Effect of Modest Caloric Restriction on Oxidative Stress in Women, a Randomized Trial

Maciej S. Buchowski¹, Nobuko Hongu², Sari Acra³, Li Wang⁴, Joshua Warolin³, L. Jackson Roberts II⁵

¹ Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America, ² Department of Nutritional Sciences, University of Arizona, Tucson, Arizona, United States of America, ³ Department of Pediatrics, Division of Gastroenterology, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America, ⁴ Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, ⁵ Department of Medicine, Department of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America

Abstract

Objectives: It is not established to what extent caloric intake must be reduced to lower oxidative stress in humans. The aim of this study was to determine the effect of short-term, moderate caloric restriction on markers of oxidative stress and inflammation in overweight and obese premenopausal women.

Materials/Methods: Randomized trial comparison of 25% caloric restriction (CR) or control diet in 40 overweight or obese women (body mass index 32±5.8 kg/m²) observed for 28 days and followed for the next 90 days. Weight, anthropometry, validated markers of oxidative stress (F₂-isoprostane) and inflammation (C-reactive protein), adipokines, hormones, lipids, interleukins, and blood pressure were assessed at baseline, during the intervention, and at follow-up.

Results: Baseline median F₂-isoprostane concentration (57.0, IQR = 40.5–79.5) in the CR group was 1.75-fold above average range for normal weight women (32.5 pg/ml). After starting of the caloric restriction diet, F₂-isoprostane levels fell rapidly in the CR group, reaching statistical difference from the control group by day 5 (median 33.5, IQR = 26.0–48.0, P<0.001) and remained suppressed while continuing on the caloric restriction diet. Three months after resuming a habitual diet, concentrations of F₂-isoprostane returned to baseline elevated levels in ~80% of the women.

Conclusions: Oxidative stress can be rapidly reduced and sustained through a modest reduction in caloric intake suggesting potential health benefits in overweight and obese women.

Trial Registration: Clinicaltrials.gov NCT00808275 http://clinicaltrials.gov/ct2/show/NCT00808275

Introduction

Obesity is associated with increased oxidative stress and chronic low-grade chronic inflammation [1–4]. Both events contribute to metabolic abnormalities occurring in the obesity-associated metabolic syndrome [1,5–7] and play a critical role in the pathogenesis of various diseases such as atherosclerosis [8,9], cancer [10,11], cardiovascular disease [12], and diabetes type 2 [13]. Recent research has shown that weight loss attenuates inflammation and leads to improvement in adipokine profiles [14–16]. Although associations of overweight and obesity with increased oxidative stress have been reported, the effects of weight loss on oxidative stress markers are rarely described in literature [17]. Moreover, many clinical studies and intervention studies with diets or supplements have employed single measurements of F₂-isoprostane before and after the intervention to estimate the oxidative stress without exploring intermittent or interval changes.

To our knowledge, no prior study has investigated the association between caloric restriction (CR) and systemic short-term changes in markers of oxidative stress.

Thus, the goal of this controlled clinical trial was to determine whether modest (25%) short-term caloric restriction-induced weight loss affects systemic oxidative stress, as measured by changes in serum F₂-isoprostane.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1. The protocol flow diagram is in Figure 1.

Subjects

Forty premenopausal overweight and obese women, [18- to 45-years-old; body mass index - BMI: 25.4 to 43.0 kg/m²], were
recruited from Nashville’s general population by advertisement in Vanderbilt University’s newspaper, mass emails, and flyers. Inclusion criteria were BMI more than 25 kg/m² and willingness to abstain from alcohol consumption for the duration of the study, complete an overnight stay at the Clinical Research Center (CRC), and come to the CRC daily to obtain study foods. Volunteers were excluded if they reported having clinically significant illnesses (including type 2 diabetes), smokers, taking lipid-lowering medications, reported recent initiation/change in hormonal birth control or hormone replacement therapy, pregnant or lactating, taking medications or dietary supplements that affect body weight, or regularly engaging in vigorous physical activities. Participants classified their own ethnicity according to investigator-defined options. At a screening visit, all volunteers were measured for height, weight, and waist circumference, and completed a lifestyle questionnaire. Approximately 150 women were screened for eligibility based on the above criteria and 40 were enrolled. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research, in accordance with the ethical principles of the Helsinki-II Declaration. The trial (Clinicaltrials.gov Identifier: NCT00808275) was approved by the Institutional Review Board at Vanderbilt University and all participants provided written informed consent.

Study Design

The study consisted of an experimental group that received a CR diet and a control group that remained on a habitual diet throughout the study. The ratio of women in CR group to the control group was 4 to 1 (allocated via blocked randomization by a statistician not involved in patient recruitment). The study was divided into a 7-day lead in period (Day -6 to Day 0), immediately followed by a 28-day intervention (Day 1 to Day 28), and a 3-month follow-up (Day 29 to Day 118). Dietary intervention in the CR group started during the luteal stage of the menstrual cycle (14–24 d) to control for confounding by menstrual cycle and any associated oxidative and/or inflammatory stress.

Anthropometrics and Body Composition

The National Health and Nutrition Examination Survey (NHANES) protocols were followed for all anthropometrical measurements [18]. Body weight was measured at baseline and
daily (for the experimental group) within 0.1 kg using a calibrated beam platform scale (Detecto-Medic, Detecto Scales, Inc, Northbrook, IL). Daily weight measurements were not revealed to participants and served as the criterion measure for dietary adherence. Height was measured at baseline within 0.5 cm using a calibrated wall-mounted stadiometer (Perspective Enterprises, Portage, MI). The average of two readings was used for analysis. All measurements were performed by the same investigator. Body composition (including fat mass and fat free mass) was measured at baseline (Day 0), during intervention (Days 14 and 28), and during the follow up visits (Days 42 and 119) using dual energy x-ray absorptiometry Lunar instrument (Lunar Prodigy, GE Medical Systems, Madison WI, adult software v. 9.15). For quality assurance and equilibration, a calibration block was scanned each morning and a spine phantom was scanned on a weekly basis (the protocol was monitored by urinary biomarkers (sodium, potassium, nitrogen) [22,23].

Resting Energy Expenditure (REE)

REE was measured at Day 0 and 29 and was defined as the average EE during a 30-min period of lying in a supine position after a 30-min rest following an overnight fast (>10 h) using a whole-room indirect calorimeter. REE was calculated from measured rates of oxygen (O₂) consumption and carbon dioxide (CO₂) production using Weir’s equation [19]. The accuracy and precision of our metabolic chamber for measuring EE as determined by routine alcohol combustion test was 99.7% (IQR = 99.5–100.0%) over 24 hours and 98.5% (IQR = 96.9–99.9%) over 30 minutes.

Table 1. Baseline demographic and anthropometric characteristics.

| Ethnicity                  | Control diet (n = 8) | CR diet (n = 32) |
|----------------------------|----------------------|------------------|
| African American           | 3                    | 9                |
| Caucasian                  | 4                    | 22               |
| Other (Asian, Hispanic)    | 1                    | 1                |

Data are presented as median (interquartile range - IQR). Abbreviations: CR diet, caloric restriction diet. doi:10.1371/journal.pone.0047079.t001

Table 2. Comparison of reported average (28 days) intake of protein, sodium, and potassium with the corresponding biological markers in urine in the caloric restriction (CR) diet group.

| Intakea | Urinary Excretionb | Ratio of Intake to Excretion | p-valuec |
|---------|--------------------|------------------------------|----------|
| Protein (g/day) | 85.0±12.0          | 87.9±3.6                    | 1.03±0.25 | 0.486   |
| Sodium (g/day)   | 3.03±0.44          | 3.18±0.83                   | 0.95±0.19 | 0.919   |
| Potassium (g/day) | 2.93±0.43          | 2.83±0.43                   | 1.04±0.32 | 0.553   |

Data are presented as mean± s.d.

aAverage daily intakes of protein, sodium, and potassium assessed by NDSR (Nutrition Diet System, St. Paul, MN).

bExcretion measured from 4 weekly 24-hour urine collections. Protein = urinary nitrogen × 7.72 [18], Potassium = urinary potassium × 0.77 [19].

cComparison of ratios of intake to excretion; one sample t-test. doi:10.1371/journal.pone.0047079.t002

Dietary Intake

**Caloric restriction diet.** Participants in the CR group received individualized energy and nutrient controlled diets provided by the CRC metabolic kitchen for consumption at home. Energy needs were calculated as the sum of resting energy expenditure (REE) and energy expenditure of physical activity. Thermic effect of food was estimated as 10% of REE. Each individualized diet, provided in daily portions divided into 3 meals and 3 snacks, contained approximately 75% (±210 kcal) of daily energy requirements, including 52–62% of energy from carbohydrates, 25–29% of energy from fat, 18–23% of energy from protein, and 22–27 g of fiber. All participants received a multivitamin supplement daily (Nature Made, Mission Hills, CA). No restrictions were imposed on the amounts of energy-free foods ingested. Each participant received a written list of foods at daily pick-up. Any unspent foods and any additional foods eaten by the participants were reported on sheets collected daily. The study dietitian met with each participant weekly to discuss the diet, resolve any barriers or concerns related to food or specimen collection, and encourage compliance. Energy and nutrient intake calculations were performed using the Nutrient Data System for Research (NDSR) software version 2009 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database [20,21]. Adherence to the protocol was monitored by urinary biomarkers (sodium, potassium, nitrogen) [22,23].

**Control diet.** Participants in the control group were asked to follow their habitual diet for the study duration and they received a multivitamin supplement daily (Nature Made, Mission Hills, CA). Their dietary intake was assessed during the intervention (Days 1–28) from three 24-dietary recalls (2 weekdays and 1 weekend day) performed using automated multi-pass method and NDS-R software [24,25].

Physical Activity

Daily physical activity was assessed using an RT3 accelerometer (StayHealthy, Monrovia, CA, US). Participants were instructed to maintain their habitual physical activity level and wore an activity monitor on their right hip while awake for the duration of the intervention and twice for 7 days during the follow-up. Total and physical activity energy expenditure was calculated using energy calculated from the monitor-measured movement and measured REE. Physical activity levels (PAL) for each monitored day were calculated by dividing total energy expenditure by REE.

Blood Pressure

Blood pressure was measured 3 times a week in the reclining position after 10 min rest with automatically inflating cuff.
outstanding performance characteristics \[27,28\]. F\(_2\)-isoprostane (F\(_2\)-isoprostane MS), a previously described and validated method \[26\] with analyzed using gas chromatography/mass spectrometry (GC/MS), each participant were included in the same assay run to avoid inter-assay variability among participants. F\(_2\)-isoprostane was stored in cryovials at –80 °C until the assays were performed in batches. Samples obtained at baseline and during the study for each participant were included in the same assay run to avoid inter-assay variability among participants. F\(_2\)-isoprostane was analyzed using gas chromatography/mass spectrometry (GC/MS), a previously described and validated method \[26\] with outstanding performance characteristics \[27,28\]. F\(_2\)-isoprostane assessing oxidation of lipids \[29\] has been shown to provide one of the most accurate assessments of oxidative stress status \[27,30\]. Other methods (not used in this study) are targeting protein oxidation by measuring carbonyl groups in serum \[31\], DNA damage by measuring hydroxyl radical-induced products of DNA bases \[32\], and activity of several antioxidative enzymes including catalase, superoxide dismutase (SOD), glutathione S-transferase, and glutathione peroxidase (GPX) \[33–35\].

A basic metabolic panel, hematological indices (hemoglobin concentration, hematocrit, red and white blood cell counts), and C-reactive protein were analyzed in the Vanderbilt University Hospital Laboratory using standard methodologies. Blood for measurement of lipids, inflammatory cytokines, and hormones (leptin, insulin, adiponectin) was centrifuged, serum was extracted, and the samples were stored at –80°C until later analyses. Plasma triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were measured using enzymatic kits from Cliniqua Corporation (San Marcos, CA). Free fatty acids (FFA) were measured using the NEFA-C kit by Wako (Neuss, Germany) and by gas chromatography. Glucose was measured using the Vitros Chemistry analyzer. Insulin and leptin measurements were performed using RIAs. Adiponectin was measured using a kit from Millipore (Billerica, MA) and Luminex multiplexing technology.

**Blood Collection and Biochemical Measurements**

Venous blood samples were drawn after an overnight fast at baseline and on days 1, 3, 5, 7, 14, 21, 29, 42, and 119, centrifuged immediately at 1 000 g for 10 minutes at 4°C, and the serum was stored in cryovials at –80°C until the assays were performed in batches. Samples obtained at baseline and during the study for each participant were included in the same assay run to avoid inter-assay variability among participants. F\(_2\)-isoprostane was analyzed using gas chromatography/mass spectrometry (GC/MS), a previously described and validated method \[26\] with outstanding performance characteristics \[27,28\]. F\(_2\)-isoprostane assessing oxidation of lipids \[29\] has been shown to provide one of the most accurate assessments of oxidative stress status \[27,30\]. Other methods (not used in this study) are targeting protein oxidation by measuring carbonyl groups in serum \[31\], DNA damage by measuring hydroxyl radical-induced products of DNA bases \[32\], and activity of several antioxidative enzymes including catalase, superoxide dismutase (SOD), glutathione S-transferase, and glutathione peroxidase (GPX) \[33–35\].

| Factor                      | Effect | 95% CI       | p-value |
|-----------------------------|--------|--------------|---------|
| Baseline age [years] - 34:26| 0.95   | 0.89–1.02    | 0.150   |
| Baseline BMI [kg/m\(^2\)] - 36.4:26.9 | 1.12 | 1.02–1.21 | 0.015   |
| Baseline F\(_2\)-isoprostane (pg/mL) - 78.2:48.8 | 1.46 | 1.36–1.55 | <0.001  |
| Day of intervention - day 21:day 3 | 0.76 | 0.69–0.84 | <0.001  |
| Group - Control : CR       | 1.60   | 1.36–1.88    | <0.001  |

Differences in F\(_2\)-isoprostane (pg/mL) serum levels between caloric restriction (CR) and control groups at baseline (Day 0), during the intervention (Days 1 to 29), and follow-up (Days 42 and 119).

**Urine Collection and Analyses**

Complete 24-h urine samples were collected once weekly. Urine volume and density was measured and a 10 mL sample was frozen at ~70°C until further analysis. Urinary calcium, sodium, and potassium were measured using Vitros 250 Analyzer (Ortho-Clinical Diagnostics, Rochester, NY, USA). Urinary nitrogen content was measured using nitrogen analyzer (Antek Instrument Nitrogen System 9000NS, Antek Instruments, Inc., Houston, TX, USA). The nitrogen excretion in the urine was used as a biological marker for protein intake by multiplying the content of nitrogen in the urine by the factor 7.72 \[22\]. The urine sodium and potassium contents were used as biological markers of sodium and potassium excretion.

**Table 4.** Differences in F\(_2\)-isoprostane (pg/mL) serum levels between caloric restriction (CR) and control groups at baseline (Day 0), during the intervention (Days 1 to 29), and follow-up (Days 42 and 119).

| Time   | N  | Control diet | CR diet | p-value*  |
|--------|----|--------------|---------|-----------|
| Baseline | 40 | 61.0 (51.8–65.0) | 57.0 (40.5–79.5) | 0.908     |
| Day 1   | 40 | 55.0 (48.8–65.0) | 44.0 (35.5–61.2) | 0.153     |
| Day 3   | 40 | 56.0 (46.8–73.5) | 39.5 (24.8–50.2) | 0.010     |
| Day 5   | 40 | 62.5 (45.0–72.5) | 33.5 (26.0–48.0) | 0.002     |
| Day 7   | 40 | 52.0 (45.0–66.0) | 33.5 (23.8–45.8) | 0.007     |
| Day 14  | 40 | 50.0 (42.0–67.5) | 34.0 (23.5–43.5) | 0.004     |
| Day 21  | 40 | 58.0 (49.2–60.2) | 30.5 (23.8–42.5) | <0.001    |
| Day 29  | 38 | 51.0 (49.2–53.5) | 29.5 (23.2–40.8) | <0.001    |
| Day 42  | 37 | 61.0 (55.0–65.0) | 33.5 (29.0–53.5) | 0.006     |
| Day 119 | 34 | 56.0 (52.0–59.5) | 56.0 (36.0–66.0) | 0.692     |

Data are presented as median (interquartile range- IQR). CR diet, caloric restriction diet.

*Wilcoxon rank sum test for differences between Control and CR diets. doi:10.1371/journal.pone.0047079.0004
intake, respectively. Urinary creatinine was measured on a Sirius Clinical Chemistry analyzer (Stanbio Laboratory, Boerne, TX).

Statistical Analysis

Descriptive statistics were presented as mean and standard deviation (SD) or median and IQR or percentage, as appropriate. Daily caloric intake and REE were expressed as the absolute and deficit number of kilocalories per day. Continuous endpoints were compared between the control and intervention group using Wilcoxon rank sum test. The change within group was assessed using Wilcoxon signed rank test. Spearman correlation coefficient was used to assess the correlation between two continuous variables. Multivariable linear model was used to assess the treatment effect at single time point while adjusting for the baseline measures. We performed a linear model using generalized least squares with autocorrelation structure of order 1 (AR1) for the within-subject correlation to assess the \( \text{F}_2\)-isoprostane change within 28 days of study period. The main effects included baseline age, BMI, and \( \text{F}_2\)-isoprostane, time, group, and time by group interaction. Time was modeled as nonlinear relationship to the \( \text{F}_2\)-isoprostane concentrations in the CR group increased \( (\text{control/CR}, 1.79, 95\% \text{ CI: } 1.50–2.13) \) and day 21 (control/CR, 1.82, 95% CI: 1.56–2.11). (Figure 2, Table 3). In participants with BMI\( >30 \) (\( n = 14 \)), plasma \( \text{F}_2\)-isoprostane concentrations decreased by more than 50% during the first week (Day 1 to Day 7). After the 3-month follow-up period, plasma \( \text{F}_2\)-isoprostane concentrations in the CR group increased and the difference between the groups was not significant (P = 0.692) (Table 4).

Changes in \( \text{F}_2\)-isoprostane Concentrations

We fit a linear model of \( \text{F}_2\)-isoprostane within the 28 days study period using generalized least squares. \( \text{F}_2\)-isoprostane was log transformed since the distribution was skewed. There were statistically significant differences between the CR and control group (P<0.001). The biggest difference appeared at day 14 (control/CR, 1.79, 95% CI: 1.50–2.13) and day 21 (control/CR, 1.82, 95% CI: 1.56–2.11). (Figure 2, Table 3). In participants with BMI\( >30 \) (\( n = 14 \)), plasma \( \text{F}_2\)-isoprostane concentrations decreased by more than 50% during the first week (Day 1 to Day 7). After the 3-month follow-up period, plasma \( \text{F}_2\)-isoprostane concentrations in the CR group increased and the difference between the groups was not significant (P = 0.692) (Table 4).

Energy Balance and Weight Changes

The average caloric intake was significantly lower in the CR than in the control group with an average intake of 17.8±2.2 kcal/kg/day vs. 28.2±4.6 kcal/kg/day (P<0.001). By the end of the dietary intervention (Day 29), total body weight and body fat weight decreased in the CR more than in the control group (difference 2.7 kg and 1.1 kg, respectively). However, differences in body weight and body fat between CR and control groups were non-significant at baseline, end of intervention, and day 119 follow-up (Table 5). There was no significant between group difference in REE adjusted for fat free mass. The total amount of physical activity-related EE (kcal/day) was lower in the CR than the control group at Day 29 (difference of 61.2 kcal/day, P = 0.057). However, there was no significant between group difference (P = 0.303) in physical

### Table 5. Body weight, BMI, and body fat mass at baseline, end of intervention (Day 29), and follow-up (Day 119).

| Variable      | Time       | Control diet | CR diet | p-value* |
|---------------|------------|--------------|---------|----------|
| Body Weight (kg) | Baseline  | 86.9 (72.2–96.2) | 85.5 (70.4–99.4) | 0.740    |
|               | Day 29    | 86.9 (71.1–97.6) | 82.8 (68.5–95.8) | 0.504    |
|               | Day 119   | 87.8 (79.7–98.7) | 84.5 (72.6–96.3) | 0.491    |
| BMI (kg/m²)   | Baseline  | 30.1 (28.1–38.3) | 32.3 (26.6–36.2) | 0.538    |
|               | Day 29    | 30.1 (27.6–38.8) | 31.5 (25.7–35.3) | 0.438    |
|               | Day 119   | 30.0 (28.5–39.0) | 32.1 (26.2–35.8) | 0.329    |
| Body Fat (kg) | Baseline  | 39.3 (33.2–44.1) | 38.5 (27.4–49.0) | 0.946    |
|               | Day 29    | 39.9 (31.0–44.0) | 38.0 (26.0–47.0) | 0.598    |
|               | Day 119   | 39.1 (33.5–45.9) | 36.7 (27.8–44.3) | 0.544    |

Data are presented as median (interquartile range - IQR).

Abbreviations: CR diet, caloric restriction diet; BMI, body mass index (kg/m²).

*Wilcoxon rank sum test for differences between Control and CR diets.

doi:10.1371/journal.pone.0047079.t005

### Table 6. Resting energy expenditure, physical activity-related energy expenditure and physical activity level (PAL) at baseline, end of intervention (Day 29), and day follow-up (Day 119).

| Variable | Time       | Control diet | CR diet | p-value* |
|----------|------------|--------------|---------|----------|
| REE (kcal/min) | Baseline  | 1.33 (1.22–1.37) | 1.22 (1.18–1.37) | 0.255    |
|           | Day 29    | 1.33 (1.21–1.38) | 1.20 (1.17–1.35) | 0.191    |
|           | Day 119   | 1.35 (1.27–1.39) | 1.28 (1.19–1.40) | 0.330    |
| PAEE (kcal/day) | Baseline  | 502.7 (471.7–514.3) | 519.5 (423.3–542.5) | 0.740    |
|           | Day 29    | 554.0 (511.0–648.1) | 492.8 (463.1–553.9) | 0.057    |
|           | Day 119   | 505.5 (476.8–540.3) | 497.8 (425.4–514.5) | 0.415    |
| PAL (kcal/min) | Baseline  | 1.31 (1.31–1.32) | 1.27 (1.25–1.32) | 0.078    |
|           | Day 29    | 1.33 (1.31–1.33) | 1.29 (1.25–1.35) | 0.303    |
|           | Day 119   | 1.32 (1.31–1.33) | 1.27 (1.25–1.31) | 0.012    |

Data are presented as median (interquartile range - IQR).

Abbreviations: REE, resting energy expenditure; PAEE, physical activity-related energy expenditure calculated as a difference between total and resting energy expenditure; PAL, physical activity level calculated as a ratio of total energy expenditure to REE, CR diet, caloric restriction diet.

*Wilcoxon rank sum test for differences between Control and CR diets.

doi:10.1371/journal.pone.0047079.t006
activity level (PAL) at the end of the intervention period (Table 6). Median intake of energy and macronutrients was lower in the CR than in control group ($P < 0.001$), as expected (Table 7).

Changes in other Disease Associated Markers

The CR diet did not have a significant effect on either systolic or diastolic blood pressure or serum concentrations of insulin, leptin, adiponectin, total cholesterol, LDL cholesterol, HDL cholesterol, CRP and triglycerides (Tables 8 and 9).

Discussion

The novelty of the present study is that we investigated serial changes in a marker of oxidative stress induced by 28-days of a caloric restriction diet. The main finding is that moderate (25%) CR and modest weight loss causes a rapid decrease in oxidative stress as measured by plasma F$_2$-isoprostane concentrations, to within the normal range exhibited by non-obese adults (35±6 pg/mL). The magnitude of this change was significant in comparison to baseline F$_2$-isoprostane plasma concentrations. This suggests that the potential benefits from reducing the oxidative stress level can be achieved rapidly without restricting caloric intake to a level that overweight and obese people might find difficult to sustain.

CR is hypothesized to lessen oxidative damage by reducing energy flux and metabolism with a consequential lowering of reactive oxygen species and rate of oxidative damage to vital tissues [36]. Several studies documented the association of CR with lowering of resting metabolic rate and thermic effect of food, and a decrease in cost of physical activity [37]. For example, Heilbronn et al [38] showed 6 months of caloric restriction (25%) significantly decreased sedentary 24-hour energy expenditure after adjusting for body composition. In the present study, we did not observe significant reductions in adjusted energy expenditure most likely because of a much shorter intervention period (1 month vs. 6 months). Other reported benefits of long-term CR, but not detected in our study, include reduction in fasting glucose and insulin, which are linked to decreased insulin resistance and risk for type 2 diabetes [39].

Our results showing decreased F$_2$-isoprostane with weight loss are consistent with a previous Davi et al [40] study involving 11 obese women who participated in a diet-induced weight loss program for a 12-week period and lost at least 5 kg of initial body weight. In eight of these women, there was a significant reduction in urinary excretion of F$_2$-isoprostane. In a recent study on the effect of CR and glycemic load on oxidative stress, Meydani et al [17] showed non-significant decreases in plasma 8-epi-prostaglandin F$_2a$ ($P = 0.09$) and protein carbonyl ($P = 0.02$), and a significant increases in plasma glutathione peroxidase activity ($P = 0.04$). In contrast, a randomized controlled trial with 42 participants with the metabolic syndrome assigned to 16 weeks of weight maintenance or a 12-week weight-loss program followed by 4 weeks of weight

### Table 7. Median daily macro- and selected micro-nutrient intake in the caloric restriction (CR) and Control diet groups.

| Nutrient               | Control diet Median | IQR       | CR diet Median | IQR       |
|------------------------|---------------------|-----------|----------------|-----------|
| Energy (kcal)          | 2062.4 (1906.1–2119.9) | 1543.1 (1410.9–1784.1) |
| Fat (g)                | 54.7 (52.8–67.6)   | 44.2 (33.5–54.6) |
| Carbohydrate (g)       | 276.1 (253.5–329.6) | 227.9 (213.1–325.9) |
| Protein (g)            | 101.2 (93.3–124.2) | 82.0 (70.9–93.4) |
| Glycemic Load (bread reference) | 213.7 (197.3–241.3) | 162.1 (141.5–183.4) |
| Dietary Fiber (g)      | 21.9 (18.8–24.3)   | 24.4 (22.1–27.3) |
| Vitamin A (retinol equivalents) (mcg) | 1886.8 (1736.8–2107.2) | 2034.7 (2202.3–6755.1) |
| Vitamin D (IU)         | 337.4 (293.4–392.8) | 325.2 (312.6–355.0) |
| Vitamin E (IU)         | 8.5 (7.1–12.0)     | 7.7 (6.4–9.3) |
| Vitamin C (ascorbic acid) (mg) | 87.0 (73.0–116.3) | 127.1 (100.9–167.1) |
| Vitamin B$_6$ (thiamin) (mg) | 1.5 (1.4–1.7)     | 1.2 (1.0–1.6) |
| Vitamin B$_6$ (riboflavin) (mg) | 2.2 (2.1–2.8)     | 1.9 (1.7–2.1) |
| Vitamin B$_6$ (mg) (piridoxal, piridoxamine, piridoxin) | 2.0 (1.8–2.1) | 1.5 (1.3–1.8) |
| Niacin (mg)            | 22.5 (20.9–24.9)   | 19.4 (15.0–22.5) |
| Folate (mcg)           | 574.3 (524.8–685.5) | 501.7 (464.5–552.4) |
| Vitamin B$_12$ (cobalamin) (mcg) | 6.9 (5.7–7.6)    | 4.6 (3.4–6.1) |
| Sodium (mg)            | 3588.8 (3319.3–4209.7) | 2604.4 (2112.0–3433.0) |
| Calcium (mg)           | 822.3 (729.1–920.5) | 1008.6 (881.6–1147.2) |
| Magnesium (mg)         | 509.2 (451.7–540.7) | 455.3 (436.8–482.1) |
| Iron (mg)              | 14.4 (12.1–15.2)   | 9.4 (7.7–11.5) |

Data are presented as median (interquartile range -IQR).

1- different from Control diet, $P < 0.001$, Wilcoxon rank sum test.

Daily multivitamin supplement was given during intervention Days 1–28 (Multi Complete, Nature Made, Mission Hills, CA) composition: vitamin A - 2 500 IU, vitamin D$_3$ - 1000 IU, vitamin E - 50 IU, vitamin C - 180 mg, vitamin B$_6$ - 15 mg, vitamin B$_12$ - 1.7 mg, niacin - 20 mg, vitamin B$_2$ - 2 mg, folate - 400 mcg, vitamin B$_12$ - 6 mcg, calcium 162 mg, magnesium - 100 mg, iron - 18 mg, selenium - 70 mcg.

doi:10.1371/journal.pone.0047079.t007
stabilization, showed different results. Relative to the weight-maintenance group, a 4-kg loss in weight resulted in a significant decrease in blood pressure but did not alter urinary or plasma F2-isoprostane [41]. The discrepancies between these studies could be explained, at least in part, by different methodology (plasma or urinary isoprostanes), various experimental designs, and diverse study populations.

Baseline F2-isoprostane correlated with body fat content. However, the F2-isoprostane decrease during the CR intervention was not correlated with the concurrent changes in body fat. Although we used a clinical trial design, strict diet control, and validated biomarkers [42,43], it was not possible to determine whether body fat itself was a source of oxidative stress. As far as we are aware, F2-isoprostanes, although present in foods, are not validated biomarkers [42,43], it was not possible to determine whether body fat itself was a source of oxidative stress. As far as we are aware, F2-isoprostanes, although present in foods, are not absorbed through the gut [44,45]. A plausible explanation is that in addition to a decrease in energy content, changes in content and amount of macronutrients consumed affected systemic oxidative balance. Lower concentrations of protein, carbohydrates, and lipids in the CR diet, when compared to habitual diets reported by Control diet participants, could have potentially shifted postprandial oxidative status towards decreased susceptibility to oxidative damage [46]. Indeed, previous research has shown that postprandial increases of lipid and carbohydrate concentrations lead to increased oxidative stress [47] and consequent hyperlipidemia and hyperglycemia [48].

Although our study did not test whether decreases in oxidative stress are linked to an improvement in risk factors for associated chronic diseases, previous data do provide such evidence. Zacardi et al [49] reported that declines in adhesion molecules and improvement in endothelial function with sustained weight loss were related to decreases in IL-6 and TNF-α, independent of change in adiposity and body fat distribution. Despite the many studies that have examined the role of oxidative stress on cardiovascular health, mechanistic studies designed to abruptly reduce plasma oxidative stress and to subsequently determine the acute effects of this intervention on antioxidative capacity, oxidative stress, and vascular function have not been extensively reported. For example, studies in young and older individuals demonstrated a dichotomous effect of antioxidant consumption on endothelial function with age [50]. Nevertheless, if the link between obesity and increased oxidative stress [47] and consequent hyperlipidemia and hyperglycemia [48].

Our findings extend those of previous studies in several ways. First, our data provide evidence from a controlled trial design...
that the decrease in oxidative stress biomarkers induced by a modest caloric restriction is rapid. This is important because obese individuals are unable to consistently comply with a long-term daily caloric reduction of 40% (consuming 60% of maintenance), as has been used in most animals studies [52]. Second, the study causally links the measured decreases in F2-isoprostane to the weight loss induced by caloric restriction. Third, we demonstrated that return to a habitual diet, with consequent gradual regaining of weight, causes an increase of F2-isoprostane to pre-study, elevated baseline levels. This observation may have clinical significance especially in weight cycling (yo-yo dieting) [53,54] and weight-loss maintenance [55]. It has been reported that weight cycling is a relatively common phenomenon in women, ranging from 19% in White [56,57] to 65% in African American women [58], as well as in approximately 10% of men [57]. Weight cycling has a negative impact on body composition and body fat distribution [59,60], and has been associated with an increased risk for metabolic syndrome [61], cardiovascular disease [62], and all-cause mortality [63]. Now we have a clear understanding of how weight cycling stimulates adjustments in energy homeostatic hormones (leptin, ghrelin, and insulin) that activate regulatory mechanisms to restore weight [64].

However, we do not know whether energy intake-induced weight cycling is affecting oxidative stress level. Our results support the notions that during caloric restriction and subsequent weight loss, oxidative stress level decreases, and in contrast, increases during a positive caloric balance and subsequent weight gain. Thus, we hypothesize that previously mentioned health consequences of weight cycling such as increased risk for metabolic syndrome and cardiovascular disease could be associated, at least in part, with changes in the oxidative stress level. Future clinical studies are necessary to explain whether these associations can be attenuated by frequent weight cycling and what mechanisms (e.g. lipids oxidation, insulin resistance, and inflammation) are involved.

A limitation of the present study is the relatively small number of participants; hence, the results need to be confirmed in larger studies. In addition, individuals in the control group ate their habitual diets, thus it was possible that their intake of antioxidants (e.g., vitamin E,) and other micronutrient would be similar in the control group. As a result, vitamin E intake was similar in the control and CR groups (∼58 international units), which was in line with the daily recommended intake of 22.4 to 50 international units for younger and older adults, respectively [65].

Although the use of F2-isoprostanes as a marker of oxidative stress is a strength [66], the study would have greatly benefited from measurement of water-soluble oxidation markers such as thiobarbituric acid reactive substances [39] and total antioxidant capacity [67,68]. However, in the study on effect of diet-induced reduction in oxidative stress it has been showed that F2-isoprostane level was well correlated with advanced oxidation protein products [69]. In addition, one should be cautious if generalizing this study’s finding to males, obese individuals, and populations with serious obesity-related diseases, such as type 2 diabetes or hyperlipidemias. Further studies will be required to determine whether caloric restriction and weight loss lead to a reduction in F2-isoprostane and other markers of oxidative stress in such conditions. Finally, we did not explore the effect of age on our results. For example, it has been shown that antioxidant consumption acutely restores endothelial function in the elderly while disrupting normal endothelium-dependent vasodilation in the young, and suggest that this age-related impairment is attributed, at least in part, to free radicals [70]. However, participants in our study were relatively healthy premenopausal women eliminating, at least in part, the potential effect of age on the results.

In summary, the results of the present study show that oxidative stress can be rapidly reduced and sustained through a modest (25%) reduction in caloric intake for a relatively short (28-day) period. Simultaneous reduction in markers of inflammation was associated with decreases in body fat and body weight. These changes suggest potential health benefits of modest caloric restriction in overweight and obese women.

Supporting Information

Checklist S1 CONSORT Checklist.

Protocol S1 Trial Protocol.

Author Contributions

Conceived and designed the experiments: MSB NH LJR. Performed the experiments: MSB LJR. Analyzed the data: MSB NH JW SA LJR LW. Contributed reagents/materials/analysis tools: NH. Wrote the paper: MSB JW SA LJR LW.

References

1. Vincent HK, Innes KE, Vincent KR (2007) Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes, Obesity and Metabolism 9: 413-439.

2. Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. The Journal of Clinical Investigation 121: 2111-2117.

3. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444: 860–867.

4. Bau S (2000) F2-isoprostanes in Human Health and Diseases: From Molecular Mechanisms to Clinical Implications. Antioxidants & Redox Signaling 10: 1405–1434.

5. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, et al. (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation 114: 1752–1761.

6. Tihanous FJ, Murri-Pierri M, Garrido-Sanchez L, Garcia-Almeida JM, Garcia-Serrano S, et al. (2006) Oxidative Stress in Severely Obese Persons Is Greater in Those With Insulin Resistance. Obesity 17: 240–246.

7. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11: 83–97.
14. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET (2002) Weight Loss Reduces C-Reactive Protein Levels in Obese Postmenopausal Women. Circulation 105: 564–569.

15. Klimavicius E, Kovačićka M, Stich V, Langin D (2010) Adipokines and dietary interventions in human obesity. Obesity Reviews 11: 446–456.

16. Rolland C, Hession M, Broom (2011) Effect of weight loss on adipokine levels in obese patients. Diabetes Metab Syndr Obes 4: 315–323.

17. Meydani M, Das S, Band M, Epstein S, Roberts S (2011) The effect of caloric restriction and caloric load on measures of oxidative stress and antioxidant enzymes in humans: results from the CALERIE Trial of Human Caloric Restriction. J Nutr Health Aging 15: 456–460.

18. (2007) National Health and Nutrition Examination Survey (NHANES): Anthropometry Procedures Manual. In: Prevention GickRa, editor. Atlanta, GA: CDC. 3: 20–23 and 30–31.

19. Weir JG (1949) New methods for calculating metabolic rate with special reference to protein metabolism. The Journal of Physiology 109: 1–9.

20. Miller PE, Mitchell DC, Harala FL, Penn TR, Smraczkowski & Wright H, et al. (2011) Development and evaluation of a method for calculating the Healthy Eating Index-2005 using the Nutrition Data System for Research. Public Health Nutrition 14: 306–313.

21. Harmaek S, Stevens M, Van H, Schakel S, Dwyer J, et al. (2008) A computer-based approach for assessing dietary supplement use in conjunction with dietary recall. J Food Compost Anal 21 (Supplement): S78–S82.

22. Bingham S, Cummings J (1985) Urine nitrogen as an independent validatory measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. The American Journal of Clinical Nutrition 42: 1279–1289.

23. McCulloh ML, Swain JF, Malarick C, Moore TJ (1991) Feasibility of outpatient electrolyte balance studies. Journal of the American College of Nutrition 10: 140–148.

24. Fudymich D, Siefall BH, Chong K, Buzzard JM (1989) Computerized collection and analysis of dietary intake information. Comput Methods Programs Bionoin 30: 47–57.

25. Johnson JK, Driscoll P, Goran MI (1996) Comparison of multiple-pass 24-hour recall estimates of energy intake with total energy expenditure determined by the doubly labeled water method in young children. J Am Diet Assoc 96: 1140–1144.

26. Morrow JD, Roberts L 2nd (1999) Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidative stress. Methods in enzymology 300: 7–12.

27. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, et al. (2003) Biomarkers of Oxidative Stress Study II: Are oxidation products of lipids, proteins, and DNA markers of CCH poisoning? Free Radical Biology and Medicine 34: 983–991.

28. Milne GL, Musick ES, Morrow JD (2005) F2-isoprostanes as markers of oxidative stress in vivo: An overview. Biomarkers 10: 16–23.

29. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, et al. (1990) A Series of Prostaglandin F2-like Compounds are Produced in vivo in Humans by a Non-Cyclooxygenase, Free Radical-Catalyzed Mechanism. PNAS 87: 9383–9387.

30. Pieper C, Redman L, Racette S, Roberts S, Bhapkar M, et al. (2011) Development of adherence metrics for caloric restriction interventions. Clin Trials 8: 155–164.

31. Field AE, Manson JE, Taylor CB, Willett WC,Colditz GA (2004) Association of weight change, weight control practices, and weight cycling among women in the Nurses’ Health Study II. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 28: 1134–1142.

32. Lahiri-Koki M, Mannisto S, Pietinen P, Vartiaenen E (2005) Prevalence of weight cycling and its relation to health indicators in Finland. Obes Res 13: 333–341.

33. Osborn R, Forbs K, Potts T, Shrocco T (2011) Yo-yo dieting in African American women: weight cycling and health. Ethn Dis 21: 274–280.

34. Cereda E, Malavaoos AE, Franco C, Giovannini M, Rossanalini M, Fattai G (2011) Weight cycling is associated with body weight excess and abdominal fat accumulation: A cross-sectional study. Clinical Nutrition 30: 718–723.

35. Gullo AG, Jacquet J, Montani JP (2012) How dieting makes some fatter: from a perspective of human body composition autoregulation. Proceedings of the Nutrition Society 71: 379–389.

36. Hooper LE, Foster-Schubert KE, Weigle DS, Sorensen B, Ulrich CM, et al. (2011) Reduction of Inflammatory Cytokine Concentrations and Improvement of Endothelial Functions in Obese Women After Weight Loss Over One Year. Circulation 105: 804–809.

37. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.

38. Gopaul N, Liugliano G, Esposito K, Martella R, et al. (2002) A MONET (Montreal Ottawa New Emerging Team) Study. Journal of the American College of Nutrition 21: 163–170.

39. Gopaul N, Liugliano G, Esposito K, Martella R, et al. (2002) A MONET (Montreal Ottawa New Emerging Team) Study. Journal of the American College of Nutrition 21: 163–170.

40. Graci S, Izzo G, Savino S, Cattani L, Lezzi G, et al. (2003) Weight cycling and mortality in a large prospective US Study. American Journal of Clinical Nutrition 88: 1495–1503.

41. Bae J-H, Paoletti K, Bailey DM, et al. (1999) The Paradox of Oxidative Stress and Exercise With Advancing Age. Exercise and Sport Sciences Reviews 27: 189–195.

42. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.

43. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.

44. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.

45. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.

46. Richelle M, Turini ME, Guidoux R, Tavazzi I, Meà®airon S, et al. (1999) Urinary epoxyeicosatrienoic acid (EET) excretion is not confounded by the lipid content of the diet. FEBS Letters 458: 259–262.
68. Apak R, Guclu K, Ozyurek M, Bektasoglu B, Bener M (2010) Cupric ion reducing antioxidant capacity assay for antioxidants in human serum and for hydroxyl radical scavengers. Methods Mol Biol 594: 215–239.

69. Nemzer BV, Rodriguez LC, Hammond L, Disilvestro R, Hunter JM, et al. (2011) Acute reduction of serum 8-iso-PGF2-alpha and advanced oxidation protein products in vivo by a polyphenol-rich beverage; a pilot clinical study with phytochemical and in vitro antioxidant characterization. Nutr J 10: 67.

70. Wray DW, Nishiyama SK, Harris RA, Zhao J, McDaniel J, et al. (2012) Acute reversal of endothelial dysfunction in the elderly after antioxidant consumption. Hypertension 59: 818–824.