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Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals

Michael Lefevre [b], Leanne M. Redman [a], Leonie K. Heilbronn [c], Julie V. Smith [d], Corby K. Martin [a], Jennifer C. Rood [a], Frank L. Greenway [a], Donald A. Williamson [a], Steven R. Smith [a], Eric Ravussin [a,*],

for the Pennington CALERIE team

[a] *Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA, United States*
[b] *Utah State University, Logan, UT, United States*
[c] *Garvan Institute of Medical Research, Darlinghurst, NSW, Australia*
[d] *William Carey University, Hattiesburgh, MS, United States*

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Abstract

Calorie restriction (CR) delays the development of age-associated disease and increases lifespan in rodents, but the effects in humans remain uncertain.

Purpose: Determine the effect of 6 months of CR with or without exercise on cardiovascular disease (CVD) risk factors and estimated 10-year CVD risk in healthy non-obese men and women.

Methods: Thirty-six individuals were randomized to one of three groups for 6 months: Control, 100% of energy requirements; CR, 25% calorie restriction; CR + EX, 12.5% CR + 12.5% increase in energy expenditure via aerobic exercise. CVD risk factors were assessed at baseline, 3 and 6 months.

Results: After 6 months, CR and CR + EX lost approximately 10% of body weight. CR significantly reduced triacylglycerol (-31 ± 15 mg/dL) and factor VIIc ($-10.7 \pm 2.3\%$). Similarly CR + EX reduced triacylglycerol (-22 ± 8 mg/dL) and additionally reduced LDL-C (-16.0 ± 5.1 mg/dL) and DBP (-4.0 ± 2.1 mmHg). In contrast, both triacylglycerol (24 ± 14 mg/dL) and factor VIIc ($7.9 \pm 2.3\%$) were increased in the Control group. HDL-cholesterol was increased in all groups while hsCRP was lower in the Controls versus CR + EX. Estimated 10-year CVD risk significantly declined from baseline by 29% in CR ($P < 0.001$) and 38% in the CR + EX ($P < 0.001$) while remaining unchanged in the Control group.

Conclusions: Based on combined favorable changes in lipid and blood pressure, caloric restriction with or without exercise that induces weight loss favorably reduces risk for CVD even in already healthy non-obese individuals.

Keywords: Caloric restriction; Exercise; Cardiovascular risk factors; Nutritional intervention; Weight loss; Aging

1. Introduction

Prolonged caloric restriction (CR) has been suggested as an anti-aging strategy in the belief that it will extend lifespan and improve quality of life. While data are convincing in shorter-lived species [1], whether calorie restriction extends lifespan in humans is not known. Heart disease and stroke are the number one and three causes of death in the USA [2], hence delaying the progression of atherosclerotic cardiovascular disease maybe one potential mechanism by which CR promotes longevity. The risk factors for CVD including blood lipids, blood pressure, hemostatic factors, inflammatory markers and endothelial function are all

* Corresponding author at: Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge, LA 70808, United States. Tel.: +225 763 3186; fax: +225 763 3030.
E-mail address: Ravusse@pbrc.edu (E. Ravussin).

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worsened with aging [3–6]. At least a portion of these age-related changes appear to be secondary to increases in adiposity and/or reductions in physical activity [7,8] and, therefore, may be amenable to improvements through prolonged caloric restriction and/or increased physical activity.

It is well established that obesity is associated with increased CVD mortality [9] and weight loss in obese individuals by CR is associated with improvements in CVD risk factors [10] and a lowering of coronary heart disease event rates [11]. Increased physical activity, primarily through aerobic exercise also reduces the risk for atherosclerotic disease, acute cardiovascular events, stroke and Type 2 diabetes mellitus [12,13]. Thus, it would be expected that increasing physical activity as a means to achieve a negative energy balance in conjunction with CR would provide benefits at least equivalent (or superior) to that of simply reducing caloric intake.

The Comprehensive Assessment of the Long Term Effects of Reducing Intake of Energy (CALERIE) study examined the potential health benefits of CR in sedentary, non-obese, healthy individuals. While the primary aim of the study was to determine the impact of CR on biomarkers of longevity and metabolic adaptation [14] the secondary aims were to evaluate the changes in risk factors for type 2 diabetes mellitus [15] and CVD. We hypothesized that 6 months of CR would improve markers for CVD and that changes in CVD risk factors would be similar whether the energy deficit was produced by combining exercise with CR or by CR alone.

2. Methods

2.1. Study participants

Forty-eight healthy, non-smoking male (25–50 years) and female (25–45 years), overweight participants ($25 \leq \text{BMI} < 30$) were recruited to participate in a 6-month intervention [14]. Participants were excluded if they had a history of CVD, elevated blood pressure (>160/90 mmHg), high fasting blood glucose (>126 mg/dL), chronic medications (except oral contraceptives), smoking, regular exercise (more than twice a week), abnormal thyroid function or abnormal ECG. The study was approved by the Pennington Center Institutional Review Board and the CALERIE Data Safety Monitoring Board. All subjects provided written informed consent.

2.2. Study design

Participants were randomized into one of four groups for 24 weeks: Control = healthy weight maintenance diet, CR = 25% caloric restriction from baseline energy requirements, CR + EX = 12.5% caloric restriction and 12.5% increase in energy expenditure through structured aerobic exercise and LCD = low calorie diet (890 kcal/day) to rapidly achieve 15% weight loss. Because of the different rate and extent of weight loss (rapid over 3 months) and different macronutrient composition, we did not include the LCD group in this analysis. Study outcomes were assessed during a 5-day inpatient stay at baseline and during weeks 12 (M3) and 24 (M6) of intervention.

2.3. Energy prescription

Individual values used to prescribe the daily energy content during the intervention were calculated at baseline from total daily energy expenditure assessed during two 14-day periods by doubly labeled water and changes in body weight during a 2-week period when participants consumed all meals prepared by our metabolic kitchen [14].

2.4. Diets and diet delivery

All diets were based on the American Heart Association guidelines; 30% fat, 15% protein and 55% carbohydrate and provided the RDA for all essential vitamins and minerals. During weeks 1–12 and 23–24 of the intervention, participants consumed only foods prepared by our metabolic kitchen. During weeks 13–22 participants self-selected a diet based on their individual calorie target. Multivitamin and mineral supplements (including calcium) were not permitted.

2.5. Exercise

Except for participants in CR + EX, other participants were not permitted to modify their physical activity pattern. The CR + EX group increased their energy expenditure by 12.5% above baseline by undergoing supervised aerobic exercise, 5 days/week. The exercise time necessary to expend the 12.5% calorie target was determined for each individual by indirect calorimetry (V-max, Sensormedics, Yorba Linda, CA) and exercise sessions were monitored by heart rate (Polar S-610, Polar Beat, Port Washington, NY) [14]. Participants self-selected their exercise intensity which ranged from 47 to 76% VO_{2max} (women: 47–70% VO_{2max} and men: 48–76% VO_{2max}). The energy expenditure target for the exercise intervention was 403 ± 63 kcal per session for women and 569 ± 118 kcal for men per session which at the self-selected exercise intensity represented 53 ± 11 and 45 ± 14 min per session for women and men, respectively.

2.6. Behavioral intervention

Commencing at baseline, participants attended weekly meetings to teach subjects how to adhere to their meal and exercise plans and to boost motivation and morale. Emphasis was placed on teaching participants the skills necessary to modify eating behavior and comply with the interventions during the out-patient phase of the study.

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2.7. Analytical methods

Serum lipids were analyzed using a Beckman-Coulter Synchron CX7 (Brea, CA). Total cholesterol (Total-C) was assayed by the cholesterol esterase/oxidase/peroxidase method, triacylglycerols (TG) by the GPO-Trinder method and HDL-cholesterol (HDL-C) by an assay from Trinity Biotech (Jamestown, NY). LDL-cholesterol (LDL-C) was calculated using the Friedwald equation. The coefficient of variation for the above assays is less than 2.0%.

Factor VII and fibrinogen were assayed on an Instrumentation Laboratory ACL 3000+ (Lexington, MA). Factor VII coagulant activity was assayed by determining the ability of test plasma to correct the clotting time of factor VII-deficiency plasma and expressed relative to a serial dilution of pooled plasma. Fibrinogen was measured following a standard protocol [16]. C-reactive protein (hsCRP) was measured by automated immunoassay with chemiluminescent detection on a DPC-2000 instrument with reagents supplied by the instrument manufacturer.

Measurements of systolic (SBP) and diastolic (DBP) blood pressure were taken twice 5 min apart in a quiet room at thermo-neutrality from the participant's right arm with a manual sphygmomanometer by a certified staff member after 10 min seated rest.

Brachial artery ultrasound images were obtained using a multi-frequency 7.5 MHz linear array transducer as previously described [17]. Images were acquired in the longitudinal plane just proximal to the olecranon process of the elbow by a single trained operator. The Doppler signal was obtained by placing the gate in the center of the vessel, and using an angle of incidence of <60°. Induction of hyperemia was accomplished by inflation of a blood pressure cuff to 300 mmHg for 5 min on the non-dominant arm. Images were analyzed using the Brachial Analyzer software package (MIA Vascular Tools, Coralville, IA) to obtain baseline artery diameter, maximum post-release change in diameter (absolute and percentage), and time to maximum post release diameter change.

2.8. Estimates of 10-year CVD risk

Ten-year CVD risk was calculated using the gender-specific equations developed by Anderson et al. [18]. These equations rely upon values for total and HDL cholesterol (expressed as their ratio), systolic blood pressure, age and gender. Because smoking, presence of diabetes, and abnormal ECG were exclusionary, these risk factors were set to zero for all participants. Relative risk estimates at months 3 and 6 were taken as the ratio of the 10-year risk at these times to the baseline 10-year risk.

2.9. Statistical analysis

SAS Version 9.12 (SAS Institute, Cary, NC) was used for data analysis. Changes from baseline at M3 and M6 were analyzed by a repeated measures approach with respect to treatment and time and treatment × time interaction, with baseline values included as covariates. A Bonferroni adjustment was used for all pair-wise comparisons to maintain an overall Type-I error rate of <5%. *S* normalizing and variance-stabilizing logarithmic transformation was applied to CRP variable.

3. Results

3.1. Study population

The baseline characteristics of the 35 men and women who completed the 6-month CALERIE trial are summarized in Table 1. As expected, the three groups were matched for weight, BMI and age. They all had normal fasting plasma glucose concentration. Furthermore, their fasting plasma insulin concentration and insulin sensitivity measured by the minimal model [15] were suggestive of normal glucose tolerance. Average baseline levels for all groups were within normal ranges for blood lipids (Total-C, HDL-C, LDL-C, TG), and blood pressure (SBP and DBP).

3.2. Body weight

Weight loss in CR and CR + EX groups continued throughout the study leading to a significantly greater reduction in body weight at M6 relative to M3 in both CR ($P < 0.01$) and CR + EX ($P < 0.001$), with no significant difference between the two groups. These differences were significant with respect to baseline and to changes in the Controls (−0.4% (NS); Table 2).

Table 1
Participant characteristics obtained during the baseline period

	Control	CR	CR + EX
Number (M/F)	5/6	6/6	5/7
Age (years)	38 ± 8	39 ± 5	36 ± 6
Race (C/AA/O)[a]	7/4/0	7/4/1	7/4/1
Weight (kg)	81.8 ± 9.3	80.9 ± 11.4	81.9 ± 10.5
BMI (kg/m^2)	27.6 ± 2.0	27.8 ± 1.4	27.5 ± 1.6
Total-C (mg/dL)	175 ± 33	177 ± 25	169 ± 33
LDL-C (mg/dL)	110 ± 32	107 ± 24	105 ± 28
HDL-C (mg/dL)	38 ± 15	41 ± 9	44 ± 8
TG (mg/dL)	134 ± 65	146 ± 113	98 ± 66
SBP (mmHg)	113 ± 12	111 ± 7	111 ± 10
DBP (mmHg)	74 ± 10	72 ± 8	72 ± 8
Factor VIIc (%)	110 ± 5	111 ± 5	106 ± 5
Fibrinogen (mg/dL)	405 ± 19	369 ± 29	354 ± 17
hsCRP (mg/dL)	0.30 ± 0.08	0.25 ± 0.07	0.15 ± 0.03
Homocysteine (μmol/L)	6.9 ± 0.5	7.5 ± 0.6	6.8 ± 0.4
Flow-mediated dilation (%)	9.8 ± 1.9	11.6 ± 2.1	11.7 ± 2.1

Data are mean ± S.D.
[a] Abbreviations for participants' race: Caucasian (C), African American (AA), Asian or Latino (O).

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Table 2
Effect of caloric restriction alone or in combination with exercise on change in weight, body composition, and risk factors for CVD

	Month 3			Month 6		
	Control	CR	CR + EX	Control	CR	CR + EX
Δ Weight, kg (% Δ)	−0.3 ± 0.7 (−0.4 ± 0.9)	−5.8 ± 0.4^{c,†} (−7.2 ± 0.4)	−4.6 ± 0.4^{c,*} (−5.6 ± 0.5)	−0.4 ± 0.9 (−0.5 ± 1.2)	−8.2 ± 0.8^{c,†,y} (−10.2 ± 0.9)	−8.1 ± 0.8^{c,†,z} (−9.9 ± 0.9)
Δ SBP, mmHg (% Δ)	0.5 ± 1.8 (0.6 ± 1.6)	1.2 ± 2.2 (1.3 ± 2.0)	−2.3 ± 2.2 (−1.9 ± 2.0)	1.70 ± 2.0 (1.5 ± 1.6)	−2.7 ± 1.7 (−2.3 ± 1.5)	−1.7 ± 2.3 (−1.1 ± 2.1)
Δ DBP, mmHg (% Δ)	−0.9 ± 1.2 (−1.2 ± 1.7)	0.3 ± 1.9 (0.8 ± 2.6)	−1.7 ± 2.3 (−1.4 ± 3.6)	−0.7 ± 1.2 (−0.9 ± 1.7)	−2.1 ± 1.7 (−2.4 ± 2.4)	−4.0 ± 2.1^{a} (−4.7 ± 2.8)
Δ Factor VIIc, % (% Δ)	2.2 ± 4.0 (2.1 ± 3.6)	−7.5 ± 2.2^{a} (−6.7 ± 2.0)	−6.5 ± 1.7 (−6.3 ± 1.6)	7.9 ± 2.3^{a} (7.1 ± 2.2)	−10.7 ± 2.3^{b,*} (−9.8 ± 2.4)	−3.3 ± 5.8 (−2.4 ± 5.9)
Δ Fibrinogen, mg/dL (% Δ)	−18 ± 24 (−2.4 ± 5.7)	−4 ± 27 (9.6 ± 15.7)	−13 ± 17 −2.8 ± 4.7)	−50 ± 22 (−12.1 ± 5.6)	−6 ± 27 (8.4 ± 15.4)	−2 ± 14 (−0.3 ± 4.2)
Δ ln(hsCRP), ln(mg/dL) (% Δ)$^{\#}$	−0.13 ± 0.20 (12 ± 31)	−0.43 ± 0.10^{b} (−31 ± 7)	−0.36 ± 0.14^{a} (−24 ± 9)	−0.39 ± 0.12^{a} (−27 ± 8)	−0.14 ± 0.22 (29 ± 53)	−0.48 ± 0.17^{b} (−27 ± 14)
Δ Homocysteine, μmol/L (% Δ)	−0.03 ± 0.18 (−1.1 ± 2.5)	0.02 ± 0.34 (1.8 ± 3.9)	0.07 ± 0.28 (1.5 ± 4.4)	0.05 ± 0.22 (2.2 ± 3.5)	−0.04 ± 0.23 (0.0 ± 2.7)	−0.07 ± 0.32 (1.0 ± 4.5)

Data are mean ± S.E.M. Percent change from baseline are shown in parenthesis; Control, $n = 11$; CR, $n = 12$, CR + EX, $n = 12$. Statistically significant from baseline: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$. Statistically significant from Control: $^{*}P < 0.01$; $^{\dagger}P < 0.0001$. Statistically significant from month 3: $^{y}P < 0.01$; $^{z}P < 0.001$.
$^{\#}$ Percent change calculated from non-transformed data.

3.3. Blood lipids

Significant reductions from baseline in LDL-C were observed in CR + EX at both M3 and M6 ($P < 0.001$). LDL-C was not significantly changed in either the Control or CR groups (Fig. 1). At M6, the reduction in LDL-C in CR + EX was significantly different compared to Controls, but not to CR. HDL-C was unchanged at M3, but was significantly increased at M6 in all groups. There were no significant differences in HDL-C between the groups at any time point. TG concentrations increased ($P < 0.05$) in the Control group but decreased at M3 ($P < 0.05$) and M6 ($P < 0.001$) in CR and CR + EX groups. At both time points, changes in TG levels in CR and CR + EX were significantly different ($P < 0.05$ to

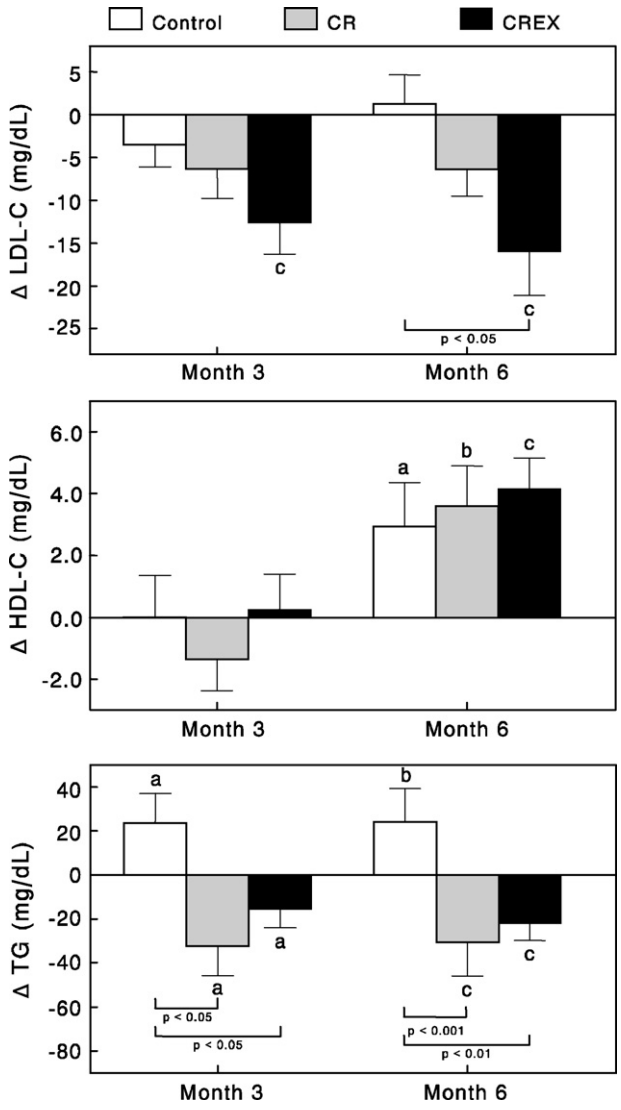

Fig. 1. Effects of caloric restriction alone or in combination with exercise on serum lipid levels. Data are changes from baseline for (Control group: $n = 11$; CR group: $n = 12$; and CR + EX group: $n = 12$) at months 3 and 6. Error bars are S.E.M. Statistically significant from baseline: a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$.

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$P < 0.001$) when compared to the Control group, but not when compared to each other.

3.4. Blood pressure

At M6, DBP was significantly reduced relative to baseline in CR + EX ($P < 0.05$; Table 2). However, there was no effect of either intervention on SBP.

3.5. Hemostasis factors, homocysteine and markers of inflammation

Factor VIIc was reduced in CR at M3 ($P < 0.05$) and M6 ($P < 0.01$), while it remained unchanged in CR + EX (Table 2). In contrast, factor VIIc in the Control group was significantly elevated from baseline at M6 ($P < 0.05$). The factor VIIc reduction in CR was significantly ($P < 0.01$) different from the Control group at M6. Fibrinogen and homocysteine concentrations were not changed in the Control or the intervention groups over the course of the study. hsCRP levels were significantly reduced in CR + EX at both time points and in CR at M3 only ($P < 0.01$). hsCRP was reduced in the Control group at M6 only ($P < 0.05$). However, there were no differences in the change in hsCRP at M3 or M6 between the two interventions and the Control groups.

3.6. Brachial artery flow-mediated dilation

Brachial artery flow-mediated dilation (BA-FMD), a marker of endothelial function, was not significantly changed relative to baseline in any group across time (Fig. 2). Furthermore, there were no significant effects of either intervention group on endothelial function.

3.7. Estimated 10-year CVD risk

Estimated 10-year CVD risk at baseline was low in all groups (Control, 3.4 ± 1.3%; CR, 2.5 ± 0.6%; CR + EX, 1.5 ± 0.6%) due primarily to the relatively young age and good health of the study population. Caloric restriction had a substantial effect on estimated CVD risk (Fig. 3). Expressed as estimated risk relative to baseline values, relative risk levels at M6 were significantly reduced in CR and CR + EX ($P < 0.001$). The reduction in relative risk was achieved more quickly in the CR + EX group in that it was already significantly reduced at M3 and tended to be larger at M6 compared to the CR group. Relative risk in the Control group was unchanged.

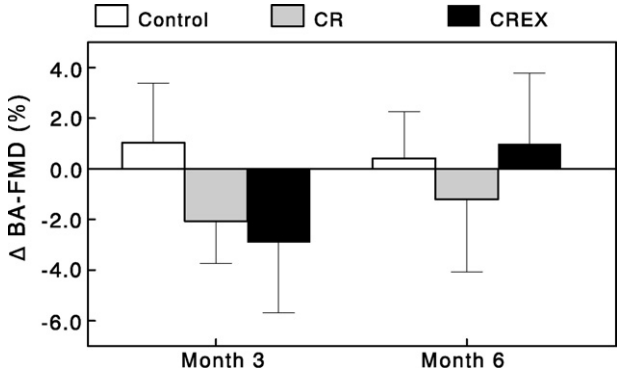

Fig. 2. Effects of caloric restriction alone or in combination with exercise on brachial artery flow-mediated dilation (BA-FMD). Data are change from baseline for the Control group ($n = 11$), CR group ($n = 12$) and CR + EX group ($n = 12$) at months 3 and 6. Error bars are S.E.M.

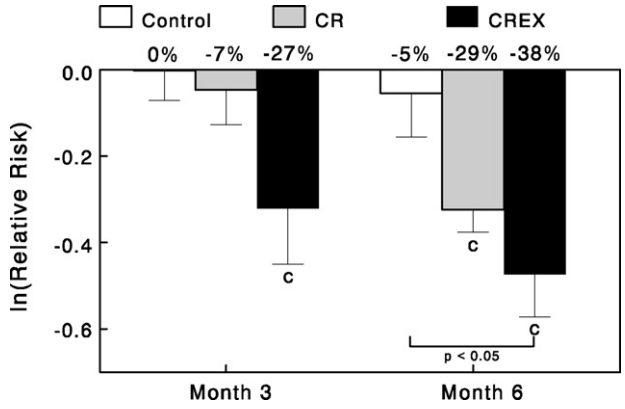

Fig. 3. Effects of caloric restriction alone or in combination with exercise on estimated 10-year CVD risk relative to baseline. Data are the log of the ratio of months 3 and 6 over baseline values for 10-year estimated CVD risk for the Control group ($n = 11$), CR group ($n = 12$) and CR + EX group ($n = 12$) at months 3 and 6. Error bars are S.E.M. Numbers at the top of each bar are estimated percent reduction in risk. Statistically significant from baseline: c, $P < 0.001$.

4. Discussion

This randomized clinical trial examined the short-term benefits of 25% caloric restriction with and without exercise on established and emerging risk factors for CVD. We observed significant reductions in triacylglycerol (TG) in both the CR and CR + EX groups. Additionally, LDL-C, blood pressure and hsCRP were reduced in CR + EX group while factor VIIc level was reduced only with CR. Fibrinogen, homocysteine and endothelial function were not changed by either intervention, despite 10% reductions in body weight in both groups. Estimated 10-year CVD risk was significantly reduced by the interventions, with a tendency towards a greater reduction in the group assigned to caloric restriction and structured exercise.

Weight loss reduces LDL-C and TG whereas active weight loss reduces HDL-C even if HDL-C is increased once weight loss is stabilized. Based on prediction equations [19] and the degree of weight loss achieved in our study at 6 months, we would expect the CR group to experience a 5.8 mg/dL reduction in LDL-C, a 10.3 mg/dL reduction in TG, and a 2.1 mg/dL reduction in HDL-C if in active weight loss (or a 2.7 mg/dL increase at weight stability). For CR, the change in LDL-C (−4.1 mg/dL) was close to that predicted while HDL-C was increased (+1.2 mg/dL). The decrease in TG

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(−31 mg/dL) was however substantially greater than predicted. Weight loss and changes in HDL-C and TG were similar in the CR + EX group versus the CR group. However, the reduction in LDL-C in the CR + EX group (−17 mg/dL versus Control) was four-times greater than that observed in the CR group and much greater than predicted by weight loss alone. Such a large effect of exercise on LDL-C has not been typically observed [20,21] and may in part reflect the carefully controlled exercise protocol.

Both atherosclerosis and CVD are recognized as inflammatory diseases [22] and consistently elevated levels of hemostatic factors (factor VIIc, fibrinogen), homocysteine and C-reactive protein (hsCRP) are associated with increased risk for CVD and cardiac events [23–25]. In our study, factor VIIc levels were significantly reduced in the CR group, but not the CR + EX group. This contrasts with an increase in factor VIIc levels in the Control group at M6 providing a significant treatment effect. Changes in factor VIIc levels are known to correlate with changes in TG [26] which may in part explain the observed changes in the Control and CR groups. Homocysteine levels were not affected by the interventions confirming other data [27]. hsCRP and fibrinogen are both acute phase reactants and markers of inflammation [25]. There was a trend for both interventions to lower hsCRP. However, the responses were inconsistent with a large degree of variability and the data suggest that the observed favorable change in inflammatory markers was a function of an improvement in diet quality, rather than an effect of CR or CR + EX.

Endothelial dysfunction, an early event in CVD, can be assessed by measures of flow-mediated endothelium-dependent vasodilation of the brachial artery (BA-FMD). Impaired BA-FMD predicts coronary endothelial dysfunction and is influenced by plasma lipoprotein levels [28], Type 2 diabetes [29], insulin resistance [30] and hypertension [31]. Thus, many of the risk factors targeted by caloric restriction could commonly impact endothelial function. However, despite generally favorable changes in many of the CVD risk factors and insulin sensitivity [15], we did not observe an improvement in BA-FMD in either intervention group. Previous studies of the effects of caloric restriction and weight loss on BA-FMD are controversial with some studies showing improved endothelial function [32] while others have not [27,33] and may be reflective of health status of the studied population. It is conceivable that an effect of the interventions could be identified with a longer intervention period and a larger sample size.

While the changes in individual risk factors were generally modest, when combined in a single prediction equation for CVD risk [18], the overall effect of caloric restriction was impressive. A 32% reduction in 10-year CVD risk was predicted based upon the combined changes in total cholesterol, HDL-C and systolic blood pressure. All participants in the CR and CR + EX groups experienced some degree of CVD risk reduction relative to baseline, while only 5 of 11 participants in the Control groups experienced such reductions. Furthermore, although not statistically significant, the addition of exercise appeared to be associated with a more favorable CVD risk profile than caloric restriction alone. By study design, we did not examine the effects of energy deficit by exercise alone, but a recent study published by Fontana et al. observed a similar reduction in CVD risk between by exercise alone as compared to CR alone [34]. Several caveats must be considered when interpreting these changes. First, our population was relatively young and included four participants under the age of 30 and therefore outside of the age range (30–74) used to generate the risk equations. Furthermore, the risk equations take into consideration only changes in serum lipids and blood pressure. Newer equations, currently available for women only [35], also consider the effects of hsCRP on CVD risks. However, when applied to women only, the equation still predicted a 25–30% reduction in 10-year CVD risk in the CR and CR + EX groups while it was essentially unchanged in the Control group (+2%).

The lack of clear independent effect of training on CVD risk factors was of surprise to us. Yates et al. [36] systematically reviewed all the controlled trials to determine the independent effect of exercise on glucose levels and risk of type 2 diabetes in people with prediabetes (IGT and/or IFG). They concluded that the contribution of physical activity independent of dietary or weight loss changes to the prevention of type 2 diabetes in people with prediabetes was equivocal. However, studies such as the Diabetes Prevention Program clearly identified that intensive lifestyle changes including increased physical activity reduced the incidence of diabetes in persons at high risk [13]. Finally in a prospective epidemiology study, low physical fitness was found to be a strong and independent predictor of CVD and all-cause mortality [37]. However, in a randomized clinical trial of graded dose of exercise training in previously sedentary, overweight or obese postmenopausal women with elevated blood pressure, Church et al. did not find exercise training to reduce blood pressure despite observing a graded increase in fitness across doses of exercise [38]. Taken together, while exercise is clearly protective against premature mortality and morbidity the mechanisms responsible for this are unclear with many studies observing common CVD risk factors not improving in response to exercise.

The design of this study was unique from several perspectives. First, care was extended in establishing baseline energy requirements and tailoring the subsequent intervention to achieve a 25% deficit in energy intake in each participant. Second, all estimates of CVD risk factors were obtained while participants were consuming diets of identical macronutrient composition, albeit at different absolute amounts. Importantly this approach allows us to isolate the effects of changes in caloric intake independent of changes in diet composition, a variable known to strongly influence several CVD risk factors. Finally, the interventions were carefully delivered and monitored. As a result, both intervention groups (CR and CR + EX) had gradual and almost identical reductions in body weight throughout the course of the

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study. However, there are some limitations that should be noted when interpreting these results. First, our study sample size was relatively small limiting our power to detect between group differences. Second, our study was only 6 months in length. Caloric restriction is viewed as a lifestyle change extending over years. However, the data are generally consistent with the benefits observed with longer caloric restriction in self-selected individuals engaging in CR [39].

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