2 years of calorie restriction and cardiometabolic risk (CALERIE): exploratory outcomes of a multicentre, phase 2, randomised controlled trial

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Summary

Background For several cardiometabolic risk factors, values considered within normal range are associated with an increased risk of cardiovascular morbidity and mortality. We aimed to investigate the short-term and long-term effects of calorie restriction with adequate nutrition on these risk factors in healthy, lean, or slightly overweight young and middle-aged individuals.

Methods CALERIE was a phase 2, multicentre, randomised controlled trial in young and middle-aged (21–50 years), healthy non-obese (BMI 22·0–27·9 kg/m²) men and women done in three clinical centres in the USA. Participants were randomly assigned (2:1) to a 25% calorie restriction diet or an ad libitum control diet. Exploratory cardiometabolic risk factor responses to a prescribed 25% calorie restriction diet for 2 years were evaluated (systolic, diastolic, and mean blood pressure; plasma lipids; high-sensitivity C-reactive protein; metabolic syndrome score; and glucose homoeostasis measures of fasting insulin, glucose, insulin resistance, and 2-h glucose, area-under-the curve for glucose, and insulin from an oral glucose tolerance test) analysed in the intention-to-treat population. This study is registered with ClinicalTrials.gov, number NCT00427193.

Findings From May 8, 2007, to Feb 26, 2010, of 238 participants that were assessed, 218 were randomly assigned to and started a 25% calorie restriction diet (n=143, 66%) or an ad libitum control diet (n=75, 34%). Individuals in the calorie restriction group achieved a mean reduction in calorie intake of 11·9% (SE 0·7; from 2467 kcal to 2170 kcal) versus 0·8% (1·0) in the control group, and a sustained mean weight reduction of 7·5 kg (SE 0·4) versus an increase of 0·1 kg (0·5) in the control group, of which 71% (mean change in fat mass 5·3 kg [SE 0·3] divided by mean change in weight 7·5 kg [0·4]) was fat mass loss. Calorie restriction caused a persistent and significant reduction from baseline to 2 years of all measured conventional cardiometabolic risk factors, including change scores for LDL-cholesterol (p<0·0001), total cholesterol to HDL-cholesterol ratio (p<0·0001), and systolic (p<0·0011) and diastolic (p<0·0001) blood pressure. In addition, calorie restriction resulted in a significant improvement at 2 years in C-reactive protein (p=0·012), insulin sensitivity index (p<0·0001), and metabolic syndrome score (p<0·0001) relative to control. A sensitivity analysis revealed the responses to be robust after controlling for relative weight loss changes.

Interpretation 2 years of moderate calorie restriction significantly reduced multiple cardiometabolic risk factors in young, non-obese adults. These findings suggest the potential for a substantial advantage for cardiovascular health of practicing moderate calorie restriction in young and middle-aged healthy individuals, and they offer promise for pronounced long-term population health benefits.

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Introduction

Cardiovascular disease is the leading cause of morbidity, disability, and death worldwide. Approximately 2200 people die each day in the USA due to cardiovascular disease—an average of one death every 40 s.[1] In rhesus monkeys, early-onset calorie restriction reduces the risk of developing and dying of cardiovascular disease by at least 50%.[2–4] In observational human studies, severe calorie restriction provides a powerful protective effect against multiple atherosclerotic risk factors.[5] These effects include less carotid artery intima-media thickening, improved left ventricular diastolic function, and increased beneficial heart rate variability than matched controls not practicing calorie restriction.[5–7] However, little is known about the extent of cardiometabolic adaptations in response to calorie restriction of longer than 1 year in healthy adults aged younger than 50 years of normal weight or who are slightly overweight. Crucially, data are needed on the effects of calorie restriction in early life on cardiometabolic health, corresponding to studies in animal models. Addressing this research gap represents an important novel aspect of this study.

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Research in context

Evidence before this study

A thorough literature search was not done by the study team before commencing this study; we instead relied on the collective experience and expertise of the study team who were not aware of any pertinent human data previously published in this area. In observational cross-sectional human studies, severe calorie restriction provides a powerful protective effect against multiple atherosclerotic risk factors: older adults practicing calorie restriction have healthier physiological cardiovascular parameters. Although we have not done a thorough literature search, the collective experience and expertise of the study team indicates that little is known about the extent of cardiometabolic adaptations in prospective human studies in response to calorie restriction of more than 1 year in healthy, normal weight or slightly overweight individuals aged younger than 50 years. In phase 1 CALERIE, the three clinical sites tested various calorie restriction interventions in relatively underpowered studies to develop preliminary data informing the phase 2 study reported here.

Added value of this study

This trial is the first study of medium term (2 years) of true calorie restriction in humans—and its effect on intermediary cardiometabolic outcomes: blood pressure, plasma lipids, C-reactive protein, metabolic syndrome, and glucose homoeostasis measures.

Implications of all the available evidence

The effects of 2 years of 13% calorie restriction on a myriad of cardiometabolic risk factors—in particular those composing the five components of metabolic syndrome, as well as glucose tolerance using an oral glucose tolerance test and derivatives thereof—raise the possibility of being able to modify the ravages of cardiovascular disease, diabetes, and obesity in high-income countries with relatively modest lifestyle interventions.

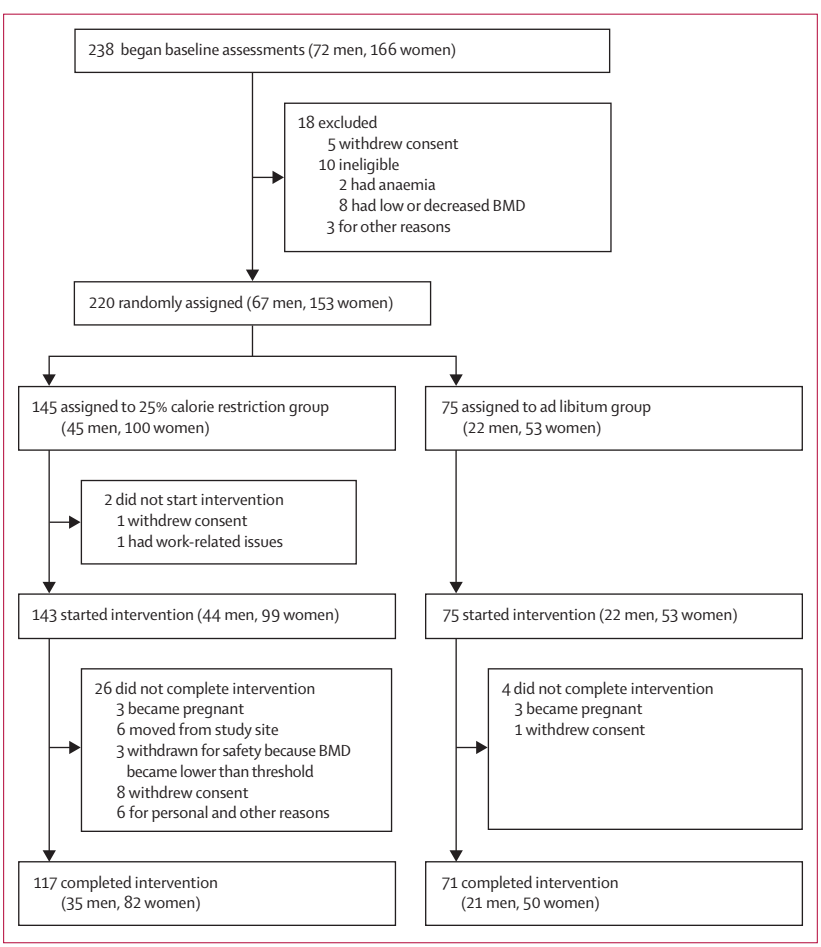

Figure 1: **Trial profile**
*Two individuals, both assigned to the calorie restriction group, dropped out before starting the intervention, resulting in an intention-to-treat cohort of 218 participants. BMD=bone mineral density.

From risk factor values well below the conventional clinical disease thresholds, cardiovascular incidence and mortality risk increase continuously for all conventional cardiometabolic risk factors,[8–13] and individuals with optimal cardiometabolic health have a substantially lower lifetime risk of developing cardiovascular disease than those with higher values.[14]

The 2-year, multicentre, randomised controlled Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) trial was designed to evaluate the potential for calorie restriction to promote anti-ageing adaptations in resting metabolic rate and body core temperature; findings for these outcomes have been previously reported.[15] In the primary analysis, baseline resting metabolic rate adjusted for weight change decreased significantly more in the calorie restriction group than the ad libitum group at 12 months, but not 24 months. Core temperature change differed little between groups. Triiodothyronine decreased more in the calorie restriction group at 12 months and 24 months, without adverse effects on quality of life. Here, our objective was to evaluate—in all individuals completing the 2-year CALERIE protocol—the short-term and long-term effects of random assignment to a 25% calorie restriction regimen on the exploratory endpoints of multiple cardiometabolic risk factors implicated in the development and progression of atherosclerotic cardiovascular disease in a healthy, young and middle-aged, non-obese population of men and women.

Methods

Study design and participants

CALERIE was a multicentre, phase 2, randomised controlled trial done at three clinical centres in the USA, aimed at evaluating the time-course effects of 25% calorie

restriction below the individual's baseline level over a 2-year period in healthy, normal weight, and slightly overweight (BMI 22·0–27·9 kg/m²) men (aged 21–50 years) and women who were premenopausal (aged 21–47 years).[16] The study protocol (NCT00427193) was approved by institutional review boards at three clinical centres (Washington University Medical School, St Louis, MO, USA; Pennington Biomedical Research Center, Baton Rouge, LA, USA; Tufts University, Boston, MA, USA) and the coordinating centre at Duke University (Durham, NC, USA). Study volunteers provided written informed consent. Study oversight was provided by a data and safety monitoring board. The CONSORT diagram for enrolled participants is shown in figure 1.

Randomisation and masking

After baseline testing, participants were randomly assigned at a ratio of 2:1 to a calorie restriction behavioural intervention designed to achieve a predicted longitudinal weight loss trajectory estimated from previous data for 25% calorie restriction in a phase 1 pilot study, or to an ad libitum control group; randomisation was stratified by site, sex, and BMI.[15] A permuted block randomisation technique was used. All study personnel doing assessments were masked to treatment assignment.

	Ad libitum group (n=75)	Calorie restriction group (n=143)
Ethnicity		
White	57 (76%)	111 (78%)
African American	11 (15%)	15 (11%)
Other*	7 (9%)	17 (12%)
Sex		
Women	53 (71%)	99 (69%)
Men	22 (29%)	44 (31%)
Age, years	37·9 (6·94)	38·0 (7·34)
Height, m	168·4 (8·31)	168·9 (8·60)
Baseline weight, kg	71·5 (8·65)	72·0 (9·49)
Baseline BMI, kg/m²	25·1 (1·64)	25·2 (1·78)
Body fat, %	33·6% (6·57)	32·9% (6·07)
Fat free mass, kg	47·6 (8·61)	48·5 (9·21)
Energy and macronutrient intake		
Energy intake, kcal per day	2390 (384·8)	2467 (405·6)
Protein, g/kg per day	1·2 (0·04)	1·2 (0·02)
Protein, % of energy	17·2 (3·48)	16·6 (3·04)
Fat, % of energy	34·7 (5·12)	33·5 (4·93)
Carbohydrates, % of energy	45·1 (6·33)	46·8 (6·48)

Data are n (%) or mean (SD). *Other includes Asian, Pacific Islander, and Native Americans.

Table 1: **Baseline characteristics**

Procedures

Extensive details of the intervention are provided in previous publications.[15–17] Briefly, participants in the calorie restriction group were prescribed a 25% restriction in calorie intake based on energy requirements estimated from doubly labelled water (individuals ingest $^{2}H_{2}{}^{18}O$ then energy expenditure is calculated as the difference in urinary excretion of ^{2}H and ^{18}O) measurements over a 4-week period at baseline. Participants were fed three meals per day each day in-house, at the three clinical centres for 1 month, during which they were instructed on the essentials of calorie restriction. They chose from one or more of six eating plans modified to suit various cultural preferences. One in-house meal was provided with intensive group and individual behavioural counselling sessions once a week. There were 24 group and individual counselling sessions over the first 24 weeks of the intervention (table 1); a detailed algorithmic strategy was applied to monitor and respond to changes in adherence (Clinical Tracking System) done by a behavioural interventionist. Adherence to the calorie restriction intervention was determined in real time by the degree to which individual weight change adhered to an individualised weight loss trajectory (15·5% weight loss at 1 year followed by weight loss maintenance). Additionally, the precise level of calorie restriction was retrospectively quantified by calculating the total daily energy expenditure by doubly labelled water and adjusting total daily energy expenditure for changes in body composition.[18] Participants assigned to the control group continued on their regular diets; they received no specific dietary intervention or counselling. They had quarterly contact with study investigators.

Outcomes

Pre-specified exploratory cardiometabolic outcomes were systolic, diastolic, and mean blood pressure; plasma lipids; high-sensitivity C-reactive protein; metabolic syndrome score; and glucose homoeostasis measures of fasting insulin, glucose, insulin resistance, and 2-h glucose, area-under-the curve for glucose, and insulin from an oral glucose tolerance test. With the participant wearing a hospital gown and no shoes, bodyweight was measured in duplicate in the morning following a 12-hour fast. Height was measured without shoes to the nearest 0·005 m (0·5 cm) and BMI calculated. Fat mass and fat-free mass was measured by dual-energy x-ray absorptiometry (DXA; Hologic 4500A, Delphi W or Discovery A scanners; Marlborough, MA, USA) with all scans analysed at University of California San Francisco (San Francisco, CA, USA). Scanner performance was monitored with baseline and longitudinal phantom cross calibrations.

Dietary intakes were established using 6-day food diaries analysed with Nutrition Data System for Research (Minneapolis, MN, USA) by a central reading centre at the University of Cincinnati (Cincinnati, OH, USA).

Venous blood was sampled for lipid and hormone concentrations after an overnight fast. Samples were collected in edetic acid plasma tubes, immediately

centrifuged to separate the plasma, aliquoted, and stored in a –80°C freezer until use. Serum lipid and lipoprotein-cholesterol concentrations were established in the Laboratory for Clinical Biochemistry at the University of Vermont (Burlington, VT, USA). Cholesterol and glycerol-blanked triglycerides were measured with automated enzymatic commercial kits (Miles-Technicon, Tarrytown, NY, USA). HDL cholesterol was measured in plasma after precipitation of apolipoprotein B-containing lipoproteins by dextran sulfate (50000 MW) and magnesium. LDL cholesterol was calculated using the Friedewald Equation.[19] High sensitivity C-reactive protein (hs-CRP) was measured using a high sensitivity ELISA kit (ALPCO Diagnostics Ltd, Windham, NH, USA).

Blood pressure was measured with an oscillometric blood pressure monitor (Dinamap Procare 200, GE Healthcare, Waukesha, WI, USA) in the morning after a 12-h fast in a seated position. Blood pressure was measured according to a common procedure; the measurement process was regularly monitored to assure protocol adherence. Three sequential measurements were obtained at each sitting. If one differed by the other two by 15 mm Hg or more, it was discarded. Otherwise the three measures were averaged.

	Ad libitum group (n=75)	Calorie restriction group (n=143)	Between-group p value
Bodyweight, kg			
Baseline	71·5 (1·0)	72·0 (0·8)	0·98
Change at month 12	–0·7 (0·4)*	–8·4 (0·3)†	<0·0001
Change at month 24	0·1 (0·5)	–7·5 (0·4)†	<0·0001
BMI (kg/m²)			
Baseline	25·1 (0·2)	25·2 (0·2)	0·94
Change at month 12	–0·2 (0·1)	–2·9 (0·1)†	<0·0001
Change at month 24	0·1 (0·2)	–2·6 (0·1)†	<0·0001
Percentage body fat, %			
Baseline	33·6% (0·8)	32·9% (0·5)	0·34
Change at month 12	–0·47 (0·3)	–5·5 (0·2)†	<0·0001
Change at month 24	0·13 (0·3)	–4·6 (0·3)†	<0·0001
Fat mass, kg			
Baseline	23·8 (0·6)	23·5 (0·4)	0·61
Change at month 12	–0·34 (0·3)	–6·1 (0·2)†	<0·0001
Change at month 24	0·38 (0·4)	–5·3 (0·3)†	<0·0001
Fat-free mass, kg			
Baseline	47·6 (1·0)	48·5 (0·8)	0·48
Change at month 12	–0·3 (0·2)	–2·2 (0·1)†	<0·0001
Change at month 24	–0·2 (0·2)	–2·2 (0·2)†	<0·0001

Baseline values are the observed mean (SE); change scores are the least-squares adjusted means (SE) from the intention-to-treat repeated measures analysis. Between-group p value tests for a significant between-group difference in the change score at the timepoint. All p values reflect Bonferroni corrections, truncated at 1·0, as appropriate (see text). *p<0·05 compared with baseline. †p<0·001 compared with baseline.

Table 2: **Body composition changes before and after intervention**

2-h, 0·075-kg oral glucose tolerance tests were done at baseline and at 12 and 24 months with blood samples collected at baseline, and 30, 60, 90, and 120 min after glucose consumption. Adequate carbohydrate intake over the previous 3 days (≥150 g per day) was ensured by prescription and subsequent interview with a study dietician. Plasma glucose was measured by the glucose oxidase method (YSI Instruments, Fullerton, CA, USA); insulin was measured by chemiluminescent immunoassay (Elecsys, Roche Diagnostics, Indianapolis, IN, USA).

Insulin resistance was calculated using homoeostasis model assessment (fasting glucose [mmol/L] × fasting insulin [mIU/L]/22·5).[20] β-cell function was calculated using homoeostasis model assessment-β; (%=[360×fasting insulin (mIU/L)]/[fasting glucose (mg/dL)–63]).[20] Area under the curve insulin and area under the curve glucose values from the oral glucose tolerance test were determined using the trapezoidal method.[21]

Insulin response was calculated as the ratio of change in plasma insulin from baseline to 30 min to the change in plasma glucose over the same period. Insulin sensitivity index was calculated as 1 divided by fasting insulin concentration (mIU/L).[22,23] The Oral Disposition Index was calculated as the product of insulin response and insulin sensitivity.[24,25]

Sex-specific metabolic syndrome scores were developed for each participant at baseline, 12 months, and 24 months using the method outlined referenced to the SD (SD_{VALUE}) of the entire CALERIE trial population at baseline.[26,27] Metabolic syndrome score was developed using mean blood pressure ([2×diastolic blood pressure+systolic blood pressure]/3), HDL cholesterol, triglycerides, waist circumference, and fasting blood glucose. For women, the metabolic syndrome score was:

$$[45–HDL]/SD_{HDLW} + [TG–150]/SD_{TG} + [WC–88]/SD_{WCW} + [FBG–100]/SD_{FBG} + [\text{mean BP}–100]/SD_{MBP}$$

in which HDLW, HDLM, WCW, and WCM refer to HDL and waist circumference in women and men, respectively; TG to triglycerides, FBG to fasting blood glucose, and BP to blood pressure. For men, the metabolic syndrome score was:

$$[40–HDL]/SD_{HDLM} + [TG–150]/SD_{TG} + [WC\text{-}102]/SD_{WCM} + [FBG\text{-}100]/SD_{FBG} + [\text{mean BP}–100]/SD_{MBP}$$

Statistical analysis

The same statistical methods used in the primary analysis of the CALERIE trial were applied.[18,28] Power calculations were done with respect to the primary study outcome variables, resting metabolic rate, and core temperature. Assuming 225 participants enrolled, with 10% dropout per year, 180 participants were expected to

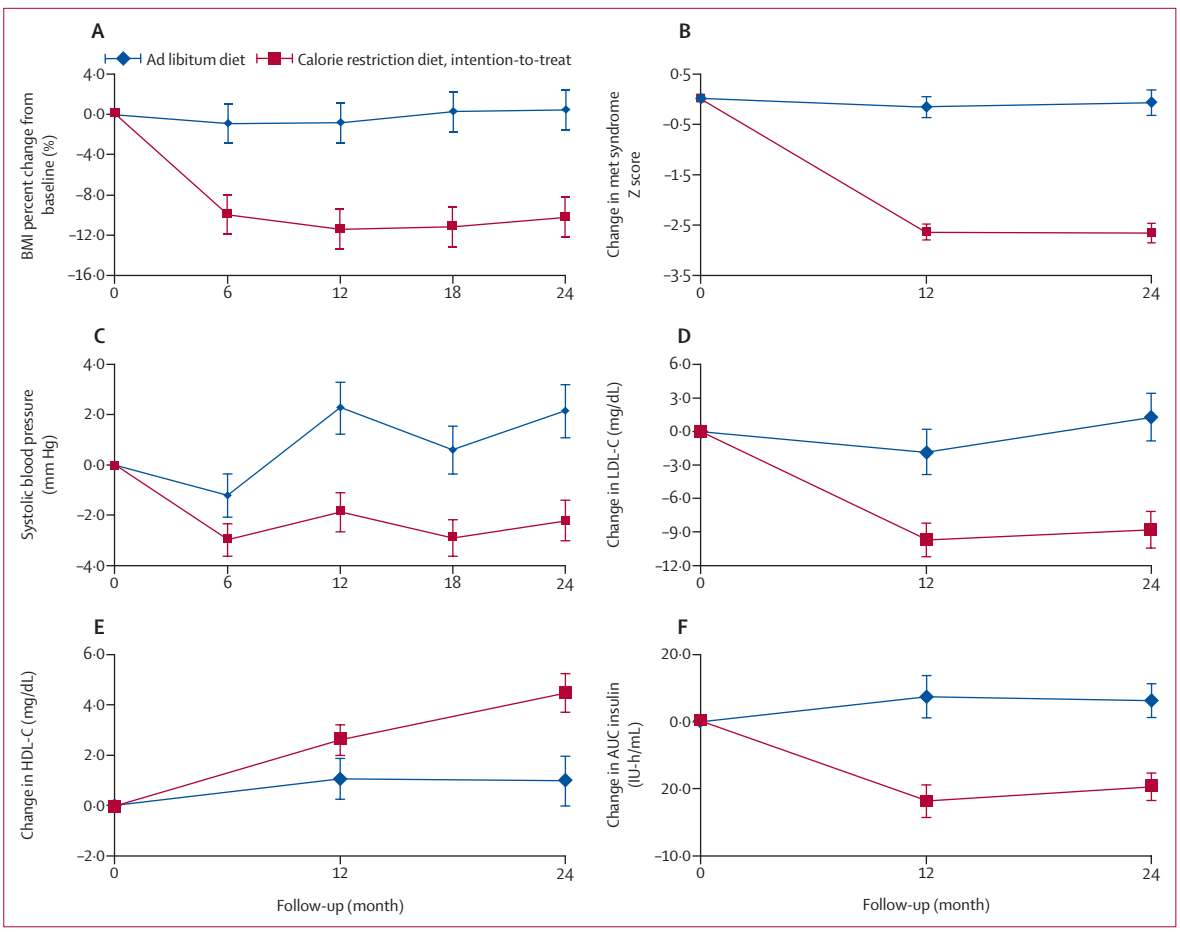

Figure 2: **Changes in cardiometabolic parameters**
The within group percent change values (mean, SE) by intervention group are shown for the cardiometabolic parameters of BMI (A), metabolic syndrome score (B), blood pressure (C), LDL-cholesterol (D), HDL-cholesterol (E), and area under the curve insulin (F).

complete the study, at 2:1 treatment allocation. On the basis of between-group differences and SDs informed by the pilot study and expert opinion, the study had more than 90% power for each of the primary outcomes. Intention-to-treat analyses were done by including all available observations in the analysis. Wilcoxon and Fisher exact tests were used to evaluate between-group differences with respect to baseline characteristics. Repeated measures ANCOVA, as implemented under mixed models,[29] was applied with change from baseline as the dependent variable, and treatment, time, and the treatment multiplied by time interaction as independent variables. The approximate normality of each outcome and of the change score of the outcome were confirmed by examination. Site, sex, BMI stratum (normal weight [22·0–24·9 kg/m²] and overweight [25·0–27·9 kg/m²]), and the baseline value were included as covariates to ensure statistical balance not captured by randomisation, and more importantly, to reduce error variance.[30] To avoid arbitrary modelling assumptions with respect to linearity, time was treated as a categorical variable; similarly, an unstructured model was applied for the covariance matrix among the repeated observations. Hypotheses of specific interest—eg, between-group differences at the individual timepoints and within-group changes over time—were tested by defining contrasts among the regression parameters; predicted mean change (SE) are the adjusted values from this model. For any outcome, type-I error was controlled using a hierarchical gatekeeping strategy.[30] The treatment-by-visit interaction term was tested first. If significant, between-group differences at each timepoint were tested at α=0·05. If not significant, the treatment main effect was tested next. Otherwise, Bonferroni correction was applied at each timepoint, with p values adjusted by multiplying the nominal p value by the number of tests (truncated at 1·0).[18]

To address the robustness of the intention-to-treat analysis (specifically with respect to the issue of non-random or missing data due to differential drop out and adherence to intervention groups) we did marginal structural modeling as previously described.[31] This

	Ad libitum group (n=75)	Calorie restriction group (n=143)	Between-group p value
Total cholesterol (mmol/L)			
Baseline	4·52 (0·10)	4·30 (0·06)	0·10
Change at month 12	−0·04 (0·06)	−0·32 (0·04)*	0·0001
Change at month 24	0·03 (0·07)	−0·25 (0·05)	0·0010
LDL-cholesterol (mmol/L)			
Baseline	2·71 (0·09)	2·51 (0·06)	0·067
Change at month 12	−0·05 (0·05)	−0·25 (0·04)*	0·0015
Change at month 24	0·03 (0·05)	−0·23 (0·04)*	0·0001
HDL-cholesterol (mmol/L)			
Baseline	1·26 (0·03)	1·26 (0·03)	0·72
Change at month 12	0·03 (0·02)	0·07 (0·02)*	0·11
Change at month 24	0·03 (0·03)	0·11 (0·02)*	0·0065
Triglycerides (mmol/L)			
Baseline	1·19 (0·08)	1·15 (0·05)	0·97
Change at month 12	−0·03 (0·05)	−0·29 (0·04)*	<0·0001
Change at month 24	−0·03 (0·05)	−0·27 (0·04)*	0·0002
Total cholesterol to HDL-cholesterol ratio			
Baseline	3·76 (0·128)	3·65 (0·097)	0·47
Change at month 12	−0·124 (0·061)	−0·505 (0·046)*	<0·0001
Change at month 24	−0·047 (0·065)	−0·532 (0·050)*	<0·0001

Baseline values are the observed mean (SE); change scores are the least-squares adjusted means (SE) from the intention-to-treat repeated measures analysis. Between-group p value tests for a significant between-group difference in the change score at the timepoint. All comparisons are controlled for baseline values. All p values reflect Bonferroni corrections, truncated at 1·0, as appropriate (see text). *p<0·001 compared with baseline.

Table 3: **Lipid and lipoproteins before and after intervention**

approach allowed us to test the effects of our findings using intention-to-treat modelling versus the effects considering full compliance and effect with 25% calorie restriction through the entire study. Analyses were done using SAS, version 9.2. This study is registered with ClinicalTrials.gov, number NCT00427193.

Role of the funding source

The CALERIE trial was done under the National Institutes of Health (NIH) U-grant mechanism, which implies that the study design and conduct was a collaborative effort between internal NIH and external study investigators. The funder for this exploratory analysis had a role in study design and data collection, but no role in data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 8, 2007, and Feb 26, 2010, participants were randomly assigned, with the final study visit on March 6, 2012. Of the 10 856 people who volunteered to participate, the screening procedures excluded 4798 (44·2%) for age or BMI; 1479 (13·6%) for health or medication reasons; and 4341 (40·0%) due to refusal to participate because of concerns about their ability to adhere to the protocol, personal, or other study-related issues.[15] Of the 238 participants who began baseline assessments, 220 were randomly assigned and 218 started the assigned intervention: 143 (66%) to the 25% calorie restriction diet and 75 (34%) to the ad libitum control diet (figure 1). 117 (82%) of 143 of the calorie restriction group and 71 (95%) of 75 of the ad libitum group completed the study (figure 1). Table 1 presents the baseline characteristics of the 218 participants. Effects of calorie restriction on resting metabolic rate, core body temperature, and hormones were reported previously.[15]

Detailed information regarding the observed adherence to the intervention has been published previously.[15] As shown in table 1, there was a mean difference of 77 kcal per day in average energy intake at baseline—assessed as total daily energy expenditure during a period of weight stability before the intervention—between the calorie restriction (2467 kcal per day [SE 34]) and ad libitum (2390 kcal per day [45]) groups. Of note, although participants were recruited to be within a BMI range of 22·0–27·9 kg/m² for this study, men had a particularly high mean percentage of body fat for men (25·9% [SE 0·4]) and women had moderately high percentage body fats for women (36·3% [0·4]). In the calorie restriction group, energy intake was reduced by a mean of 19·5% (SE 0·8, 480 kcal per day) during the first 6 months; decreased over time as might be expected to a mean of 9·1% (0·7) after 6 months, but averaged 11·9% (0·7) over the entire, 2-year intervention. In the control group, average daily energy intake was unchanged over the period (data not shown).

Changes in body composition by intervention group and by sex are detailed in a previous report.[32] In summary, weight loss was from a baseline mean of 8·4 kg (SE 0·3, 11·5%) at 1 year and 7·5 kg (0·3, 10·4%) at 2 years in the calorie restriction group (p<0·0001); it did not change significantly in the ad libitum group (table 2, figure 2). Fat mass decreased from baseline by a mean of 6·1 kg (SE 0·2) at 1 year and 5·3 kg (0·3) at 2 years in the calorie restriction group (p<0·0001); it did not change in the ad libitum group. Percentage weight loss did not differ by sex (data not shown). Percent responses in waist circumference, BMI, total body fat, and appendicular fat to calorie restriction did not differ by sex.[32] Fat loss at 2 years accounted for 71% of the weight loss in the calorie restriction group (table 2).

On the basis of 7-day food records, the calorie restriction group significantly restricted their energy intake by a mean of −279 kcal per day (SE 29; p<0·0001) at 1 year and −216 kcal per day (33; p<0·0001) at 2 years; the ad libitum group maintained its intake at 1 year (−83 kcal per day [SE 38]) and reported a reduction of −121 kcal per day (43) at 2 years (p=0·020).

Total cholesterol and LDL-cholesterol decreased significantly both at 1 and 2 years in the calorie restriction group, but did not decrease significantly in the ad libitum group (table 3, figure 2). HDL-cholesterol was increased

by calorie restriction at 1 and 2 years, but the change was significantly different from the ad libitum group only at year 2. Accordingly, the ratio of total cholesterol to HDL-cholesterol decreased significantly and persistently in the calorie restriction group (table 3). Calorie restriction, but not the ad libitum diet, caused a drop in serum triglycerides concentrations of 24% (table 3).

Baseline blood pressure values were low-normal in the individuals enrolled in the trial; nevertheless, calorie restriction but not the ad libitum diet resulted in a significant reduction in systolic, diastolic, and mean blood pressure (table 4, figure 2). The lowering of blood pressure was evident as early as 6 months; however, it reached statistical significance only at 1 year and persisted for the entire duration of the study (table 4).

Fasting and area under the curve insulin were both significantly reduced in the calorie restriction group compared with the ad libitum group at 1 and 2 years (table 5, figure 2). Fasting glucose was significantly reduced by calorie restriction at year 1, but not at year 2. By contrast, no significant reduction in the area under the curve glucose was observed in this study (table 5, figure 2). Nonetheless, the calorie restriction group had improvements in insulin sensitivity, as reflected in significantly lower homoeostasis model assessment-insulin resistance, insulin response (at 2 years only), and increased insulin sensitivity index (table 5). Oral disposition increased in the calorie restriction group more than in the ad libitum group although the difference between groups was not significant. Plasma hs-CRP concentrations were significantly reduced in the calorie restriction group, but not in the control group, at 2 years (table 5). Calorie restriction also resulted in a major and persistent reduction in the metabolic syndrome score (table 5). Calorie restriction-induced responses in clinical cardiometabolic risk variables when controlling for simultaneous changes in bodyweight are shown in the appendix (p 2). In summary, there is substantial residual and significant dose–response effects of calorie restriction on cardiometabolic risk factors when controlling for weight loss.

The findings of the marginal structural modelling are presented in the appendix (pp 3–9). This analysis allows us to account for biases created by unbalanced drop out and non-adherence to the intervention by inferring a 25% calorie restriction to all calorie restriction participants using trial data and weighted by data obtained from individuals that achieved the goal calorie restriction.[31] We found that assuming full compliance and effect in the calorie restriction group had marginal effects on the p values and essentially no effect on the conclusions of the trial on the cardiometabolic factors of interest, with the exception of hs-CRP, for which the between-group difference was not significant at 2 years in the marginal structural modelling analysis.

There were no serious adverse events in this study. There were numerous adverse events, published in an accompanying manuscript dedicated to safety.[33] However, there was no significant difference in the rate of adverse events among the two study groups.

	Ad libitum group (n=75)	Calorie restriction group (n=143)	Between-group p value
Systolic pressure (mmHg)			
Baseline†	111·2	112·1	0·48
Change at month 6	−1·21 (0·86)	−2·97 (0·65)‡	0·092
Change at month 12	2·26 (1·03)*	−1·87 (0·77)*	0·0011
Change at month 18	0·60 (0·95)	−2·89 (0·74)‡	0·0031
Change at month 24	2·15 (1·06)*	−2·20 (0·82)†	0·0011
Diastolic pressure (mmHg)			
Baseline†	71·2	72·1	0·50
Change at month 6	−2·70 (0·76)‡	−3·84 (0·57)‡	0·22
Change at month 12	1·30 (0·74)	−3·38 (0·55)‡	<0·0001
Change at month 18	−0·58 (0·79)	−3·31 (0·61)‡	0·0052
Change at month 24	1·55 (0·80)*	−3·40 (0·62)‡	<0·0001
Mean blood pressure (mmHg)			
Baseline†	84·5	85·4	0·48
Change at month 6	−2·20 (0·74)†	−3·57 (0·55)‡	0·12
Change at month 12	1·62 (0·76)*	−2·90 (0·57)‡	<0·0001
Change at month 18	−0·19 (0·77)	−3·20 (0·60)‡	0·0017
Change at month 24	1·75 (0·81)*	−3·02 (0·63)‡	<0·0001
Pulse pressure (mmHg)			
Baseline†	40·0	40·1	0·63
Change at month 6	1·62 (0·63)*	0·99 (0·47)*	0·41
Change at month 12	1·15 (0·75)	1·63 (0·55)†	0·60
Change at month 18	1·35 (0·71)	0·56 (0·55)	0·36
Change at month 24	0·79 (0·77)	1·32 (0·59)*	0·58

Baseline values are the observed mean (SE); change scores are the least-squares adjusted means (SE) from the intention-to-treat repeated measures analysis. Between-group p value tests for a significant between-group difference in the change score at the time point. All comparisons are controlled for baseline values. All p values reflect Bonferroni corrections, truncated at 1·0, as appropriate (see text). *p<0·01 compared with baseline.†p<0·05 compared with baseline. ‡p<0·001 compared with baseline.

Table 4: **Blood pressure before and after intervention**

See **Online** for appendix

Discussion

Little is known about the effects of prolonged calorie restriction on cardiometabolic health in normal weight, young individuals. Because the studies of calorie restriction on lifespan in animal models start early in the lifespan in normal weight individuals, to understand similar effects in humans, it is essential to do calorie restriction experiments in similarly young, healthy individuals. In this 2-year, multicentre, randomised clinical trial, we tested—for the first time to the best of our knowledge—the time-course of cardiometabolic adaptations to 2 years of moderate calorie restriction in healthy non-obese young and middle-aged individuals, having clinically normal risk factors at baseline (percentage changes in six parameters of cardiometabolic risk are shown in figure 2). Results of this trial provide evidence that calorie restriction with adequate nutrition

	Ad libitum group (n=75)	Calorie restriction group (n=143)	Between-group p value
Fasting insulin (μIU/mL)			
Baseline	5·79	5·38	0·27
Change at month 12	−0·14 (0·24)	−1·59 (0·18)*	<0·0001
Change at month 24	0·14 (0·21)	−1·71 (0·16)*	<0·0001
Fasting glucose (mg/dL)			
Baseline	4·64	4·55	0·12
Change at month 12	0·02 (0·03)	−0·07 (0·02)*	0·0096
Change at month 24	−0·01 (0·03)	−0·05 (0·02)†	0·26
Homoeostatic model assessment-insulin resistance			
Baseline	1·20	1·11	0·15
Change at month 12	−0·031 (0·050)	−0·347 (0·038)*	<0·0001
Change at month 24	0·027 (0·046)	−0·364 (0·035)*	<0·0001
Homoeostatic model assessment-β (%)			
Baseline	107·4	128·1	0·92
Change at month 12	−17·30 (9·09)	−33·01 (6·78)*	0·16
Change at month 24	−16·71 (6·25)	−43·92 (4·85)*	0·0004
Area under the curve insulin (μIU-h/mL)			
Baseline	98·8	96·2	0·68
Change at month 12	7·33 (6·25)	−23·61 (4·86)*	<0·0001
Change at month 24	6·25 (4·98)	−19·34 (4·08)*	<0·0001
Area under the curve glucose (mg-h/mL)			
Baseline	260·8	260·1	0·97
Change at month 12	4·52 (4·70)	−4·70 (3·72)	0·23
Change at month 24	2·89 (4·82)	0·10 (3·88)	1·0
Insulin response			
Baseline	0·93	0·93	0·83
Change at month 12	0·037 (0·149)	−0·055 (0·111)	1·0
Change at month 24	0·096 (0·055)	−0·143 (0·045)‡	0·0014
Insulin sensitivity			
Baseline	0·22	0·24	0·27
Change at month 12	0·018 (0·015)	0·078 (0·012)*	0·0013
Change at month 24	−0·013 (0·020)	0·099 (0·015)*	<0·0001
Oral disposition			
Baseline	0·19	0·24	0·44
Change at month 12	−0·036 (0·052)	0·042 (0·039)	0·46
Change at month 24	−0·027 (0·030)	0·035 (0·024)	0·18
High sensitivity C-reactive protein (nmol/L)			
Baseline	0·114	0·155	0·91
Change at month 12	0·030 (0·037)	−0·045 (0·028)	0·105
Change at month 24	0·002 (0·023)	−0·068 (0·018)‡	0·012
Metabolic syndrome score			
Baseline	−7·9	−8·3	0·35
Change at month 12	−0·156 (0·212)	−2·646 (0·160)*	<0·0001
Change at month 24	−0·064 (0·252)	−2·669 (0·193)*	<0·0001

Baseline values are the observed mean (SE); change scores are the least–squares adjusted means (SE) from the intention-to-treat repeated measures analysis. Between-group p value tests for a significant between-group difference in the change score at the time point. All comparisons are controlled for baseline values. All p values reflect Bonferroni corrections, truncated at 1·0, as appropriate. *p<0·001. †p<0·05. ‡ p<0·01.

Table 5: **Glucose tolerance, insulin action, and inflammation before and after intervention**

leads to improvements in multiple cardiometabolic risk factors—even when implemented in healthy young and middle-aged men and women with normal baseline values; further, we found that calorie restriction in this population improved already normal risk factors, implying improvement in long-term cardiovascular risk. Moreover, these data indicate that sustained calorie restriction over 2 years exerts beneficial effects on cardiometabolic health

over and above those conferred by the concordant weight loss.

The development of atherosclerotic disease is a multistage process modulated by several cardiometabolic risk factors, of which elevated LDL-cholesterol and low HDL-cholesterol are among the most important triggers and predictors of future cardiovascular disease events.[12,34–36] According to the guidelines of the National Cholesterol Education Program ATP-III, plasma LDL-cholesterol concentrations should be less than 2·56 mmol/L.[37] However, data from randomised, placebo-controlled trials using statins for primary prevention of cardiovascular disease events indicate that optimal LDL-cholesterol concentrations should be much lower than 2·56 mmol/L; and starting as low as 1·46 mmol/L. There is a linear relationship between serum LDL-cholesterol concentration and the risk of developing cardiovascular disease.[12] In our study, moderate calorie restriction, but not the ad libitum diet, reduced serum LDL-cholesterol concentrations by 7% from 2·51 to 2·33 mmol/L. Importantly, the reduction in LDL-cholesterol was accompanied by a significant increase in serum HDL-cholesterol concentration (from 1·26 to 1·36 mmol/L) and a substantial reduction in serum triglyceride concentration (from 1·15 to 0·90 mmol/L)—two factors modulating cardiovascular risk independently of LDL-cholesterol.[8,38,39]

Elevated blood pressure is another key risk factor for the development of myocardial infarction, stroke, heart, and renal failure. According to the new American Heart Association/American College of Cardiology blood pressure guidelines, optimal blood pressure is lower than 120/80 mm Hg in people aged between 30 and 59 years, and blood pressure values between this level and 130/85 mm Hg are considered elevated.[40] In fact, data from observational studies involving more than 1 million people clearly indicate that in all age groups, the risk of dying from both coronary heart disease and stroke rises drastically starting from a blood pressure as low as 115/75 mm Hg.[10] The death risk from coronary heart disease and stroke doubles for every 20 mm Hg systolic or 10 mm Hg diastolic increase in blood pressure.[10] In our study, individuals randomly assigned to the calorie restriction diet had a rapid and significant reduction in systolic, diastolic, and mean blood pressure, whereas blood pressure increased in the control group eating an ad libitum diet. The mechanisms mediating this rapid and persistent drop in blood pressure induced by calorie restriction are not completely known; it could be due to a calorie restriction-mediated reduction in oxidative stress, inflammation, and preservation of endothelial nitric oxide bioavailability and function.[41]

Insulin resistance is a powerful risk factor for the development of type 2 diabetes, coronary heart disease, and some forms of cancer.[9,42] Insulin resistance predicts the risk of developing type 2 diabetes as early as 13 years before diagnosis. Further and relevant, a rise in plasma glucose concentrations also predict the development of diabetes as early as 12 years before diagnosis; they are normally still within the normal, non-diagnostic range until 2–5 years before diagnosis, when a rapid deterioration of insulin secretion (β-cell function) and a parallel elevation of glycaemia occurs.[43] Moreover, insulin resistance and the compensatory hyperinsulinaemia play a role in the pathogenesis of hypertension; in the inhibition of fibrinolysis; and in the stimulation of vascular smooth muscle proliferation and migration, all leading to atherosclerosis.[42] In our study, calorie restriction did not significantly change glucose tolerance at a point in the development of diabetes where it might still be in the normal range; however, it profoundly improved insulin sensitivity, and reduced plasma fasting glucose and glucose-stimulated insulin concentration. This reduction in circulating quantities of insulin and the improvement of the metabolic syndrome score could exert beneficial effects not only in lowering the risk of atherosclerosis but also in the primary and secondary prevention of some common cancers, such as breast cancer.[44,45]

Low-grade chronic inflammation is also implicated in the pathogenesis of coronary heart disease, but also of cancer, cognitive impairment, and in the biology of ageing itself.[46,47] According to the guidelines of the American Heart Association, individuals with circulating hs-CRP concentrations less than 9·5 mmol/L have a 50% less risk of coronary heart disease than individuals with hs-CRP between 9·5 and 28·6 mmol/L.[48] In our study, individuals randomly assigned to calorie restriction, but not the control group, had a reduction of hs-CRP from 14·1 to 8·38 mmol/L over the course of the 2-year study. Changes in hs-CRP were only significant after 2 years of calorie restriction even though there was some weight re-gain between 1 and 2 years of calorie restriction. This finding emphasises the benefits of sustained calorie restriction over short-term weight loss for cardiovascular disease risk reduction. Of further interest, in a pharmacological interventional clinical trial with atorvastatin of 502 patients with angiographically documented coronary disease, a similar reduction of hs-CRP from 27·6 to 21·9 mmol/L resulted in a significant regression of atherosclerosis as determined by intravascular ultrasonography.[11]

We believe these results have a profound public health relevance for the lifetime reduction of atherosclerotic risk for at least two reasons. First, in the Framingham Heart study,[13] individuals with optimal cardiometabolic risk factors aged 50 years have a 13-times lesser risk of developing cardiovascular disease during their remaining lifetime than do men and women with two or more abnormal risk factors, despite a much longer lifespan. Second, early reduction in cardiometabolic risk is much more effective than late reductions in preventing coronary events. A 30% reduction of serum LDL-cholesterol with statins results in a 30% lesser risk of

coronary events; indeed, a similar 30% reduction of LDL-cholesterol in individuals born with the rare PCSK9 genetic variant have a reduced coronary artery disease risk by approximately 90%.[11]

To our knowledge, this trial is the first, adequately powered 2-year dietary randomised clinical trial to show such a profound effect on lowering all cardiometabolic risk factors beyond normal levels, even in rather young, lean individuals. There are no pharmacological agents with such a profound effect on such a broad range of cardiometabolic risk factors. These findings should provide a new tool for clinicians in fighting the ravages of poor and unhealthy diets.

Major strengths of this study include the large sample size for an intensive physiological intervention study of this type, the intention-to-treat randomised controlled trial design minimising the potential for selection bias, and the long duration of the study. Moreover, in all the participants we carefully measured their energy intake and energy expenditure using both nutrition assessment software and direct analysis through doubly-labelled water. Our study had a high retention rate of enrolled participants, and good adherence to the study interventions as shown by the successful weight reduction over 2 years. A major limitation of this study is the lack of a clinical measurement of atherosclerotic plaque modifications.

In conclusion, the results of this large, randomised controlled trial provide evidence that a moderate calorie restriction-induced negative energy balance improves multiple cardiometabolic risk factors—waist circumference, blood pressure, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin resistance and glucose control, metabolic syndrome, and chronic inflammatory tone—well below the conventional risk thresholds used in clinical practice. These findings are of substantial public health importance even when started in people who are healthy, young and middle-aged, and not obese. These data combined with previously published safety data for calorie restriction,[33] indicate that inexpensive and safe dietary interventions, such as moderate calorie restriction, can be implemented early in life to optimise cardiometabolic health and reduce the lifetime risk of developing some of the most common, disabling, and expensive chronic diseases, such as hypertensive and atherosclerotic cardiovascular disease. It will be important to do additional research in this area to understand the physiological and molecular biological factors by which the described adaptations are achieved. Such understanding could lead to pharmacological therapies of use for improving human health.

Contributors

WEK, CFP, SKD, LMR, DTV, JR, SBR, ER, and JOH were responsible for study design and collection of study data. WEK drafted and revised the manuscript. KMH, LF, and CFP contributed to drafting of the manuscript. MB, CFP, and JP did statistical analyses, reviewed and contributed to the editing of the manuscript. WEK, MB, KMH, CFP, SKD, LMR, DTV, JR, SBR, ER, JOH, and LF reviewed the manuscript and approved the contents.

Declaration of interests

We declare no competing interests.

Data sharing

Data from CALERIE are publicly available at the CALERIE Research Network web site at http://calerie.duke.edu.

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References

1 Mozaffarian D, Benjamin EJ, Go AS, et al. Executive summary: heart disease and stroke statistics—2016 update: a report from the American Heart Association. *Circulation* 2016; **133:** 447–54.

2 Colman RJ, Anderson RM, Johnson SC, et al. Calorie restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009; **325:** 201–04.

3 Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Calorie restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun* 2014; **5:** 3557.

4 Mattison JA, Colman RJ, Beasley TM, et al. Calorie restriction improves health and survival of rhesus monkeys. *Nat Commun* 2017; **8:** 14063.

5 Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci USA* 2004; **101:** 6659–63.

6 Meyer TE, Kovacs SJ, Ehsani AA, Klein S, Holloszy JO, Fontana L. Long-term calorie restriction ameliorates the decline in diastolic function in humans. *J Am Coll Cardiol* 2006; **47:** 398–402.

7 Stein PK, Soare A, Meyer TE, Cangemi R, Holloszy JO, Fontana L. Calorie restriction may reverse age-related autonomic decline in humans. *Aging Cell* 2012; **11:** 644–50.

8 Barter P, Gotto AM, LaRosa JC, et al. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007; **357:** 1301–10.

9 Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med* 1999; **341:** 1097–105.

10 Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; **360:** 1903–13.

11 Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med* 2005; **352:** 29–38.

12 O'Keefe JH Jr, Cordain L, Harris WH, Moe RM, Vogel R. Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better and physiologically normal. *J Am Coll Cardiol* 2004; **43:** 2142–46.

13 Tirosh A, Shai I, Tekes-Manova D, et al. Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med* 2005; **353:** 1454–62.

14 Lloyd-Jones DM, Leip EP, Larson MG, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation* 2006; **113:** 791–98.

15 Ravussin E, Redman LM, Rochon J, et al. A 2-year randomized controlled trial of human calorie restriction: feasibility and effects on predictors of health span and longevity. *J Gerontol A Biol Sci Med Sci* 2015; **70:** 1097–104.

16 Rochon J, Bales CW, Ravussin E, et al. Design and conduct of the CALERIE study: comprehensive assessment of the long-term effects of reducing intake of energy. *J Gerontol A Biol Sci Med Sci* 2011; **66:** 97–108.

17 Rickman AD, Williamson DA, Martin CK, et al. The CALERIE study: design and methods of an innovative 25% calorie restriction intervention. *Contemp Clin Trials* 2011; **32:** 874–81.

18 Racette SB, Das SK, Bhapkar M, et al. Approaches for quantifying energy intake and %calorie restriction during calorie restriction interventions in humans: the multicenter CALERIE study. *Am J Physiol Endocrinol Metab* 2012; **302:** E441–48.

19 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chem* 1972; **18:** 499–502.

20 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28:** 412–19.

21 Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in diabetes research. *Diabetes Care* 1995; **18:** 245–50.

22 Lee HW, Muniyappa R, Yan X, et al. Comparison between surrogate indexes of insulin sensitivity/resistance and hyperinsulinemic euglycemic glucose clamps in rhesus monkeys. *Endocrinology* 2011; **152:** 414–23.

23 Mohd Nor NS, Lee S, Bacha F, Tfayli H, Arslanian S. Triglyceride glucose index as a surrogate measure of insulin sensitivity in obese adolescents with normoglycemia, prediabetes, and type 2 diabetes mellitus: comparison with the hyperinsulinemic-euglycemic clamp. *Pediatric Diabetes* 2016; **17:** 458–65.

24 Bergman RN, Ader M, Huecking K, Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. *Diabetes* 2002; **51** (suppl 1)**:** S212–20.

25 Utzschneider KM, Prigeon RL, Faulenbach MV, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care* 2009; **32:** 335–41.

26 Bateman LA, Slentz CA, Willis LH, et al. Comparison of aerobic versus resistance exercise training effects on metabolic syndrome (from the Studies of a Targeted Risk Reduction Intervention Through Defined Exercise-STRRIDE-AT/RT). *Am J Cardiol* 2011; **108:** 838–44.

27 Johnson JL, Slentz CA, Houmard JA, et al. Exercise training amount and intensity effects on metabolic syndrome (from Studies of a Targeted Risk Reduction Intervention through Defined Exercise). *Am J Cardiol* 2007; **100:** 1759–66.

28 Dmitrienko A, Millen BA, Brechenmacher T, Paux G. Development of gatekeeping strategies in confirmatory clinical trials. *Biom J* 2011; **53:** 875–93.

29 Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982; **38:** 963–74.

30 Group IEEW. Statistical principles for clinical trials: ICH harmonized tripartite guideline. *Stats in Med* 1999; **18:** 1905–42.

31 Rochon J, Bhapkar M, Pieper CF, Kraus WE. Application of the marginal structural model to account for suboptimal adherence in a randomized controlled trial. *Contemp Clin Trials Commun* 2016; **4:** 222–28.

32 Das SK, Roberts SB, Bhapkar MV, et al. Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: a 2-y randomized controlled trial of calorie restriction in nonobese humans. *Am J Clin Nutr* 2017; **105:** 913–27.

33 Romashkan SV, Das SK, Villareal DT, et al. Safety of two-year calorie restriction in non-obese healthy individuals. *Oncotarget* 2016; **7:** 19124–33.

34 Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015; **161:** 161–72.

35 McGill HC Jr, McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation* 2008; **117:** 1216–27.

36 Pletcher MJ, Bibbins-Domingo K, Liu K, et al. Nonoptimal lipids commonly present in young adults and coronary calcium later in life: the CARDIA (Coronary Artery Risk Development in Young Adults) study. *Annals Intern Med* 2010; **153:** 137–46.

37 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106:** 3143–421.

38 Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Low triglycerides-high high-density lipoprotein cholesterol and risk of ischemic heart disease. *Arch Intern Med* 2001; **161:** 361–66.

39 Tirosh A, Rudich A, Shochat T, et al. Changes in triglyceride levels and risk for coronary heart disease in young men. *Ann Intern Med* 2007; **147:** 377–85.

40 Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* 2018; **71:** 1269–324.

41 Donato AJ, Walker AE, Magerko KA, et al. Life-long calorie restriction reduces oxidative stress and preserves nitric oxide bioavailability and function in arteries of old mice. *Aging Cell* 2013; **12:** 772–83.

42 Kraemer FB, Ginsberg HN, Reaven GM. Demonstration of the central role of insulin resistance in type 2 diabetes and cardiovascular disease. *Diabetes Care* 2014; **37:** 1178–81.

43 Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012; **379:** 2279–90.

44 Berrino F, Villarini A, Traina A, et al. Metabolic syndrome and breast cancer prognosis. *Breast Cancer Res Treat* 2014; **147:** 159–65.

45 Bhandari R, Kelley GA, Hartley TA, Rockett IR. Metabolic syndrome is associated with increased breast cancer risk: a systematic review with meta-analysis. *Int J Breast Cancer* 2014; **2014:** 189384.

46 Libby P, Hansson GK. Inflammation and immunity in diseases of the arterial tree: players and layers. *Circulation Res* 2015; **116:** 307–11.

47 Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* 2013; **339:** 166–72.

48 Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107:** 499–511.