Special Article

Effects of caloric restriction on human physiological, psychological, and behavioral outcomes: highlights from CALERIE phase 2

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Caloric restriction (CR) is a strategy that attenuates aging in multiple nonhuman species. The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) trials are part of a research program aiming to test the effects of CR on aging and longevity biomarkers in humans. Building on CALERIE phase 1, CALERIE phase 2 (CALERIE 2) was the largest study to date to assess sustained CR in healthy humans without obesity. In a 24-month randomized controlled trial comprising 218 participants at baseline, CALERIE 2 showed that moderate CR, 11.9% on average, induced improvements in aging-related biomarkers without adversely affecting psychological or behavioral outcomes. The objectives of this report are to summarize and review the highlights of CALERIE 2 and report previously unpublished results on eating disorder symptoms and cognitive function. This article specifically summarizes the physiological, psychological, aging, behavioral, and safety results of the trial. Also provided are research directions beyond CALERIE 2 that highlight important opportunities to investigate the role of CR in aging, longevity, and health span in humans.

INTRODUCTION

Life expectancy is increasing worldwide. For the first time in history, there are more individuals older than 65 years than younger than 5 years, and by 2050, it is projected that 1 in 6 individuals will be 65 years old or older.[1] Aging will pose major challenges, such as reductions in health and independence, with corresponding increases in age-related diseases that lead to financial and societal burdens.[1] Strategies that maintain or attenuate declines in health and independence with advancing age will become increasingly important for relieving these burdens.

Caloric restriction (CR) may be a viable strategy for improving longevity and attenuating the age-related increase in chronic disease risk. CR is defined as a reduction in energy intake below the amount that would be consumed ad libitum (AL) while maintaining adequate intake of essential nutrients.[2] Studies in animals and nonhuman primates have reported that sustained CR

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beginning in early adulthood or midlife can extend both the length of life and health span—the latter considered the length of life free of chronic disease.[3] Observational reports on the effects of long-term CR in humans come from individuals in the Calorie Restriction Society International, a group who voluntarily and habitually implements CR via a nutrient-dense, whole-foods–based diet.[4] Compared with individuals consuming a typical standard American/Western diet, individuals in this group who had been practicing CR for a mean of 15 years have reduced body fat, markers of inflammation, and cardiovascular disease (CVD) risk.[4] Although long-term data on longevity and mortality are not yet available, the lower risk for the development of metabolic disease and the apparent long-term adherence to CR displayed by this group are potentially significant.[5]

Clinical studies performed with healthy participants without obesity demonstrated that CR (~30%) improves metabolic health.[6,7] These studies, however, were relatively short ($\leq$6 mo), and it is likely that weight maintenance had not been reached. Additionally, because of postulated reductions in quality of life, libido, and mood,[8] combined with the potential for adverse eating behaviors,[9] the long-term applicability of CR has been questioned. Thus, in 2001, the National Institute of Aging of the US National Institutes of Health initiated longer-term studies in humans to explore the age-related benefits and prolonged applicability of CR. This research program came to be known as the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) consortium. To inform the design of the larger definitive study, CALERIE phase 1 (CALERIE 1) tested the effects of 6[10] and 12 months[11–14] of 10% to 30% energy-deficit diets on biomarkers of aging among healthy, middle-aged adults who were overweight but not obese. In these studies, energy deficits were achieved through reduced food intake, increased exercise energy expenditure, or a combination of the two. The results have been discussed extensively elsewhere[11,12,15] and are highlighted where relevant in this review.

Following CALERIE 1, CALERIE phase 2 (CALERIE 2) was designed, with the critical goal of establishing whether CR-induced changes in end points become stable or improve further during weight stability after weight loss.[16,17] Using a randomized controlled trial performed at 3 sites, the CALERIE 2 trial became the largest and most systematic examination of prolonged CR in humans. The aim of this review is to summarize and highlight the scientific information generated from CALERIE 2. By incorporating numerous end points, we cover novel, collective insights into the key aging-related, psychological and behavioral findings, which can be used by nutrition scientists, scholars, clinicians, and practitioners interested in the influence of CR in humans. Moreover, we present new data that detail the changes in eating-disorder symptoms and cognitive function during the trial. Examinations into the influence of CR on these end points are important, particularly in individuals who have undergone CR for a prolonged period and are not obese. Indeed, some have suggested that dietary restriction leads to maladaptive changes in eating-disorder symptoms[18] and cognitive function[19] that could undermine many positive effects of such regimens. Finally, we identify areas of current scientific inquiry that will enhance CALERIE 2 findings and aid understanding of the potential of CR and other nutritional-based strategies to promote healthy aging in humans.

THE CALERIE 2 STUDY

CALERIE 2 was conducted at Pennington Biomedical Research Center (Baton Rouge, Louisiana), Tufts University (Boston, Massachusetts), and Washington University School of Medicine (St. Louis, Missouri); the Duke Clinical Research Institute (Durham, North Carolina) served as the coordinating center. Investigators of the clinical sites, the coordinating center, and the National Institute on Aging constituted the steering committee. The institutional review boards at the clinical sites and the coordinating center approved the protocol, and participants provided written informed consent before enrolment.[16]

Healthy men (aged 21–50 y) and premenopausal women (aged 21–47 y) with a body mass index (BMI) ranging from 22.0 to $<$28.0 kg/m^2 were eligible. The upper age limit was chosen on the basis of evidence from animal studies showing that CR beyond 50% of the average life span evokes equivocal benefits and is associated with reduced hormonal-induced changes in disease risk with menopause.[16] The lower limit of BMI was based on safety concerns that a BMI $<$18.5 kg/m^2 might be harmful; the upper limit ensured participants without obesity were recruited, reducing the likelihood of confounding complications linked with this condition.[16] Exclusion criteria included cardiometabolic diseases, abnormal laboratory markers, psychiatric or behavioral problems, and regular use of medications other than oral contraceptives. Individuals engaging in $\geq$30 minutes of physical activity $\geq$5 per week were also excluded to mitigate the effects of high physical activity levels or change in physical activity levels on energy expenditure.[16]

Study design

CALERIE 2 was a parallel-group, randomized controlled trial in which the effects of 24 months of sustained CR in humans were assessed. After completing baseline assessments, participants were randomly assigned into either a CR group that aimed to enact immediate and sustained 25% CR or an AL control group that was instructed to maintain habitual dietary patterns. Although animal studies indicate that higher levels of CR improve age-related biomarkers, long-term adherence to CR >25% was deemed unfeasible in humans, especially in participants who live in obesogenic Western settings and who are not obese.[16] As a result, 25% CR represented a compromise between the attainment of optimal physiological adaptations and perceived feasibility.

Randomization was applied in a 2:1 ratio favoring CR and was stratified by site, sex, and BMI (normal weight or overweight). Overall, 220 participants were randomly assigned to a study arm: 145 to the CR group and 75 to the AL group. Two CR participants dropped out before the intervention; thus, 143 and 75 participants in the CR and AL groups, respectively, began the trial.

A thorough description of the CR intervention has been published.[20] An intensive dietary and behavioral regimen was implemented, with the aim to achieve 25% CR without malnutrition throughout the 24-month intervention. Dietary patterns were introduced early during the intervention; thereafter, participants were free to modify dietary patterns as they desired to ensure adequate adherence to the CR protocol. Participants were provided a daily multivitamin and mineral supplement and a calcium supplement to ensure micronutrient requirements were met.

The intervention was administered by behaviorists and nutritionists. Materials given to the CR group were largely based on strategies used in the Look AHEAD trial,[21] the Diabetes Prevention Program,[22] and CALERIE 1.[10–12] Individual counselling sessions mostly were delivered weekly during the first month, twice monthly until month 12, and then monthly until the end of the trial. Additional sessions were arranged to promote adherence if required, and sessions were customized in line with the participants' dietary requirements and weight goals. In addition to individual sessions, group sessions were initiated after week 4 and occurred twice monthly.[20]

A variety of evidence-based techniques were used to assist the CR group.[20] Specifically, during the first 27 days, participants were supplied all meals and instructed to consume only the foods and beverages provided. Three different dietary patterns—low glycemic load, Mediterranean, and low fat—were provided during this initial phase to educate participants on the appropriate foods and portion sizes for CR prescription. Each participant received an individualized caloric prescription and was instructed to self-monitor food intake daily and to provide these data to an interventionist at each meeting for review. This was facilitated by the provision of food scales, measuring cups and spoons, and portion-size training. Other topics covered throughout the intervention included maintaining motivation, managing food cravings, managing hunger, goal setting, and social support.[20]

Baseline energy requirements were calculated over 2 consecutive 14-day periods by the intake-balance method, with total daily energy expenditure (TDEE) assessed through doubly labelled water (DLW).[16,17] Average %CR throughout the trial was estimated using 2 objective measures. First, average %CR was quantified during each 6-month interval using the intake-balance method.[23] Akin to baseline energy requirements, DLW was used to assess TDEE, with adjustments made for CR-provoked changes in body composition.[23,24] Second, to provide a real-time assessment of adherence, the intervention used an innovative computer tracking system (CTS) to objectively estimate adherence on the basis of weekly weight change.[20,25,26] The CTS applied an algorithm to project the 24-month weight trajectory for each participant, assuming adherence to the 25% CR goal. Frequent weight measurements were then recorded in the CTS during the trial, providing participants and interventionists a gauge of success in achieving CR.[25] In instances where weight was outside the range consistent with the CR goal, adherence was deemed suboptimal and the CTS triggered various toolbox options that reinforced psychological, nutritional, and behavioral support strategies.[20]

Many end points were assessed throughout CALERIE 2.[16] Changes in the resting metabolic rate residual, defined as changes in resting metabolic rate (RMR) not attributable to changes in fat mass and fat-free mass (FFM), and core temperature were primary end points of the trial. Secondary outcomes comprised changes in levels of circulating triiodothyronine and tumor necrosis factor-α.[16] Exploratory aims included assessments of biomarkers of primary aging (inflammation and oxidative stress), cardiometabolic disease risk markers, bone health, quality of life, eating behaviors and attitudes, and cognition. Furthermore, CALERIE 2 examined the safety and tolerability of prolonged CR through a series of assessments.[16] An exhaustive summary of the changes in these end points is detailed in Table 1.

With few exceptions, the same intent-to-treat approaches were applied by the CALERIE Study Group

Table 1 **Responses in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy 2 trial from baseline relative to control.**

Outcome responses	Change	Reference
Metabolic adaptation, oxidative stress, and core temperature		
Total energy expenditure residual	↓	Ravussin et al (2015)[17]; Redman et al (2018)[27]
Resting metabolic rate residual	↔	Ravussin et al (2015)[17]
F_2-isoprostanes	↓	Il'yasova et al (2018)[28]
24-h core temperature	↔	Ravussin et al (2015)[17]
Sleep core temperature	↓	Redman et al (2018)[27]
Biological aging		
Klemera-Doubal method	↓	Belsky et al (2018)[29]
Homeostatic dysregulation	↓	Belsky et al (2018)[29]
Body composition		
Body weight	↓	Ravussin et al (2015)[17]; Das et al (2017)[30]
Fat mass	↓	Ravussin et al (2015)[17]; Das et al (2017)[30]
Lean body mass	↓	Das et al (2017)[30]
Trunk fat	↓	Das et al (2017)[30]
Bone mineral density	↓	Villareal et al (2016)[31]
Cardiometabolic risk markers		
Fasting insulin	↓	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Insulin sensitivity	↑	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Blood pressure	↓	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Triglyceride	↓	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Total cholesterol	↓	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Low-density lipoproteins	↓	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
High-density lipoproteins	↑	Ravussin et al (2015)[17]; Kraus et al (2019)[32]
Hormones		
Leptin	↓	Ravussin et al (2015)[17]; Meydani et al (2016)[33]
Triiodothyronine	↓	Ravussin et al (2015)[17]
Thyroid-stimulating hormone	↔	Ravussin et al (2015)[17]
Dehydroepiandrosterone-sulfate	↔	Ravussin et al (2015)[17]
Cortisol	↔	Fontana et al (2016)[34]
Adiponectin	↑	Redman et al (2018)[27]
Parathyroid hormone	↔	Villareal et al (2016)[31]
25-hydroxyvitamin D	↑	Villareal et al (2016)[31]
Sex hormone-binding globulin	↑	Martin et al (2016)[35]
Testosterone	↔	Martin et al (2016)[35]
Luteinizing hormone	↔	Martin et al (2016)[35]
Follicle-stimulating hormone	↔	Martin et al (2016)[35]
Growth factors		
Insulin-like growth factor-1	↔	Fontana et al (2016)[34]
Insulin-like growth factor-1 binding protein	↓	Fontana et al (2016)[34]
Transforming growth factor β-1	↔	Fontana et al (2016)[34]
Platelet-derived growth factor-AB	↔	Fontana et al (2016)[34]
Markers of inflammation/oxidative stress		
Tumor necrosis factor-α	↓	Ravussin et al (2015)[17]; Meydani et al (2016)[33]
C-reactive protein	↓	Ravussin et al (2015)[17]; Meydani et al (2016)[33]
Intercellular adhesion molecule-1	↔	Meydani et al (2016)[33]
Immune function		
Lymphocyte count	↓	Meydani et al (2016)[33]
Delayed-type hypersensitivity to vaccine	↔	Meydani et al (2016)[33]
Nutritional intake		
Protein	↔	Villareal et al (2016)[31]
Carbohydrates	↔	Villareal et al (2016)[31]
Fat	↓	Villareal et al (2016)[31]
Calcium	↔	Villareal et al (2016)[31]
Vitamin A	↔	Villareal et al (2016)[31]
Vitamin D	↔	Villareal et al (2016)[31]
Vitamin K	↑	Villareal et al (2016)[31]
Magnesium	↑	Villareal et al (2016)[31]
Phosphorus	↔	Villareal et al (2016)[31]
Physical performance		
Maximal oxygen uptake	↑	Racette et al (2017)[36]
Strength	↔	Racette et al (2017)[36]

(continued)

Table 1 **Continued**

Outcome responses	Change	Reference
Mood, stress and quality of life		
Mood	↑	Martin et al (2016)[35]
Depression	↔	Martin et al (2016)[35]
Quality of life	↑	Martin et al (2016)[35]
Eating behaviors, eating attitudes, and disordered eating		
Self-efficacy for regulating food intake	↑	Dorling et al (2019)[37]
Dietary restraint	↑	Dorling et al (2019)[37]
Dietary disinhibition	↔	Dorling et al (2019)[37]
Eating-disorder symptoms	↔	
Concern with body shape	↓	
Appetite and food cravings		
Appetite	↔	Dorling et al (2019)[37]; Dorling et al (2020)[26]
Satisfaction with foods	↔	Dorling et al (2020)[26]
Food cravings	↔	Dorling et al (2019)[37]
Cognition, sexual function, and sleep		
Working memory	↑	Leclerc et al (2019)[38]
Attention/concentration	↔	
Reaction time	↔	
Recall	↔	
Sexual relationship and drive	↑	Martin et al (2016)[35]
Sexual function	↔	Martin et al (2016)[35]
Sleep quality	↔	Martin et al (2016)[35]
Physical activity	↔	Ravussin et al (2015)[17]; Racette et al (2017)[36]; Villareal et al (2016)[31]

Abbreviations: ↑, increase; ↓, decrease; ↔, no change.

for trial end points.[16,17]Repeated-measures analyses, as performed under mixed models, were used, with change from baseline as the dependent variable and treatment and time as the independent variables. Covariates included sex, site, BMI stratum and baseline, along with any variables that were deemed to affect the tested dependent variable. Hypotheses of interest, which were primarily between-group comparisons over time, were tested by defining contrasts among regression parameters, and a gatekeeping strategy and Bonferroni corrections prevented inflation of the type 1 error. These procedures were used in this report to examine the effects of CR versus AL on eating-disorder symptoms and cognitive function, as assessed by the Multifactorial Assessment of Eating Disorder Symptoms (MAEDS) and Cambridge Neuropsychological Test Automated Battery, respectively.

RESULTS

Nutritional intake

The intake-balance method demonstrated that energy intake in the CR group declined from baseline by an average of 480 kcal/d (19.5% CR) during the first 6 months of the intervention. Energy intake then stabilized at 234 kcal/d below baseline (9.5% CR) for the remainder of the trial,[17] resulting in a mean %CR of 11.9% over the entire 24-month period. This is broadly in line with %CR data from sophisticated models,[39] which indicate that %CR was approximately 15% at month 12 and approximately 11% at month 24. Although less than targeted, participants in the CR group achieved a level of CR that was below the upper limit of adherence at month 12 (∼13% CR) and month 24 (∼10% CR), as defined by the CTS.[25] Moreover, the %CR reached by the CR group was greater than that of the AL group, which displayed no change in energy intake during the trial.

Prolonged changes in macronutrient intake were not imposed, but the CALERIE 2 trial included food diaries to estimate changes. Although there are drawbacks of inferring data from self-reported methods of food intake,[40] data from this instrument indicated that the decrease in energy intake in the CR group was primarily attributable to a reduction in fat. Relative to baseline, fat intake was reduced at month 12 and month 24 by 20.2 g/d and 15.3 g/d, respectively; these values were lower than in the AL group.[31] Protein and carbohydrate intake, by contrast, were not altered when the CR and AL groups were compared.[31] Moreover, there were, on balance, few changes in micronutrient, vitamin, and mineral intake.[31]

Safety and monitoring

Four participants were withdrawn from the AL group and 26 participants were withdrawn from the CR group

(8 voluntarily).[41] Of interest, 3 were withdrawn for safety reasons, with 2 withdrawn for reductions in hematocrit and 1 for excessive losses in bone mineral density (BMD).[41] Another 2 participants experienced a potentially adverse reduction in BMD and temporarily discontinued the trial until the loss of BMD returned to the acceptable range. The proportion of adverse events reported by CALERIE 2 participants did not differ between groups. Abnormalities in safety laboratory tests were mostly small, with no between-group differences in the proportion of abnormal values; plus, there were no clinically significant changes in electrocardiogram readings, physical examinations, or vital signs during the trial.[41] Collectively, these results suggest the CALERIE 2 CR regimen was safe and tolerable.

Physiological outcomes

Weight, body composition, and bone density. Reductions in adiposity constitute the major component of body composition changes during CR and are associated with improvements in metabolic health.[30] Nonetheless, dietary restriction can result in losses of skeletal muscle mass and BMD,[42] which can negatively affect physical function and increase mortality risk.[43] During CALERIE 2, the AL group experienced no change in weight, but the CR group lost on average 7.1 kg (9.9%) of body weight at 6 months, 8.3 kg (11.5%) at 12 months, and 7.6 kg (10.4%) at 24 months.[17,30] Additional analyses from the CTS weight data showed that active weight loss occurred until approximately 60 weeks in the CR group, with weight maintenance occurring thereafter.[26] In accord with CALERIE 1,[11,44] the majority of body mass loss in CR participants was from fat mass (5.4 kg), with smaller losses in FFM (2.0 kg). Besides the reduction in total adiposity in the CR group, large reductions in central adiposity were observed at 24 months, with a 6.1 cm and 2.8 kg decrease in waist circumference and trunk fat, respectively. The CR group also had reductions in skeletal muscle lipid deposition, lipid transport, and lipogenesis-related gene expression, illustrating CR-induced reductions in ectopic fat.[45] This is noteworthy because ectopic fat deposition in skeletal muscle[46] and visceral organs[47] is strongly associated with metabolic syndrome and all-cause mortality.

Participants in the CR group lost BMD (∼2%) in clinically important sites, including the spine and hip.[31] Consistent with the reductions in FFM, and proportionally to weight loss, reductions in BMD occurred at month 12 and subsequently stabilized. The changes in BMD occurred along with increases in the bone-resorption markers C-terminal telopeptide of type I collagen and tartrate-resistant acid phosphatase isoform-5b.[31] Responses in other factors related to BMD, namely parathyroid hormone and insulin-like growth factor-1 (IGF-1), were not significantly different between groups, but responses in 25-hydroxyvitamin D concentrations were greater in CR participants. Parathyroid hormone and IGF-1 prevent bone loss,[48] and sufficient 25-hydroxyvitamin D levels are important in attenuating reductions in BMD;[49] as such, these are unlikely to serve as explanatory factors for the CR-induced attenuation of BMD.[31] Taken together, these findings suggest additional approaches, such as resistance training, may be needed to mitigate BMD losses during CR.[50] Indeed, decreases in BMD were mitigated when participants performed exercise during CR in CALERIE 1,[51] and greater activity-related energy expenditure was the strongest predictor of FFM maintenance during CR in CALERIE 2.[52] Nevertheless, modest BMD changes notwithstanding, bone quality, which is an additional factor influencing fracture risk, was not directly measured in CALERIE 2.[51] Thus, consistent with data from individuals who practice prolonged CR,[53] it is plausible that bone quality was preserved during the trial.

Energy expenditure, core temperature, oxidative stress, and biological aging. Defined as the rate at which the body uses energy at complete rest, RMR accounts for approximately 60% to 70% of TDEE in most individuals.[54] An elevated RMR per unit of lean body mass is considered a key risk factor for accelerated aging.[55] However, as outlined in the "rate of living" theory of aging, CR is thought to slow the aging process by decreasing RMR beyond what is expected for the concomitant losses in FFM and fat mass.[27] Termed *metabolic adaptation*, this may lead to a decrease in core body temperature and oxidative damage, which attenuates DNA, lipid, and protein damage, and ultimately slows the aging process.[3,56] RMR, which was measured for 30 minutes via a ventilated hood, decreased significantly in the CR group from baseline to month 12 (5.9%) and remained repressed at month 24 (5.0%).[17] The change in RMR residual (or metabolic adaptation) was nevertheless similar in the CR and AL groups at month 24, implying that CR-induced metabolic adaptation is prominent during the weight-loss phase but does not persist when weight stability is achieved.[17]

Even when changes in body composition were statistically considered, TDEE, as assessed by the DLW technique, remained significantly lower than baseline after 24 months of CR compared with the control.[17] Thus, given that the RMR residual was similar between groups at the end of the trial, the between-group differences in TDEE were likely the result of a decrease in nonresting energy expenditure.[17] This was elegantly supported in an ancillary per-protocol analysis of 34 CR

and 19 AL control participants from the Pennington Biomedical Research Center.[27] Here, 24-hour energy-expenditure assessments in a respiratory chamber showed that reductions in sleep energy expenditure not attributable to changes in body composition were greater at month 24 in the CR group versus the AL group, indicating that CR-induced metabolic adaptation is prominent during sleeping hours.[27] Additional work is required to determine if reductions in low-intensity physical activity (eg, less standing, less walking around, more sitting, less fidgeting) and improvements in the metabolic efficiency of physical activity are also evident.[17]

Decreases in core temperature in response to CR may be key in the CR-related improvements in aging. Similar to findings from primates,[57] among individuals who practiced CR for an average of 6 years, 24-hour core body temperatures were less than that of matched control subjects consuming a Western diet.[58] Though core temperature reductions were observed in CALERIE 1 after CR,[10] core temperature changes were not different between CALERIE 2 groups at months 12 and 24.[17] In the Pennington ancillary study, Redman et al.[27] showed that mean core temperature during sleep was 0.10°C less in the CR group at month 24, but there was only a tendency for between-group differences.

There were CR-induced reductions in urinary F_2-isoprostanes, which are considered reliable markers of nonenzymatic lipid peroxidation and, thus, of tissue oxidative damage.[28] More specifically, there were 13% and 27% decreases in 2,3-dinor-iPF(2a)-III and iPF(2a)-II levels, respectively, at month 24 in the CR group, and values were significantly lower than the AL group.[28] In the Pennington Biomedical participants, change in 2,3-dinor-iPF(2a)-III levels was associated with 24-hour energy expenditure metabolic adaption, tentatively suggesting that CR in humans may slow aging via a biological link between reductions in energy expenditure and oxidative stress.[27] Additional studies are required to determine the long-term changes in these end points and molecular pathways underlying these alterations.

Although the chief end points of CALERIE 2 were conceived on the basis of key theories of aging, other approaches have been advocated as holistic metrics of aging in humans. In this regard, a series of algorithms have been proposed to assess *biological aging*, which is defined as the gradual deterioration in the integrity of the bodily systems over time, where the loss of molecular fidelity in tissues exceeds repair capacity.[59] By definition, the rate of chronological aging increases at the same pace for everyone, whereas the pace of biological aging can be decelerated or accelerated as a consequence of lifestyle.[59] Integration of various circulating biomarkers—such as cholesterol, glycated hemoglobin, systolic blood pressure, white blood cell count, uric acid, and C-reactive protein—using algorithms delivers metrics of biological age.[60] In observational studies, these metrics have been associated with all-cause mortality; [61] thus, the rationale for investigating biological aging in CALERIE 2 was strong. Using 2 established algorithms of biological aging, namely the Klemera-Doubal method[62] and the Homeostatic Dysregulation algorithm,[63] the effects of CR on biological aging were assessed in CALERIE 2. The Klemera-Doubal method indicated that biological age increased by an average of 0.11 years per 12-month period in the CR group versus 0.71 years in the AL group. These results were comparable for the Homeostatic Dysregulation algorithm, with a significant suppression in homeostatic dysregulation shown in the CR group but not the AL group. Sensitivity analyses also demonstrated that the beneficial effects of CR on longevity markers were not related to weight loss, challenging the viewpoint that weight loss is a prerequisite for all CR-related improvements in longevity.[29]

Inflammation. Reduced inflammation is considered an important mechanism by which CR mediates its beneficial effects on aging.[56]Low-grade systemic inflammation is associated strongly with accelerated aging and the development of age-related metabolic disease.[64] After 24 months of CR, decreases in inflammatory markers were seen relative to AL. Though between-group differences in intercellular adhesion molecule-1 were not observed at this point, sustained 40%–50% reductions in circulating concentrations of the pro-inflammatory cytokines tumor necrosis factor-α and C-reactive protein were revealed; plus, relative to AL, CR improved total white blood cell, lymphocyte, and monocyte counts.[17,33] The mechanisms underlying the anti-inflammatory effects of CR are not fully understood, although because there were concomitant changes in circulating leptin and adiponectin, these responses may relate to nutrient-sensing pathways affecting inflammatory gene-activation and redox status.[65]

Glucose tolerance and insulin sensitivity. Insulin resistance is associated with decreased longevity[66] and increased risk for type 2 diabetes, CVD, and cancer.[67] In agreement with CALERIE 1,[15,68] 20%–30% reductions in the homeostatic model assessment of insulin resistance, fasting insulin, and insulin area under the curve were observed in response to CR, and these were greater than in the control group.[32] Furthermore, circulating adiponectin concentrations increased in the CR group only,[31] which likely is related to the increase in insulin sensitivity with CR.[69] There were no differential

responses in fasting glucose or glucose tolerance between groups, however. Although ostensibly at odds with the insulin resistance and insulin sensitivity results, these findings are not wholly unexpected, given baseline glucose values were well within normal limits in this cohort.

Endocrine function. Excessive activation of the IGF-1 pathway is implicated in accelerated aging.[70] In rodents, CR reduces circulating IGF-1 and increases corticosterone concentrations, potentially exerting longevity benefits via autophagic and apoptotic pathways.[71]Twenty-four months of CR did not affect circulating IGF-1 levels in CALERIE 2.[34] Additionally, at month 24, there were no between-group differences in cortisol or growth factors (i.e., transforming growth factor-β1 and platelet-derived growth factor-AB), which have been linked to the pathogenesis of cancer.[72] There was nevertheless a 21% increase in circulating IGF-binding protein-1 (IGFBP-1) in the CR group at month 24.[34] Circulating IGF-1 binds to IGFBP-1, which in turn can inhibit IGF-1 activity. Therefore, the 42% decrease in the IGF-1-to-IGFBP-1 ratio at 24 months in the CR group indicates that free IGF-1 was suppressed because of CR.[73] The mechanisms explaining the increased IGFBP-1 levels are unclear, but CR-induced reductions in circulating insulin may have increased hepatic production of IGFBP-1.[34]

Whether leptin has a direct impact in promoting longevity remains unknown, yet animal models suggest an important role for intestinal, skeletal muscle, and brain leptin signaling in aging via the modulation of inflammation, glucose homeostasis, and mitochondrial efficiency.[74] Consistent with CALERIE 1,[75] no responses in circulating leptin concentrations were seen in the AL group. However, in the CR group, a decrease in circulating leptin of 11.0 ng/mL was observed at 12 months, followed by stable concentrations until 24 months (–9.7 ng/mL from baseline).[27,33] These findings are not surprising because leptin is secreted predominantly from adipose tissue, and circulating levels are more or less proportional to adipose tissue.[76,77] A central role in modulating energy homeostasis has been ascribed to leptin, with some positing that this adipokine primarily counteracts excessive losses of energy stores during energy deficits.[78] In the context of CALERIE 2, the decrease in leptin in the CR group, in part, may drive the metabolic adaptation seen in this group.[17] Such findings may be the result of blunted leptin signaling in the hypothalamus[78] and/or the periphery, with the latter likely occurring through an increase in mitochondrial efficiency.[79] This is tentatively supported by a CALERIE 2 ancillary study in which those with "more coupled" (i.e., higher functioning) mitochondria at baseline had a larger increase in muscle mitochondrial adenosine triphosphate synthesis rates and mitochondrial coupling than those with uncoupled mitochondria.[45]

As with leptin, lower levels of triiodothyronine were observed at 12 and 24 months of CR when compared with the AL group (between-group difference at month 24: –10.9 ng/dL),[17] although no significant changes were seen in thyroid-stimulating hormone (between-group difference at month 24: –0.1 uIU/mL).[17] The thyroid axis is associated with longevity in animal models[80] and is involved in the regulation of metabolic rate and core temperature,[81] in conjunction with insulin, leptin, glucocorticoids, and reproductive hormones.[82] Longevity associated with low levels of thyroid hormones has likewise been attributed to lower reactive oxygen species generation and oxidative stress as a result of lower thermogenesis.[83] In this respect, the findings from 53 CALERIE 2 participants may demonstrate that the thyroid axis influences these pathways in humans, given CR stimulated a reduction in core body temperature during sleep and lowered oxidative stress.[27] That said, a strong mechanistic link between reduced thyroid function and longevity remains to be elucidated.

A concern of sustained CR is negative alterations in reproductive hormones that can manifest as decreased libido, loss of strength, and/or decreased quality of life.[8] A mean 0.76 µg/mL increase in sex hormone binding globulin (SHBG) levels were found in men after 12 months of CR versus AL, and this was maintained at 24 months (between-group difference: 0.92 µg/mL).[35] SHBG binds to sex hormones and subsequently inhibits function,[84] yet SHBG may have biological effects beyond regulation of free sex-hormone levels because levels are inversely associated with insulin resistance[85] and obesity.[86] As highlighted, there were substantial improvements in insulin sensitivity in the CR group that coincided with reductions SHBG levels. However, because insulin is an inhibitor of SHBG secretion in vitro,[87] work is required to ascertain how changes in SHBG and insulin sensitivity during CR are connected. Compared with the AL group, there was also a reduction in free testosterone levels at 12 months in the CR group (between-group difference: –2.98 ng/dL), but this was no longer present at 24 months (between-group difference: –0.96 ng/dL).[35] No group differences were observed in levels of total testosterone, luteinizing hormone, follicle-stimulating hormone, or dehydroepiandrosterone, and there were no reports of amenorrhea, suggesting CR had no impact on reproductive function.[35] Though it must be acknowledged that most women were taking an oral contraceptive, this is consonant with research showing that CR does not influence the hypothalamic-pituitary-gonadal axis.[88]

Cardiometabolic disease risk markers. The Global Burden of Disease Study researchers estimated that poor dietary patterns were responsible for 10.9 million deaths in 2017, with CVD related to 9.5 million of these deaths.[89] Although dietary strategies to prevent future CVD events are predominantly aimed at those who show conventional CVD risk factors (e.g., high level of low-density lipoprotein–cholesterol [>100 mg/dL], low level of high-density lipoprotein–cholesterol [<40 mg/dL], elevated total cholesterol level [>200 mg/dL], high triglyceride levels [>150 mg/dL]), less consideration has been given to individuals with clinically healthy values. In CALERIE 1, CR improved these risk markers over 6–12 months in healthy individuals,[90,91] but CALERIE 2 offered the chance to determine if these positive shifts were maintained or improved with sustained CR.

At 24 months, in the CR group, total cholesterol levels were reduced from 166 to 156 mg/dL, serum low-density lipoprotein–cholesterol concentrations were reduced from 97 to 90 mg/dL, and serum high-density lipoprotein–cholesterol concentration increased from 49 to 53 mg/dL.[17,32] These changes were significantly different from the AL group, which experienced virtually no changes in blood lipid concentrations. Adverse blood cholesterol profiles are chief in triggering and predicting CVD[92]; thus, these improvements in response to CR are encouraging. There was also a CR-induced reduction in serum triglyceride concentrations from 102 to 88 mg/dL, which was greater than the decrease in the control group and is clinically important because lower triglycerides are associated with decreased CVD risk independent of cholesterol levels.[93] In addition, the triglyceride-to-high-density lipoprotein–cholesterol ratio, which reflects atherogenic small, dense, low-density lipoprotein particles[93] and is associated with CVD risk,[94] improved from 2.1 to 1.4.

Elevated blood pressure is another key risk factor for the development of CVD.[95] Attenuations in blood pressure, therefore, could lead to clinically meaningful changes in CVD risk in those characterized with healthy baseline readings. In CALERIE 2, after 24 months, the CR group showed a significant drop in systolic blood pressure and diastolic blood pressure of 2.2 mm Hg and 3.4 mm Hg, respectively, with significant differences versus the control group.[17,32] Considered as a whole, these data imply that CR exerted robust improvements in CVD risk markers. This is corroborated by analysis of the metabolic syndrome score, which showed that CR provoked a decrease in metabolic syndrome risk from month 12 until the end of the trial.[32] Though the mechanisms by which CR mediates these improvements are not clear, it may be that concomitant reductions in inflammation and oxidative stress play a role.[96]

Physical performance. Because of the documented decrease in FFM with CR, a decline in absolute aerobic capacity and physical strength are likely to occur. Such changes are worrisome for CR researchers because poorer aerobic capacity and strength have been associated with increased risk of death.[97,98] In CALERIE 2, maximal oxygen uptake relative to lean mass did not change, whereas maximal oxygen uptake relative to body mass increased in the CR group by 5% after 24 months, and this was greater than the change seen in the AL group (–3%).[36] Moreover, exercise time during the maximal-oxygen-uptake treadmill test increased to a greater extent in the CR group (2.9 min) compared with the AL group (1.8 min), suggesting aerobic performance was enhanced.

With respect to strength, there was a 3%–7% decline in knee extensor and flexor strength in the CR group, which was unsurprising considering the loss of lean mass and the lack of structured resistance exercise in the CR regimen. Nevertheless, 4%–6% improvements were demonstrated when strength was expressed relative to body mass, implying that functionality was not compromised.[36] Future studies that incorporate resistance training into CR regimens must ascertain if strength training can ameliorate CR-induced reductions. Likewise, given evidence linking protein intake with the preservation of muscle functionality,[99] studies are needed to investigate if losses in strength are attenuated during CR when protein intake is augmented.

Psychological and behavioral outcomes

The CALERIE 2 trial used several strategies to augment participant adherence and decrease attrition, on the basis of evidence from previous behavioral intervention trials.[21,22] Continuous assessments of outcomes associated with these strategies are consequently needed to ascertain intervention efficacy. Furthermore, studying psychological and behavioral responses to CR determines the applicability of CR and identifies pivotal constructs that assist individuals in adhering to CR for sustained periods.[100]

Mood, stress, and quality of life. The Beck Depression Inventory was administered to monitor mood disturbances throughout the CALERIE 2 trial. Five participants from the CR group and 1 from the AL group presented scores indicative of severe depression (scores of $\geq$30 on the inventory). Per the surveillance protocol,[16,101] these participants subsequently completed a clinical interview and none exhibited depression symptoms that warranted discontinuation.[41]

Table 2 **Changes in Multifactorial Assessment of Eating-Disorder Symptoms and Cambridge Neuropsychological Test Automated Battery outcomes from baseline in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy 2 trial.**

Variable	Visit	AL (n = 75)		CR (n = 143)		*P*
		Mean	SE	Mean	SE	
Multifactorial assessment of eating-disorder symptoms						
Depression (T-score)	Baseline	38.09	0.72	38.32	0.48	0.58
	Δ month 12	0.38	0.58	0.63	0.44	0.72
	Δ month 24	1.21	0.56	0.06	0.43	0.09
Binge eating (T-score)	Baseline	43.99	0.99	45.09	0.78	0.44
	Δ month 12	−0.82	0.74	−1.58	0.56	0.40
	Δ month 24	0.39	0.81	−0.47	0.62	0.38
Purgative behavior (T-score)	Baseline	44.47	0.31	45.22	0.29	0.11
	Δ month 12	−0.15	0.35	−0.36	0.26	0.62
	Δ month 24	−0.49	0.36	0.18	0.27	0.13
Fear of fatness (T-score)	Baseline	44.61	0.97	44.04	0.81	0.47
	Δ month 12	0.75	0.72	1.53	0.54	0.36
	Δ month 24	0.73	0.78	2.52	0.59	0.06
Restrictive eating (T-score)	Baseline	42.76	0.73	44.59	0.60	0.10
	Δ month 12	−0.66	0.56	0.86	0.43	0.03
	Δ month 24	−0.83	0.72	0.87	0.56	0.06
Avoidance of forbidden foods (T-score)	Baseline	50.72	1.19	51.80	0.74	0.40
	Δ month 12	1.50	0.85	8.01	0.64	<0.01
	Δ month 24	2.70	0.83	7.98	0.64	<0.01
Cambridge Neuropsychological Test Automated Battery						
Reaction time (ms)	Baseline	339.15	6.69	331.91	3.87	0.50
	Δ month 12	12.83	4.84	0.32	3.81	0.03
	Δ month 24	6.82	4.89	−0.09	3.92	0.24
Rapid visual information processing (signal detection measure)	Baseline	0.93	0.01	0.94	<0.01	0.91
	Δ month 12	0.02	<0.01	0.02	<0.01	0.84
	Δ month 24	0.02	<0.01	0.02	<0.01	0.66
Delayed matching to sample (% correct)	Baseline	90.41	1.99	90.80	1.17	0.64
	Δ month 12	−2.85	1.74	0.24	1.34	0.15
	Δ month 24	−4.63	1.87	−1.75	1.48	0.22
Verbal recognition memory (no. correct)	Baseline	8.22	0.21	8.27	0.15	0.87
	Δ month 12	−0.34	0.24	−0.03	0.19	0.28
	Δ month 24	−0.20	0.24	−0.17	0.19	0.92
Intra-extra dimensional shift (total errors)	Baseline	27.32	4.15	21.94	2.27	0.24
	Δ month 12	−7.45	1.83	−8.14	1.43	0.75
	Δ month 24	−7.54	1.82	−8.20	1.45	0.76

For baseline values, data are observed mean ± SE and *P* values were determined by Wilcoxon test. Estimated change in outcome measures and *P* values were determined through an intent-to-treat approach to determine whether change on the outcome variables differed between groups. *Abbreviations*: AL, ad libitum; CR, calorie restriction.

In CALERIE 1, mood was not modified by CR,[102] but CR enhanced mood in CALERIE 2, with a modest 0.76-point decrease on the Beck Depression Inventory mood disturbance subscale at month 24 in the CR group relative to the AL group.[35] The CR group similarly exhibited improvements in the tension-anxiety subscale of the Profile of Mood States[103] compared with the AL group at month 24 (between-group difference: –0.79 points).[35] Though effect sizes were modest, these improvements are noteworthy considering participants scored low on the mood disturbance subscale at baseline. Furthermore, some have suggested that energy restriction can exert negative effects on mood,[104] but CALERIE 2 showed that sustained CR had no adverse impact on mood even in healthy individuals without obesity.

Assessment of the Rand 36-Item Short Form[105] revealed that the CR group had an increase in quality of life compared with the AL group, though this was only evident for the general health perceptions (between-group difference: 6.45 points). Despite no between-group differences, unpublished intent-to-treat analyses showed that in the CR group, there were no reductions in MAEDS depression T-score (Table 2) and perceived stress.[35] Additional analyses suggested that the degree of weight loss attained was linked to changes in aspects of mood and quality of life, with significant correlations found between percent weight loss and vigor, mood disturbance, and the general health subscale on the Rand 36-Item Short Form.[35] Overall, these results demonstrate that CR does not adversely influence quality of life. Some findings, in fact, demonstrate that CR may

actually enhance mood and quality of life, and that greater decreases in weight may predict improvements in such outcomes.[35] Changes of this nature are extremely encouraging and support the feasibility of sustained CR in individuals without obesity.

Eating behaviors, eating attitudes, and disordered eating. Changes in habitual eating patterns and attitudes are expected in dietary interventions. Such alterations are often the focus of the intervention and assist individuals in meeting dietary, weight, and health targets.[102,106] In this regard, self-efficacy in the context of managing food intake is important in response to dietary interventions, with an increase routinely seen in weight loss trials.[107] According to responses on the Weight Efficacy Lifestyle Questionnaire,[108] the CR group experienced an increase in self-efficacy for regulating food intake compared with the AL group (between-group difference: 8.64 points), particularly during situations of social pressure and physical discomfort.[37] This is a notable success of the behavioral and nutritional strategies implemented in the CR intervention because an enhancement of self-efficacy was an important goal.[20] Additional analyses showed that greater elevations in self-efficacy were associated with larger reductions in weight, possibly implying that an increase in self-efficacy for controlling food intake was effective in facilitating adherence to the CR intervention.[37] Research is needed to determine if elevations in self-efficacy can be attained in settings where less intervention support is available. Indeed, though between-group variations were detected at month 12, differences were larger at month 24, implying that, potentially, prolonged interventional training and support may be required to sufficiently enhance self-efficacy to assist CR-related goals.

The CR group experienced a large and expected 4.70–4.80 point increase in dietary restraint,[37] which is defined as the cognitive intent and ability to limit food intake.[109] Similarly, original MAEDS data from CALERIE 2 (Table 2) show that at 12-month intervals, there was an average 7.98- to 8.01-point increase in the avoidance of forbidden foods subscale. These findings are in line with CALERIE 1[106] and trials involving individuals with obesity,[110] and likely are attributable to the intervention strategies that advocated the avoidance of energy-rich foods and consumption of smaller food portions. It is interesting, though, that changes in restraint and avoidance of forbidden foods were not associated with %CR or weight loss.[37] Therefore, although CALERIE 2 findings illustrate that restraint and avoidance for forbidden foods are elevated in periods of both weight loss and weight maintenance, contrary to some research in individuals with obesity,[111] these constructs are not likely to mediate the changes in dietary intake and weight seen in CALERIE 2.

There was a significant 0.68-point rise in reported dietary disinhibition at months 12 and 24 in the CR group,[37] which some claim could lead to maladaptive eating behaviors and eating disorders such as anorexia nervosa and bulimia.[109] However, the small increase in disinhibition compared with the control group, is unlikely to be clinically meaningful because, importantly, CALERIE 2 participants reported low scores at baseline (average score, 4.8) and scores were within normal limits at the end of the trial.[112] It is also notable that no participants were removed due to the eating-disorders screening protocol,[41] which used the MAEDS[113,114] and, if scores in this instrument were abnormal, an Interview for the Diagnosis of Eating Disorders-IV to determine if significant eating-disorder pathology was present.[16,115] Furthermore, as shown by novel comparisons (Table 2), the CR group and the AL group exhibited no differences on the binge-eating subscale of the MAEDS. Elevations in binge eating, especially in tandem with restraint, are symptomatic of eating disorder pathology; hence, these findings illustrate that the CR regimen did not increase susceptibility to eating disorders. This is underlined by other specific between-group comparisons on the MAEDS subscales, which showed that changes in purgative behavior, fear of fatness, and restrictive-eating scores were similar between the CR and AL groups (Table 2). Additionally, the CR group displayed a 5.51- to 8.51-point decrease in the Body Shape Questionnaire[116] score versus the AL group, indicating that concerns with body shape were reduced as a result of the CR intervention. From the perspective of eating-disorder symptoms, this is notable because high body dissatisfaction, as assessed via the Body Shape Questionnaire, is symptomatic of eating-disorder pathology[116] and may drive the association between dietary restraint and eating disorders.[117] Thus, these findings suggest that the increase in dietary restraint and the avoidance of forbidden foods subscales in the CR group were benign and reflective of the cognitive and behavioral changes necessary to restrict energy intake for 24 months in an obesogenic environment.

Appetite and food cravings. An increase in subjective appetite during energy restriction interventions is a frequently cited reason for substandard adherence and weight regain.[118] Energy deficits are hypothesized to initiate compensatory mechanisms that attempt to defend body weight, particularly when deficits occur for prolonged periods.[118] Along with dietary restraint and disinhibition, the Eating Inventory[112] examines perceived hunger, and this was unchanged in both groups

during CALERIE 2.[37] Likewise, examinations of the state hunger and desire to eat constructs of the Food Craving State Questionnaire[119] demonstrated clinically unimportant between-group variations, with differences of < 1 point on a 15-point scale.[37] These results are consistent with a recent analysis that investigated changes in retrospective (over the previous week) appetite of the CR group periodically throughout the intervention.[26] Polynomial regressions revealed that although there was a gradual increase in hunger during the intervention, the total increase was approximately 3 mm on a 100-mm visual analogue scale; hence, the clinical relevance is questionable.[26] There were also no changes in other appetite constructs, namely fullness, prospective food consumption, and satisfaction, bolstering the view that the CALERIE 2 intervention did not trigger alterations in appetite perceptions.[26]

Although increases in appetite are common during energy restriction, a surprising phenomenon seen during behavioral weight-loss interventions in individuals with obesity is a decrease in food cravings.[120] This is postulated to occur through a deconditioning mechanism, whereby restriction of certain foods extinguishes the association between the intake of those foods and particular stimuli.[120] Given this evidence, it was hypothesized that the CR group would display a decrease in food cravings for unhealthy foods, yet no significant changes were observed.[37] Although it is unclear why food cravings were not reduced, it is likely that the low food cravings documented at baseline in CALERIE 2 participants (1.42–2.00 points) left little opportunity for a decrease.[37] Regardless, it is encouraging that 24 months of sustained CR provoked no elevation in food cravings in individuals without obesity who had low food cravings at the interventions onset.

Cognition, sexual function, and sleep. Cognitive performance was evaluated in CALERIE 2 because CR-induced impairments would jeopardize the feasibility of sustained CR. Additionally, the influence of CR on cognition is equivocal, with some reports associating CR with a decrement in cognitive performance[19] and others finding no relationships.[121] The CALERIE 2 trial used the Cambridge Neuropsychological Test Automated Battery, a computerized touch-screen test, to measure changes in cognitive function. Six modules were assessed: reaction time, verbal recognition memory, the intra-extra dimensional shift (a module that tests the ability of the test taker to attend to the specific attributes of compound stimuli and shift attention), rapid visual information processing (a module focused on sustained attention and working memory), delayed matching to sample (a test that requires participants to recall complex visual patterns), and spatial working memory.[122] In an analysis of spatial working memory, Leclerc et al[38] used generalized estimating equation models to show that the CR group had greater improvements in the spatial working memory error count than the AL group at month 24; however, the CR group scored lower at baseline, potentially confounding this finding. Besides this observation, intent-to-treat analyses of these data revealed largely no between-group differences in the cognitive function end points at months 12 and 24 (Table 2). Furthermore, compared with the AL group, the CR group experienced no significant negative changes in scores on a Stroop color-naming task, Polysemous Words Test, and a Word Stem Completion task, suggesting that CR did not adversely affect attentional, selective interpretation, and memory bias for items or words related to depression, body size or shape, and food. Such findings are notable because some cognitive impairment as a result of dietary restriction may be caused by obsessive preoccupations with food and body weight.[123] Together, these results illustrate that sustained CR did not result in decrements in cognitive performance, supporting results from CALERIE 1.[121]Longer-term studies are needed to ascertain if CR alters the age-related decline in cognition or the development of degenerative diseases in light of findings from rodent models.[124]

In addition to cognitive performance, CALERIE 2 examined changes in sexual function using the Derogatis Interview for Sexual Function-Self report, an instrument that assesses sexual cognition and fantasy, sexual arousal, sexual behavior and experience, orgasm, and sexual drive and relationship.[125] Generally, despite changes in SHBG and free testosterone levels, no changes were observed in these Derogatis constructs. The CR group, however, had a small 1.06-point improvement in the sexual drive and relationship subscale compared with the AL group at month 24.[35] Previous evidence from individuals with obesity has documented improvements in sexual function with weight loss.[126] The findings from CALERIE 2 extend this body of work by showing that CR in individuals without obesity induces no negative changes in sexual function and may enhance desire for sexual activity and relationships.

The impact of CR on sleep has received relatively little attention, evidence linking poor sleep with negative physical and mental health notwithstanding.[127] In CALERIE 2, sleep quality was measured with the Pittsburgh Sleep Quality Index, which produces 7 subscales of sleep quality, including overall sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction.[128] Although the CR group showed an improvement in sleep duration relative to the AL group

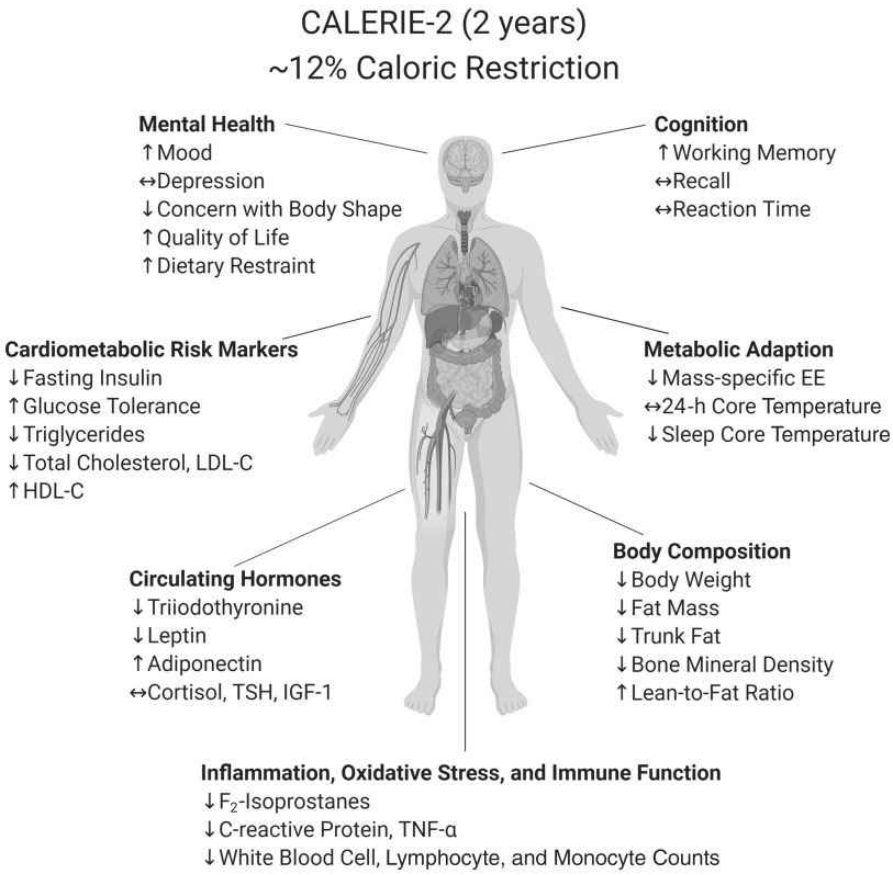

Figure 1 **Highlights of the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) 2 trial**. *Abbreviations:* EE, energy expenditure; HDL-C, high-density lipoprotein–cholesterol; IGF-1, insulin-like growth factor-1; LDL-C, low-density lipoprotein–cholesterol; TNF-α, tumor necrosis factor-α; TSH, thyroid-stimulating hormone.

at month 12 (between-group difference: −0.26 points), between-group variations in other subscales were not evident at this point and no significant differences were observed for any subscales at month 24.[35] Therefore, it appears that prolonged CR does not adversely influence sleep quality.

Physical activity. Hypothetically, it is possible that compensatory reductions in physical activity may have attenuated the energy deficits imposed by CR. All CALERIE 2 participants were informed at baseline of the health guidelines recommending 30 min/d of moderate physical activity for a minimum of 5 d/wk, although physical activity was not prescribed for either group.[16]Self-reported physical activity decreased to a greater extent in the CR group than the AL group when assessed by the Stanford 7-day Physical Activity Recall.[31] Although accelerometry was not used, objectively measured physical-activity energy expenditure, which was calculated by subtracting RMR and the thermic effect of food from DLW-derived TDEE, was also assessed. This indicated that activity energy expenditure was reduced by 64–84 kcal/d in the CR group at months 12 and 24 relative to baseline, though no between-group differences were observed.[36] However, there are limitations in self-report measures of physical activity[40] and there are confounding effects of RMR reductions on physical-activity energy expenditure values during weight loss.[23,36] Therefore, DLW-determined physical-activity energy expenditure also was expressed relative to RMR, and revealed no significant changes in either the CR or AL group.[17,36] This finding is encouraging because physical activity has a plethora of health benefits and may mitigate CR-induced reductions in muscle and bone mass.

CONCLUSION

The results of CALERIE 2 provide evidence that sustained, moderate (11.9%) CR in individuals without obesity improves a multitude of physiological, psychological, and behavioral outcomes (Figure 1). Strikingly, these changes transpired when several traditional clinical risk factors were already well below the risk thresholds. There were reductions in FFM and BMD that, though expected during prolonged CR,[31] should be

considered and monitored; nonetheless, CR over 24 months appears safe. Overall, CALERIE 2 illustrates that in healthy individuals without obesity, CR is an accessible and safe dietary intervention that can be implemented early in life to improve longevity biomarkers and reduce the lifetime risk of developing cardiometabolic conditions.

There are, however, 2 crucial areas of scientific inquiry needed to bolster the evidence yielded from CALERIE 2. First, studies are needed to explore the relative importance of diet composition in optimizing the benefits of CR. In CALERIE 2, the sole objective was to induce a fixed %CR and no stringent stipulations regarding macronutrient and micronutrient composition were implemented. Although derived from flawed measures,[40] the CR group reduced fat intake, and such changes could have altered the effects of CR. With limited long-term studies, the influence of macronutrient and micronutrient intake on markers of aging remains controversial. Some investigators, for instance, have shown that ketogenic diets, which aim to elevate endogenous ketogenesis by severely restricting carbohydrate intake, can improve mortality rates in rodents.[129] Likewise, a collection of researchers posit that protein restriction is pivotal in mediating the aging-related benefits of CR,[130] whereas others suggest that increased dietary protein intake with aging is necessary to stimulate tissue regeneration, offset muscle loss, and increase longevity.[131] Variability in findings and beliefs are rife in the fields of aging and health, necessitating high-quality, well-powered randomized trials. Second, it is essential that carefully conducted intervention studies, such as CALERIE 2, continue to follow cohorts to investigate the legacy effects of relatively short-term CR interventions. Although observational studies indicate that voluntary long-term CR is viable,[4] a drawback of most dietary restriction and weight loss regimens is a lack of sustained adherence that ultimately leads to weight regain.[132] Prospective assessments are needed in individuals before they adopt CR and for years after CR is initiated in order to identify strategies that optimize adherence. Additionally, studies into new dietary approaches that modify patterns of intake throughout the day are needed, given these may be more beneficial for long-term adherence and, therefore, may be applicable to more people, with a greater yield in terms of health benefits.[133]

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Declaration of interest. The authors have no relevant interests to declare.

DATA AVAILABILITY

Data from CALERIE 2 can be accessed at https://calerie.duke.edu/.

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