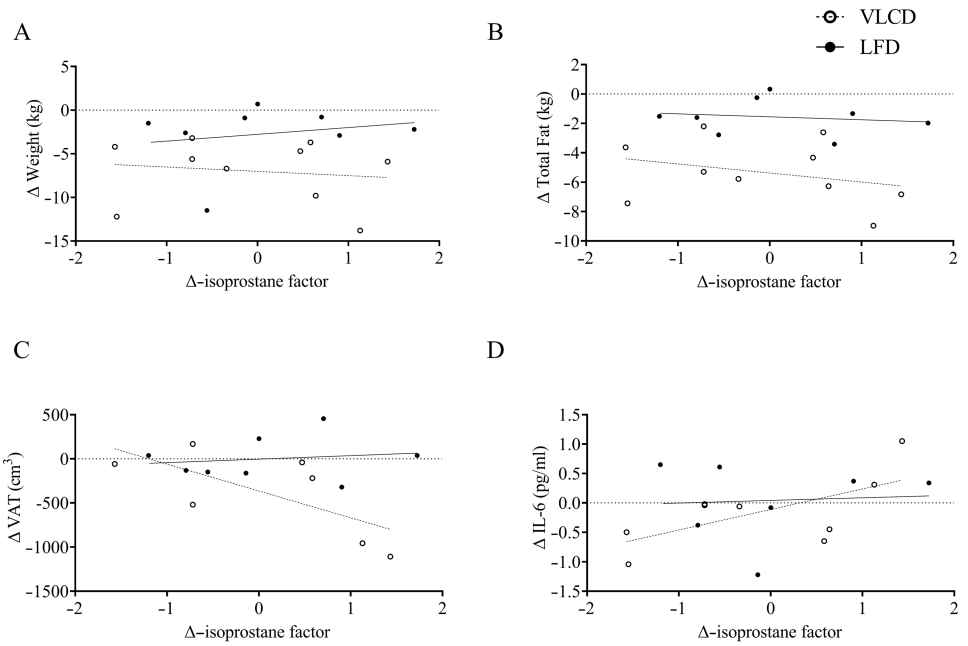



Δ measured as follow-up – baseline values.

B

| Diet Group | Pearson's $r$ |
|---|---|
| Total | r = -0.372<br>$p$ = 0.211 |
| VLCD | r = -0.778<br>$p$ = 0.069 |
| LFD | r = 0.107<br>$p$ = 0.819 |

**FIGURE 1** (A) Linear relations and (B) simple correlations between change in IL-6 with change in VAT. LFD, low-fat diet; VAT, visceral adipose tissue; VLCD, very-low-carbohydrate diet.




E

| Variables | Pearson's Correlation Coefficient | | |
|---|---|---|---|
| | Total | VLCD | LFD |
| Δ Weight (kg) | 0.040 | −0.142 | 0.203 |
| Δ Total Fat (kg) | −0.118 | −0.304 | −0.160 |
| Δ VAT ($cm^3$) | −0.340 | −0.690* | 0.155 |
| Δ IL-6 (pg/ml) | 0.397 | 0.642* | 0.068 |

Δ measured as follow-up – baseline values.
* $P < 0.10$.

**FIGURE 2** Linear relations between Δ-isoprostane factor and (A) change in weight, (B) change in total fat, (C) change in VAT, and (D) change in IL-6. (E) Simple correlations of Δ-isoprostane factor with fat distribution and inflammatory markers. LFD, low-fat diet; VAT, visceral adipose tissue; VLCD, very-low-carbohydrate diet.

ated with change in VAT ($r = -0.690$, $P = 0.086$), and positively with change in IL-6 ($r = 0.642$, $P = 0.062$). No significant relations were observed between Δ-$F_2$-isoprostane factor and changes in weight or total fat. There were no significant associations between Δ-$F_2$-isoprostane factor and VAT, IL-6, weight, or total fat in the LFD group. These relations were supported by individual isomers shown in Supplemental Table 1. Briefly, within the VLCD but not the LFD group, changes in $F_2$isoP1 and $F_2$isoP4 were inversely associated with change in VAT ($r = -0.761$, $P = 0.047$ and $r = -0.693$, $P = 0.085$, respectively), and changes in $F_2$isoP2 and $F_2$isoP3 were significantly related to changes in IL-6 ($r = 0.607$, $P = 0.083$ and $r = 0.618$, $P = 0.076$, respectively). No isomers were related to changes in hsCRP or TNF-$\alpha$.

## Discussion

Results from this pilot study supported our hypotheses: specifically, reduction in VAT was associated with increases in IL-6, whereas increases in urinary $F_2$-isoprostanes were related to increases in IL-6 and reduction in VAT. These relations, however, were diet specific. Following the VLCD but not the LFD, individuals with the greater loss of VAT showed an increase in IL-6 and the lowest decrease (or increase) in the Δ-$F_2$-isoprostane factor. These preliminary findings suggest that within a VLCD, IL-6 could be involved in VAT mobilization and oxidation, and urinary $F_2$-isoprostanes could in turn reflect this greater fat oxidation. We propose a diet-specific underlying mechanism (**Figure 3**): within the context of negative energy balance, greater levels of dietary fatty acids induce the increase in circulating IL-6, which in turn intensifies the lipolysis and release of fatty acids from VAT with subsequent oxidation that can be tracked by changes in urinary F2-isoprostanes. Our hypothesis is supported by the associations among VAT, IL-6, and F2-isoprostanes observed in the present study but needs to be directly tested in a larger cohort.

Although weight loss interventions typically induce a reduction of inflammatory markers, reduction in IL-6 is not always observed and is often not different from baseline (37, 38). It is possible that changes in

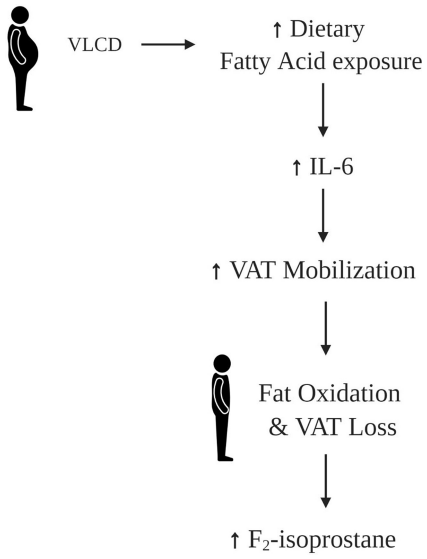



**FIGURE 3** Proposed diet-specific underlying mechanism. VAT, visceral adipose tissue; VLCD, very-low-carbohydrate diet.

IL-6 depend on whether participants are in negative energy balance. The existing literature has suggested that IL-6 signaling mediates fatty acid mobilization and fat oxidation in humans and animal models (18, 39–46), particularly within VAT (14). IL-6 stimulation of lipolysis and fat oxidation has been observed in the context of an exercise-induced increase in IL-6 (42, 45, 46), as well as in response to IL-6 infusion (18, 39). However, metabolic influences of different diets on IL-6 remain largely unexplored. In a mouse model (20), the ketogenic diet in combination with exercise had a greater effect on IL-6 mRNA induction compared with the unpurified diet plus exercise, with the effect of the diet being specific to slow-twitch muscle fibers, which are known to have high oxidative capacity and a preference for fatty acids as a substrate for ATP production (47). It is therefore possible that negative energy balance in conjunction with increased fatty acid exposure from a VLCD increases IL-6, which could in turn be partially involved in VAT mobilization and lipolysis.

Previously published cross-sectional studies have reported a direct association between systemic concentrations of $F_2$-isoprostanes as well as inflammatory markers with greater measures of total and regional adiposity (32, 48, 49). In contrast, our prospective analyses provide insight into how changes in urinary $F_2$-isoprostanes might relate to changes in adiposity, specifically to VAT loss. It is known that urinary $F_2$-isoprostane concentrations drop in response to negative energy balance, reflecting the metabolic slowing (50–53). Our findings suggest that in the context of a VLCD, $F_2$-isoprostane concentrations are maintained (or increased) despite the weight loss, possibly reflecting greater fat oxidation. However, more prospective evidence in larger cohorts is needed to confirm the observed associations and fully elucidate the connection between systemic $F_2$-isoprostanes and response to different dietary interventions.

The major limitation of this exploratory analysis was a small sample size resulting in inadequate power to detect robust associations, and use of an $\alpha$ level of 0.1. Moreover, it is possible results were influenced by selective dropout rates, because the results reflect only a small number of individuals who completed the 24-h urine collection at baseline and after the 8-wk diet intervention. We present our results as hypothesis-generating, and findings should be interpreted with caution. Therefore, a larger study is needed to confirm these pilot findings. Other limitations are related to the intervention framework. Participants were allowed to self-regulate intake and were provided with food lists, sample menus, and recipes. Consequently, we were unable to examine the effect of equivalent weight loss in the low-fat group in $F_2$-isoprostane outcomes. Moreover, to increase dietary adherence, study visit attendance, and participant retention, participants within the VLCD group were provided whole eggs, and participants in the LFD were provided breakfast bars for daily consumption. It is possible that the egg consumption in the present study influenced urinary $F_2$-isoprostane outcomes (54); however, these effects could not be disentangled. Although it was not feasible to blind participants or study staff to diet assignment, staff performing MRI analysis were blinded to diet assignment, and intervention measurements were performed as objectively as possible.

In conclusion, reduction in VAT was related to increases in IL-6, whereas changes in urinary $F_2$-isoprostanes were inversely related to changes in VAT and directly related to change in IL-6 within the VLCD group. These results suggest that in the context of a VLCD, IL-6 might be partially involved in VAT mobilization and oxidation, and urinary $F_2$-isoprostanes reflect this fat oxidation (Figure 3). These pilot findings are important to inform future studies elucidating short-term markers of successful VAT loss during diet interventions.

## Acknowledgments

We gratefully acknowledge the help of Maryellen Williams, Cindy Zeng, and Heather Hunter of the UAB Metabolism Core Laboratory (Nutrition Obesity Research Center, Diabetes Research Center, Center for Clinical and Translational Science) with laboratory analyses, and of the Nursing staff of the UAB Center for Clinical and Translational Science with experimental design.

AMG, BAG, KRF: designed research; AMG, IS: conducted research; SEH: analyzed data; SEH, DI: wrote the paper; SEH: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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