

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

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## 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  $32 \pm 5.8$  kg/m<sup>2</sup>) observed for 28 days and followed for the next 90 days. Weight, anthropometry, validated markers of oxidative stress (F<sub>2</sub>-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 F<sub>2</sub>-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, F<sub>2</sub>-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 F<sub>2</sub>-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>

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## 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 F<sub>2</sub>-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 F<sub>2</sub>-isoprostane.

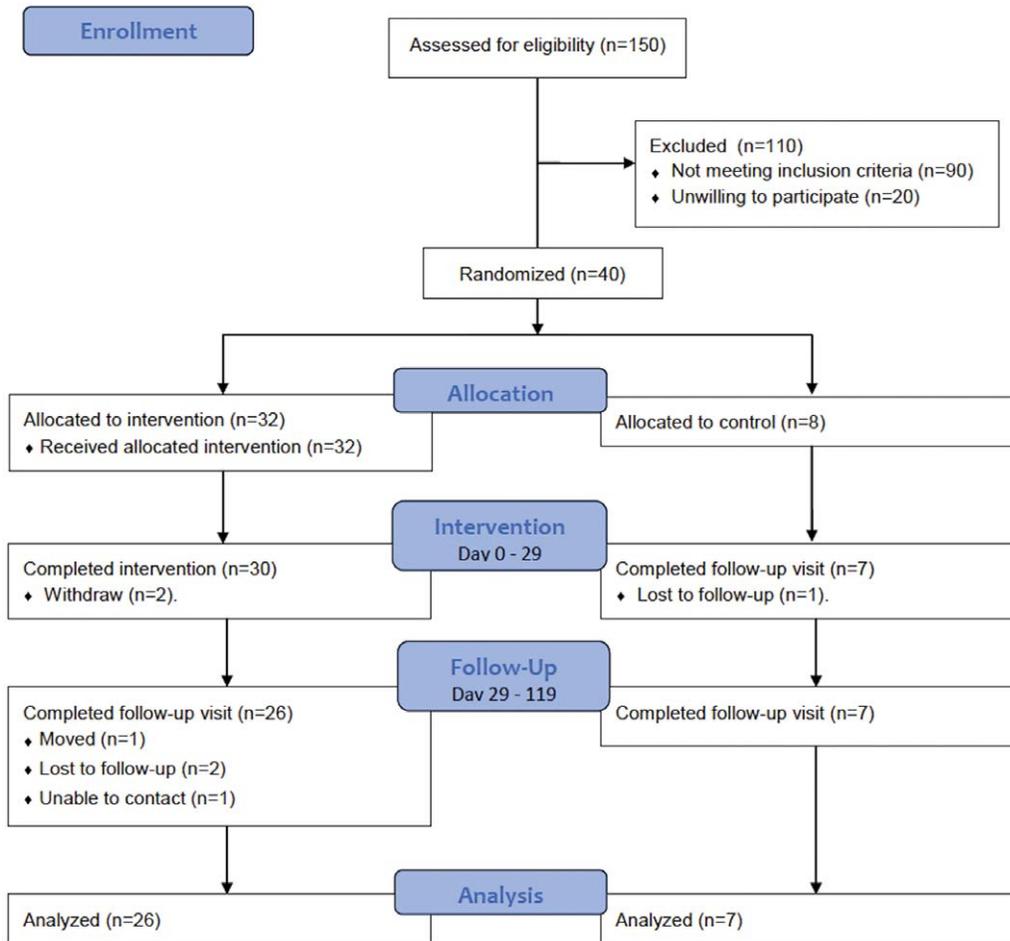
## Methods

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/m<sup>2</sup>), were

## CONSORT 2010 Flow Diagram



**Figure 1. CONSORT flow diagram.**  
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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/m<sup>2</sup> 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

**Table 1.** Baseline demographic and anthropometric characteristics.

	Control diet (n = 8)	CR diet (n = 32)
Age (years)	29.5 (26.8–33.3)	30.5 (26.0–34.0)
Body weight (kg)	86.9 (72.2–96.2)	85.2 (70.4–99.4)
Body-mass index (kg/m <sup>2</sup> )	30.1 (28.1–38.3)	32.3 (26.6–36.3)
Body fat mass (kg)	39.3 (33.2–44.1)	38.6 (27.5–49.0)
<b>Ethnicity</b>		
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.  
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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 coefficient of variation was 0.7%).

### 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 (O<sub>2</sub>) consumption and carbon dioxide (CO<sub>2</sub>) 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.

### 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% ( $\pm$ 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 uneaten 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

**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.

	Intake <sup>a</sup>	Urinary Excretion <sup>b</sup>	Ratio of Intake to Excretion	p-value <sup>c</sup>
Protein (g/day)	85.0 $\pm$ 12.0	87.9 $\pm$ 3.6	1.03 $\pm$ 0.25	0.486
Sodium (g/day)	3.03 $\pm$ 0.44	3.18 $\pm$ 0.83	0.95 $\pm$ 0.19	0.919
Potassium (g/day)	2.93 $\pm$ 0.43	2.83 $\pm$ 0.43	1.04 $\pm$ 0.32	0.553

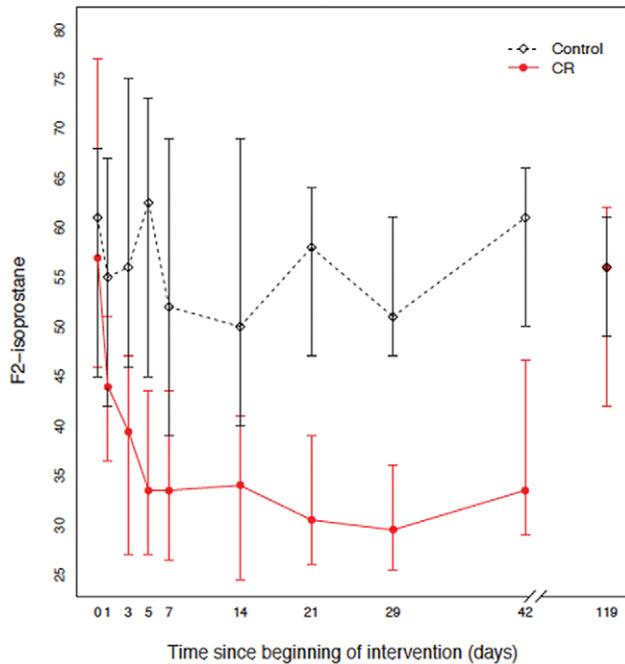
Data are presented as means  $\pm$  s.d.

<sup>a</sup>Average daily intakes of protein, sodium, and potassium assessed by NDSR (Nutrition Diet System, St. Paul, MN).

<sup>b</sup>Excretion measured from 4 weekly 24-hour urine collections. Protein = urinary nitrogen \*7.72 (18), Potassium = urinary potassium \*0.77 (19).

<sup>c</sup>Comparison of ratios of intake to excretion; one sample t-test.

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**Figure 2. Median plasma concentrations of F<sub>2</sub>-isoprostane (prostaglandin F<sub>2</sub>-like compound) in overweight and obese women at baseline (Day 0), during dietary intervention (Days 1–29), and after 90-day follow-up (Days 42 and 119) in the CR (caloric restricted) diet (75% of energy requirement) group (n = 32) and in the control group (n = 8) without diet restrictions (100% of energy requirement).** Vertical bars represent 95% bootstrap confidence intervals.  
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(Dynamap, General Electric, Milwaukee, WI, USA) using a standard protocol.

### 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<sub>2</sub>-isoprostane was analyzed using gas chromatography/mass spectrometry (GC/MS), a previously described and validated method [26] with outstanding performance characteristics [27,28]. F<sub>2</sub>-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

**Table 3. Multivariable linear regression results for change in F<sub>2</sub>-isoprostane serum levels during the 28-day dietary intervention.**

Factor	Effect	95% CI	p-value
Baseline age [years] - 34:26	0.95	0.89–1.02	0.150
Baseline BMI [kg/m <sup>2</sup> ] - 36.4:26.9	1.12	1.02–1.21	0.015
Baseline F <sub>2</sub> -isoprostane (pg/mL) - 78.2:44.8	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

Effect shown is the F<sub>2</sub>-isoprostane ratio. BMI - body mass index; CR diet- caloric restriction diet. Adjusted to: Day of intervention = 7, Group = CR.  
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(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 (Nneuss, 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.

### 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

**Table 4. Differences in F<sub>2</sub>-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 <sup>a</sup>
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.

<sup>a</sup>Wilcoxon rank sum test for differences between Control and CR diets.

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**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 <sup>a</sup>
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/m <sup>2</sup> )	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/m<sup>2</sup>).

<sup>a</sup>Wilcoxon rank sum test for differences between Control and CR diets.

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intake, respectively. Urinary creatinine was measured on a Sirmu 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 F<sub>2</sub>-isoprostane change within 28 days of study period. The main effects included baseline age, BMI, and F<sub>2</sub>-isoprostane, time, group, and time by group interaction. Time was modeled as nonlinear relationship to the F<sub>2</sub>-isoprostane using restricted cubic splines. Residual plot and quantile-quantile (QQ) plots were used to check the model assumptions. The level of statistical significance was set at  $p < 0.05$ . All analyses were done with STATA 11 (StataCorp, College Station, TX) and the statistical programming language R, version 2.13.1 (R Development Core Team, Vienna, Austria).

## Results

### Baseline Characteristics and Compliance

Thirty of 32 participants in the CR group and all controls completed the intervention part of the study (i.e., returned for the Day 29 visit). The reasons for study dropout were work related ( $n = 1$ ) and a family emergency ( $n = 1$ ). Twenty-six CR and 7 control group participants completed the entire study. The reasons for study dropout were work-related (CR,  $n = 2$ ; control,  $n = 1$ ), pregnancy (CR,  $n = 1$ ), and an unspecified reason (CR,  $n = 1$ ). The participants who did not complete the CR protocol did not differ from the completers in regards to age, body weight, body fat, CRP, or insulin, but did have lower baseline F<sub>2</sub>-isoprostane

**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 <sup>a</sup>
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	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.

<sup>a</sup>Wilcoxon rank sum test for differences between Control and CR diets.

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plasma concentrations (38.5, IQR = 20.5–48.2 vs. 63.5, IQR = 45.8–79.0;  $P = 0.01$ ). Baseline demographic and anthropometric characteristics are shown in Table 1. Adherence to the protocol in completers was good as measured by urinary biomarkers calculated as ratios of reported intake and excretion for protein ( $1.03 \pm 0.25$ ), sodium ( $0.95 \pm 0.19$ ) and potassium ( $1.04 \pm 0.32$ ) (Table 2).

### Changes in F<sub>2</sub>-isoprostane Concentrations

We fit a linear model of F<sub>2</sub>-isoprostane within the 28 days study period using generalized least squares. F<sub>2</sub>-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 F<sub>2</sub>-isoprostane concentrations decreased by more than 50% during the first week (Day 1 to Day 7). After the 3-month follow-up period, plasma F<sub>2</sub>-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  $17.8 \pm 2.2$  kcal/kg/day vs.  $28.2 \pm 4.6$  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 7.** Median daily macro- and selected micro-nutrient intake in the caloric restriction (CR) and Control diet groups.

Nutrient	Control diet		CR diet	
	Median	IQR	Median	IQR
Energy (kcal)	2062.4	(1906.1–2119.9)	1543.3 <sup>1</sup>	(1410.9–1784.1)
Fat (g)	54.7	(52.8–67.6)	44.2 <sup>1</sup>	(33.5–54.6)
Carbohydrate (g)	276.1	(253.5–329.6)	227.9 <sup>1</sup>	(213.1–253.9)
Protein (g)	101.2	(93.3–124.2)	82.0 <sup>1</sup>	(70.9–93.4)
Glycemic Load (bread reference)	213.7	(197.3–241.3)	162.1 <sup>1</sup>	(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 <sub>1</sub> (thiamin) (mg)	1.5	(1.4–1.7)	1.2	(1.0–1.6)
Vitamin B <sub>2</sub> (riboflavin) (mg)	2.2	(2.1–2.8)	1.9	(1.7–2.1)
Vitamin B <sub>6</sub> (mg) (pyridoxal, pyridoxamine, pyridoxin)	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 <sub>12</sub> (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).

<sup>1</sup> - 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<sub>3</sub> - 1000 IU, vitamin E - 50 IU, vitamin C - 180 mg, vitamin B<sub>1</sub> - 15 mg, vitamin B<sub>2</sub> - 1.7 mg, niacin - 20 mg, vitamin B<sub>6</sub> - 2 mg, folate - 400 mcg, vitamin B<sub>12</sub> - 6 mcg, calcium 162 mg, magnesium - 100 mg, iron - 18 mg, selenium - 70 mcg.

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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<sub>2</sub>-isoprostane concentrations, to within the normal range exhibited by non-obese adults ( $35 \pm 6$  pg/ml). The magnitude of this change was significant in comparison to baseline F<sub>2</sub>-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<sub>2</sub>-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<sub>2</sub>-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<sub>2a</sub> ( $P = 0.09$ ) 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 8.** Blood pressure, hormones, and lipids at baseline, end of intervention (Day 29), and follow-up (Day 119).

Variable	Time	Control diet	CR diet	p-value <sup>1</sup>
Systolic blood pressure (mmHg)	Baseline	121.5 (120.1–126.5)	128.5 (122.8–132.3)	0.052
	Day 29	125.0 (123.0–126.0)	123.0 (117.3–129.0)	0.648
	Day 119	126.0 (124.5–128.0)	125.0 (121.5–131.5)	0.897
Diastolic blood pressure (mmHg)	Baseline	79.5 (76.5–82.5)	77.5 (74.0–82.0)	0.206
	Day 29	79.0 (78.0–81.5)	77.0 (73.0–81.0)	0.116
	Day 119	77.0 (75.0–80.0)	77.0 (75.0–79.8)	0.983
Insulin (uU/mL)	Baseline	13.8 (7.0–16.8)	11.7 (8.9–17.0)	0.803
	Day 29	11.1 (8.1–17.0)	9.8 (6.8–12.6)	0.514
	Day 119	11.5 (7.5–16.1)	10.1 (8.9–15.2)	0.830
Leptin (ng/mL)	Baseline	19.5 (16.5–25.6)	21.1 (15.4–28.0)	0.974
	Day 29	19.6 (16.4–31.1)	17.8 (11.6–24.2)	0.250
	Day 119	17.8 (15.9–25.9)	22.6 (15.4–26.8)	0.784
Adiponectin (ng/mL)	Baseline	9.1 (5.5–10.2)	10.0 (7.7–13.8)	0.235
	Day 29	7.2 (5.9–11.3)	12.0 (7.8–15.7)	0.114
	Day 119	8.1 (7.0–9.9)	12.1 (8.7–17.7)	0.243
Triglyceride (mg/dL)	Baseline	134.5 (131.5–154.0)	138.0 (122.0–153.2)	0.595
	Day 29	142.0 (131.0–157.0)	132.0 (118.0–144.0)	0.125
	Day 119	126.0 (124.5–149.0)	135.0 (121.5–145.0)	0.587
Total cholesterol (mg/dL)	Baseline	214.5 (189.5–241.2)	208.0 (181.0–226.2)	0.454
	Day 29	214.5 (188.8–227.8)	190.5 (174.2–210.8)	0.378
	Day 119	215.0 (205.0–231.5)	209.0 (186.0–220.0)	0.391
LDL (mg/dL)	Baseline	148.8 (110.0–167.8)	132.0 (105.8–150.2)	0.377
	Day 29	152.0 (122.2–159.2)	118.5 (107.2–143.8)	0.208
	Day 119	149.0 (137.5–153.5)	125.0 (117.0–142.5)	0.132
HDL (mg/dL)	Baseline	45.5 (36.0–53.5)	46.0 (42.0–54.2)	0.752
	Day 29	42.0 (35.0–46.0)	44.0 (35.0–54.0)	0.515
	Day 119	48.0 (40.5–50.5)	46.0 (41.0–52.0)	0.573
Free fatty acids (mmol/L)	Baseline	0.41 (0.32–0.60)	0.53 (0.37–0.73)	0.262
	Day 29	0.41 (0.31–0.53)	0.52 (0.42–0.67)	0.108
	Day 119	0.38 (0.35–0.55)	0.49 (0.40–0.65)	0.156

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

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

<sup>1</sup>Wilcoxon rank sum test for differences between Control and CR diets.

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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 F<sub>2</sub>-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 F<sub>2</sub>-isoprostane correlated with body fat content. However, the F<sub>2</sub>-isoprostane decrease during the CR intervention

**Table 9.** Inflammatory markers at baseline, end of intervention (Day 29), and follow-up (Day 119).

Variable	Time	Control diet	CR diet	P-value <sup>1</sup>
Tumor necrosis factor- $\alpha$ (ug/dL)	Baseline	5.67 (1.65–9.25)	2.88 (0.00–5.22)	0.310
	Day 29	5.90 (1.40–9.11)	3.08 (0.00–8.00)	0.555
	Day 119	7.67 (2.48–8.81)	4.96 (0.00–5.68)	0.340
Interleukin -12 (pg/mL)	Baseline	6.47 (5.30–9.52)	2.75 (0.00–5.68)	0.027
	Day 29	9.53 (5.70–10.39)	3.19 (0.00–5.81)	0.027
	Day 119	8.33 (7.48–9.39)	4.26 (0.00–6.94)	0.007
Interleukin -8 (pg/mL)	Baseline	8.55 (8.20–17.73)	5.02 (3.04–8.37)	0.003
	Day 29	9.72 (7.32–39.30)	6.48 (4.87–8.29)	0.019
	Day 119	7.41 (7.16–25.88)	5.94 (2.65–8.55)	0.075
C-reactive protein (ug/mL)	Baseline	6.00 (3.00–9.50)	3.50 (1.00–6.00)	0.157
	Day 29	4.20 (2.65–7.25)	2.60 (1.00–6.25)	0.274
	Day 119	4.70 (1.12–9.10)	3.00 (1.00–5.07)	0.776

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

<sup>1</sup>Wilcoxon rank sum test for differences between Control and CR diets.

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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, F<sub>2</sub>-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. Zaccardi 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- $\alpha$ , 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 is confirmed, the potential for antioxidant therapy to decrease the risk of obesity-related co-morbid conditions such as cardiovascular disease warrants additional studies in diverse populations [51].

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 F<sub>2</sub>-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 F<sub>2</sub>-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 63% 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 below the recommended allowances. To prevent this possibility, participants in both groups received a vitamin/mineral supplement daily. 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 F<sub>2</sub>-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 F<sub>2</sub>-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 F<sub>2</sub>-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.**  
(DOC)

**Protocol S1 Trial Protocol.**  
(DOCX)

## 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.

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