Effect of 2-year caloric restriction on organ and tissue size in nonobese 21- to 50-year-old adults in a randomized clinical trial: the CALERIE study

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ABSTRACT

Background: Sustained calorie restriction (CR) promises to extend the lifespan. The effect of CR on changes in body mass across tissues and organs is unclear.

Objectives: We used whole-body MRI to evaluate the effect of 2 y of CR on changes in body composition.

Methods: In an ancillary study of the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) trial, 43 healthy adults [25–50 y; BMI (kg/m^2): 22–28] randomly assigned to 25% CR ($n = 28$) or ad libitum (AL) eating ($n = 15$) underwent whole-body MRI at baseline and month 24 to measure adipose tissue in subcutaneous, visceral, and intermuscular depots (SAT, VAT, and IMAT, respectively); skeletal muscle; and organs including brain, liver, spleen, and kidneys but not heart.

Results: The CR group lost more adipose tissue and lean tissue than controls ($P < 0.05$). In the CR group, at baseline, total tissue volume comprised 32.1%, 1.9%, and 1.0% of SAT, VAT, and IMAT, respectively. The loss of total tissue volume over 24 mo comprised 68.4%, 7.4%, and 2.2% of SAT, VAT, and IMAT, respectively, demonstrating preferential loss of fat vs. lean tissue. Although there is more muscle loss in CR than AL ($P < 0.05$), the loss of muscle over 24 mo in the CR group comprised only 17.2% of the loss of total tissue volume. Changes in organ volumes were not different between CR and AL. The degree of CR (% decrease in energy intake vs. baseline) significantly ($P < 0.05$) affected changes in VAT, IMAT, muscle, and liver volume (standardized regression coefficient ± standard error of estimates: 0.43 ± 0.15 L, 0.40 ± 0.19 L, 0.55 ± 0.17 L, and 0.45 ± 0.18 L, respectively).

Conclusions: Twenty-four months of CR (intended, 25%; actual, 13.7%) in young individuals without obesity had effects on body composition, including a preferential loss of adipose tissue, especially VAT, over the loss of muscle and organ tissue. This trial was registered at www.clinicaltrials.gov as NCT02695511. *Am J Clin Nutr* 2021;114:1295–1303.

Keywords: aging, caloric restriction, body composition, magnetic resonance imaging, organ, brain

Introduction

Caloric restriction (CR) is defined as the long-term restriction of dietary energy intake while still fulfilling nutrition requirements and is a promising lifestyle intervention to increase the lifespan. The innate progression of aging is defined as primary aging (1). Secondary aging is the acceleration of the aging process by extrinsic factors such as morbidities (1). In healthy, nonobese humans, it has been found that prolonged CR may delay primary aging via enhancing resting energy efficiency and lowering systemic oxidative damage (2). CR has been proposed to attenuate secondary aging by lowering risks for chronic disease (1).

Until now, body composition in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) trial have been described using DXA in 3 compartments, including fat mass, fat-free mass (FFM), and bone (3). Fat tissue, however, is not a homogeneous depot. Visceral adipose tissue (VAT) and intermuscular adipose tissue (IMAT) are closely related to cardiovascular disease and type 2 diabetes and hence secondary aging (4, 5). Prolonged CR in healthy, nonobese adults has been shown to reduce body fat mass (3) as well as VAT (6) and liver fat (7). However, the effect of long-term CR on IMAT
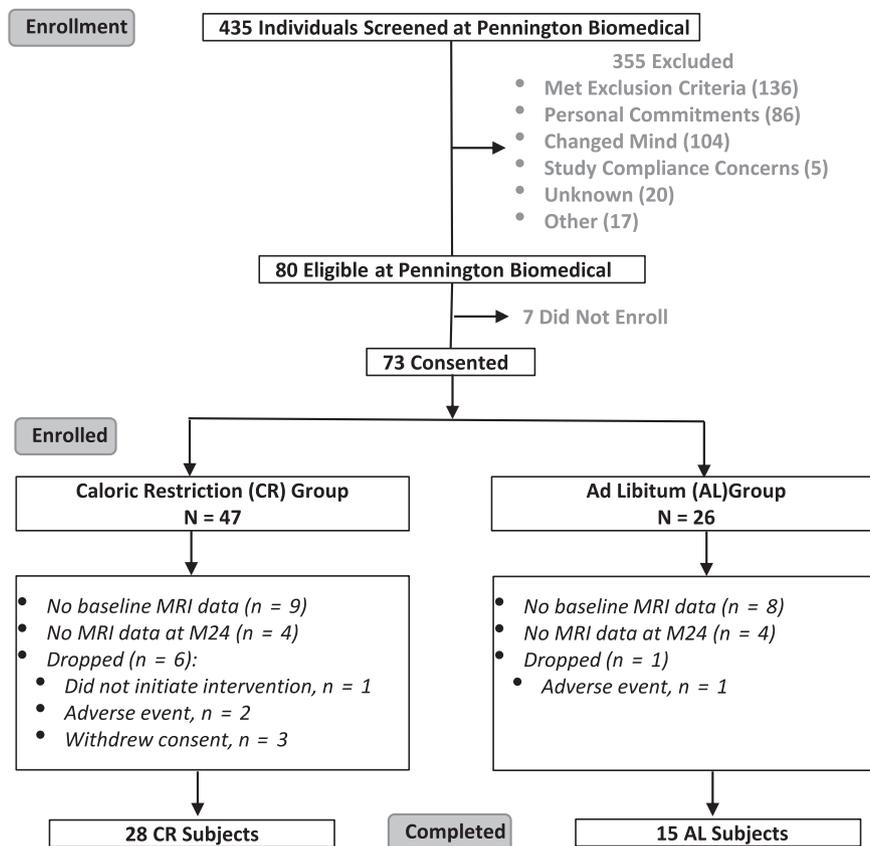
FIGURE 1   Study participants throughout from enrollment ($n = 73$) to data analysis ($n = 43$). M24, month 24.

is unclear. Furthermore, FFM is a mixture of muscle, organs, bone, tendons, etc. Muscle is related to quality of life and the conservation of muscle mass later in life can attenuate the adverse effects of aging (8). Each organ has a distinct physiological function. As the metabolic rates of organs, including brain, heart, liver, and kidneys, at rest are ∼15 to 30 higher than muscle (9, 10), organ mass influenced weight-loss–induced metabolic adaption in obese individuals (11–15). It is currently unknown how much muscle mass loss and organ mass loss occurs during sustained CR in individuals without obesity.

Whole-body MRI has the ability to quantify organ mass that is related to primary aging, as well as body components (e.g., VAT, IMAT, and muscle) that are related to secondary aging. The aim of the present study was to examine changes in body composition [i.e., subcutaneous adipose tissue (SAT), VAT, IMAT, skeletal muscle, and organs, including brain, liver, kidneys, and spleen] with 24 mo of sustained CR, and to understand how the level of CR (% CR) and participant characteristics (baseline weight, age, sex, and race) modulate these changes. Understanding the composition of changes in body mass with CR may help to explain the benefits for primary and secondary aging and potential caveats with losses of lean tissues.

Methods

Study design and study participants

This was an ancillary study conducted from 8 May 2007 to 26 February 2010 in a subset of participants enrolled in

the CALERIE 2 ancillary trial (NCT02695511) at Pennington Biomedical Research Center. CALERIE 2 was a multicenter randomized clinical trial that tested the efficacy of a 24-mo intervention targeting a sustained 25% CR compared with a control group that ate ad libitum (AL) (16–18). The ancillary study protocol was approved by the institutional review boards at Pennington Biomedical Research Center (Baton Rouge, LA) and Columbia University Irving Medical Center (New York, NY). All participants provided written, informed consent for ancillary study procedures. Details on the study recruitment and screening process and exclusion criteria can be found elsewhere (16, 19) and are summarized in the Consolidated Standards of Reporting Trials (CONSORT) diagram in **Figure 1**.

In CALERIE 2, individuals were healthy with a BMI (kg/m^2) between 22.0 and 28.0 at enrollment (16, 19). Men were aged between 21 and 50 y and women were aged between 21 and 47 y to avoid menopause onset throughout the trial. Exclusion criteria included a history of significant medical conditions (e.g., cardiovascular disease and diabetes), abnormal laboratory markers, psychiatric or behavioral problems, or regular use of medications except for oral contraceptives (16). Participants were randomly assigned with a 2:1 allocation to the CR group. Randomization was stratified by sex and BMI. Details about the intervention have been reported (16, 18, 20). The CR intervention targeted an immediate and sustained 25% restriction of energy intake from baseline energy requirements as determined by doubly labeled water (16, 21). Participants in the CR group followed an intensive behavioral intervention that resulted in weight loss through week 60 followed by a plateau

(22). A mathematical model with predicted weekly changes in body weight was used to guide adherence to the intervention (18, 23).

Anthropometrics, demographics, % CR, and DXA measurements were collected as part of the parent study. Race was self-reported by participants as part of a demographic questionnaire during screening (19). The average % CR over 6-mo intervals was calculated retrospectively by the intake-balance method with the 6-, 12-, 18-, and 24-mo measures of total daily energy expenditure from doubly labeled water and changes in body composition measured by DXA between the time periods and relative to baseline (20, 23–25). The primary outcomes of the CALERIE 2 ancillary study are change in 24-h sedentary energy expenditure and sleeping energy expenditure from baseline. The secondary outcome measures are change in oxidative stress from baseline and change in organ/tissue size from baseline (2). The primary outcomes have been reported previously (2). The present study is part of the secondary aims of the CALERIE 2 ancillary study.

MRI-measured body composition

At baseline and month 24, whole-body MRI scans were acquired in the Biomedical Imaging Core at Pennington Biomedical using a 3.0-T Sigma Excite system (General Electric). MRI images were acquired from tips of the fingers acquired with arms stretched above the head to the bottom of the feet (26–28). A 3D gradient echo sequence with a repetition time of 3.5 ms and an echo time of 1.7 ms was used for whole-body MRI scans using a torso phase array coil. The acquisition matrix is 380×192 and the slice thickness of the contiguous axial slice is 3.4 mm. For brain imaging, axial contiguous brain MRI scans were acquired using a spin echo sequence with a slice thickness of 5 mm, a repetition time of 3500 ms, and an echo time of 98 ms. Following acquisition, images were segmented for SAT, VAT, IMAT, skeletal muscle, brain, liver, spleen, and kidneys at the Image Analysis Core Laboratory of the New York Obesity Nutrition Research Center by a blinded experienced MRI technician using the image analysis software SliceOmatic 5.0 (Tomovision Inc.) (29). Tissue compartment volume was calculated as previously described (30). Organs include brain, liver, spleen, and kidneys. The residual lean tissue included bones, tendons, connective tissue, heart, digestive tract, but not the lungs as lung regions are primarily composed of air. Since residual lean tissue is a mixture of bone and other soft tissues, there is not a universal density that can be used to convert residual lean tissue volume to lean tissue mass. Therefore, for consistency across organs and tissues, we report MRI-measured tissue volume for all body components.

The intraclass correlation coefficient for volume rendering of brain, liver, spleen, kidneys, skeletal muscle, and adipose tissue for the same scan by the same analysts at the Image Analysis Core Laboratory of the New York Obesity Nutrition Research Center is 0.95–0.99 (26, 31, 32).

Statistical analysis

The primary analysis was to evaluate *1*) body-composition changes in the CR and AL groups at month 24 and *2*) difference in body composition changes between the CR and AL groups. The secondary analysis was to evaluate how % CR, baseline weight, baseline body composition, age, sex, and race modulate the changes in body composition. Data are presented as the mean ± SEM unless otherwise noted.

Regression models were used for comparisons between baseline and month 24 and for comparisons between the CR group and AL group, with the covariates tested including age, sex, race, and baseline body composition. In the CR group, to evaluate the effect of % CR on body composition, additional regression models were established for the changes in each body component over the 24-mo intervention as dependent variables and % CR, sex, race, age, and baseline weight as potential independent variables. In the % CR effect regression analysis, baseline weight was replaced by corresponding baseline body-composition values (e.g., baseline SAT was included in the model with ΔSAT as the dependent variable). Biologically plausible 2-way interactions were included in the regression models. Multivariable regression models were built using stepwise regression, with $P = 0.05$ for entry and retention.

Shapiro-Wilk test was applied to test the normality of the residual distributions. When necessary, variable values were mathematically transformed to normalize the residual distributions. Log transformations were applied initially and followed by Box-Cox transformations if necessary (33).

All statistical analyses were carried out using SAS 9.4 package (SAS Institute, Inc.). Two-tailed ($\alpha = 0.05$) tests of significance were used.

Results

Baseline

Study participants with MRI scans were predominantly female (69.8%) and White (72.1%) (**Table 1**). The age range of the study participants with MRI scans was 25 to 50 y, similar to the age range of the overall participants in the parent study (Table 1) (3). The AL group and the CR group did not differ in baseline demographics, body weight, BMI, or body composition, including individual organ sizes measured by MRI (Table 1 and **Table 2**).

Total adipose tissue volume from MRI was highly correlated with fat mass measured by DXA ($r = 0.961$, $P < 0.0001$). Similarly, total lean tissue volume was strongly correlated with FFM measured by DXA ($r = 0.983$, $P < 0.0001$).

MRI-measured tissue volume changes

CR group vs. AL group.

The 24-mo CR intervention resulted in a 11.6% weight loss (i.e., -8.4 ± 0.5 kg); this was significantly different from the AL group, which had a small (i.e., 2.8%, 1.9 ± 0.8 kg) and not statistically significant weight gain. In contrast to the AL group (Table 2), there were significant reductions in SAT, VAT, IMAT, and muscle from baseline observed in the CR group (all $P < 0.05$). The differences between AL and CR groups were significant for reductions in SAT, VAT, IMAT, muscle, but not for the reductions in liver volume.

Adipose vs. lean tissue loss during 24-mo CR.

In the CR group, SAT, VAT, and IMAT comprised 32.1%, 1.9%, and 1.0% of total tissue volume at baseline. The reduction in SAT, VAT, and IMAT comprised 68.4%, 7.4%, and 2.2% of the

TABLE 1 Characteristics at baseline for the participants with whole-body MRI and all participants[1]

	Group			
	Participants with MRI (*n* = 43)		Overall (*n* = 218)	
	AL (*n* = 15)	CR (*n* = 28)	AL (*n* = 75)	CR (*n* = 143)
Demographics				
Age, y	39.7 ± 1.28	40.1 ± 1.29	37.9 ± 0.80	38.0 ± 0.61
Male, *n* (%)	5 (33.3)	8 (28.6)	22 (29.3)	44 (30.8)
Race, *n* (%)				
White	9 (60.0)	22 (78.6)	57 (76.0)	111 (77.6)
Black	5 (33.3)	5 (17.9)	11 (14.7)	15 (10.5)
Other	1 (6.7)	1 (3.6)	7 (9.3)	17 (11.9)
Anthropometrics				
Weight, kg	70.9 ± 2.18	72.4 ± 1.78	71.5 ± 1.00	72.0 ± 0.79
BMI, kg/m^2	25.0 ± 0.38	25.3 ± 0.31	25.1 ± 0.19	25.2 ± 0.15
Body composition				
Body fat, %	32.4 ± 1.65	33.3 ± 1.14	32.9 ± 0.51	32.9 ± 0.51
Fat mass, kg	22.6 ± 1.15	23.9 ± 0.91	23.8 ± 0.58	23.5 ± 0.36
Fat-free mass, kg	48.0 ± 2.41	48.2 ± 1.64	47.6 ± 0.99	48.5 ± 0.77

[1]Values are means ± SEMs unless otherwise indicated. For continuous variables, *P* values (data not shown) were not significant for treatment group (i.e., AL vs. CR) as calculated using regression models with adjustment for age, gender, and race, when applicable. For categorial variables, *P* values (data not shown) were not significant for treatment group (i.e., AL vs. CR) as calculated using Fisher's exact test. AL, ad libitum; CR, calorie restriction.

total tissue volume reduction (**Figure 2**A). In contrast, lean tissue in skeletal muscle, organs, and residual lean tissue comprised 37.0%, 4.8%, and 23.2% of total tissue volume at baseline and made up 17.2%, 1.3%, and 3.5% of the total tissue volume reduction at 24 mo (Figure 2A). Adipose tissue loss contributed more to the total tissue loss than did lean tissue loss.

Composition of adipose tissue loss during 24-mo CR.

In the CR group, VAT comprised 5.5% of total adipose tissue volume at baseline, but the loss of VAT amounted to 9.5% of the total adipose tissue volume reduction (Figure 2B). IMAT and SAT comprised 2.7% and 91.8% of total adipose tissue volume at baseline and the loss of IMAT and SAT comprised 2.9% and 87.7%, respectively, of total adipose tissue volume reduction.

Composition of lean tissue loss during 24-mo CR.

Among the lean tissue components, skeletal muscle comprised 56.9% of total lean tissue volume at baseline, but 78.4% of total lean tissue volume reduction came from loss of skeletal muscle (Figure 2C). Organs and residual lean tissue comprised 7.4% and 35.7% of total lean tissue volume at baseline and the loss of organ and residual lean tissue comprised 5.6% and 16.0%, respectively, of total lean tissue volume reduction.

Organ volume loss during 24-mo CR.

In the AL group, there was no significant reduction in any organ volume at 24 mo. In the CR group, the reduction in individual organ volumes at 24 mo from baseline was significant for the liver (−0.078 ± 0.030 L, *P* = 0.02) but not for brain, kidneys, and spleen (*P* = 0.10–0.73) (Table 2).

Predictors of MRI-measured body-composition changes

% CR.

From baseline to month 24, % CR in the AL group (−0.5% ± 2.5%; range: −19.8% to 16.4%) was significantly lower (*P* < 0.05) than in the CR group (13.7% ± 1.5%; range: −3.8% to 27.9%). In regression analyses, % CR significantly (*P* < 0.05) contributed to changes in VAT, muscle, and organ volumes (**Table 3**) after adjustment for sex. Percentage CR also influenced the change in SAT but with borderline significance (*P* = 0.05) after adjustment for sex (Table 3). Race, age, and baseline weight were tested as covariates in each model but did not significantly enter any model.

Sex and race.

In the CR group, women lost more SAT than men (−6.38 ± 0.52 L vs. −4.45 ± 0.42 L, *P* < 0.05); men lost more VAT than women (−0.97 ± 0.12 L vs. −0.50 ± 0.13 L, *P* < 0.05). Even after the adjustment for baseline SAT and baseline VAT, sex was a significant determinant of the change in SAT and VAT. There was no significant difference in the loss of muscle, organ, and residual lean tissue between sexes or racial groups (Table 3).

Age.

In regression models, age did not significantly contribute to changes in any body-composition components (Table 3).

Baseline weight and baseline body composition.

In regression analyses, baseline weight did not significantly contribute to changes in any body-composition components (Table 3). When each tissue component at baseline was tested as independent variables in the regression models, higher

TABLE 2 Comparison of whole-body MRI-measured body composition ($n = 43$) at baseline and month 24 between the AL control and CR groups[1]

	AL ($n = 15$)	CR ($n = 28$)	P: AL vs. CR
Weight, kg			
Baseline	70.9 ± 2.18	72.4 ± 1.78	0.35
Δ Month 24	1.95 ± 0.78	− 8.45 ± 0.53	<0.0001
P: month 24 vs. baseline	0.48	<0.0001	—
Subcutaneous adipose tissue, L			
Baseline	18.9 ± 1.35	20.2 ± 1.14	0.36
Δ Month 24	1.22 ± 0.51	− 5.83 ± 0.42	<0.0001
P: month 24 vs. baseline	0.36	<0.0001	—
Visceral adipose tissue, L			
Baseline	0.93 ± 0.18	1.20 ± 0.16	0.25
Δ Month 24	0.17 ± 0.07	− 0.63 ± 0.11	<0.0001
P: month 24 vs. baseline	0.96	<0.0001	—
Intermuscular adipose tissue, L			
Baseline	0.55 ± 0.07	0.60 ± 0.05	0.34
Δ Month 24	0.04 ± 0.02	− 0.19 ± 0.02	<0.0001
P: month 24 vs. baseline	0.62	0.01	—
Skeletal muscle, L			
Baseline	23.4 ± 1.67	23.3 ± 1.04	0.87
Δ Month 24	0.23 ± 0.23	− 1.47 ± 0.21	<0.0001
P: month 24 vs. baseline	0.99	0.003	—
Brain, L			
Baseline	1.262 ± 0.030	1.273 ± 0.023	0.76
Δ Month 24	− 0.005 ± 0.004	− 0.006 ± 0.003	0.35
P: month 24 vs. baseline	0.66	0.73	—
Liver, L			
Baseline	1.232 ± 0.048	1.319 ± 0.045	0.05
Δ Month 24	− 0.059 ± 0.039	− 0.078 ± 0.030	0.88
P: month 24 vs. baseline	0.27	0.02	—
Spleen, L			
Baseline	0.167 ± 0.021	0.181 ± 0.015	0.94
Δ Month 24	− 0.004 ± 0.006	− 0.016 ± 0.004	0.13
P: month 24 vs. baseline	0.81	0.10	—
Kidneys, L			
Baseline	0.302 ± 0.018	0.273 ± 0.011	0.15
Δ Month 24	− 0.003 ± 0.007	− 0.005 ± 0.004	0.21
P: month 24 vs. baseline	1.00	0.49	—
Residual lean tissue, L			
Baseline	14.5 ± 0.62	14.6 ± 0.50	0.61
Δ Month 24	0.16 ± 0.18	− 0.30 ± 0.13	0.06
P: month 24 vs. baseline	0.64	0.32	—

[1] Values are means ± SEMs. P values for timepoints (i.e., month 24 vs. baseline) or treatment group (i.e., AL vs. CR) were calculated using regression models with adjustment for age, gender, and race. AL, ad libitum; CR, calorie restriction; Δ, change.

baseline VAT contributed to higher loss of VAT (standardized regression coefficient ± SEE: 0.81± 0.11; $P < 0.0001$); higher baseline IMAT significantly contributed to higher loss of IMAT (standardized regression coefficient ± SEE: 0.59 ± 0.18; $P < 0.0001$). SAT, muscle, organs, and residual lean tissue at baseline did not significantly contribute to the changes in these tissue components.

Determinants of individual organ volume change.

In regression analyses, sex, race, age, and baseline weight did not significantly contribute to changes in any organ. Percentage CR significantly (standardized regression coefficient ± SEE: 0.45 ± 0.18; $P < 0.05$) contributed to liver-size change over the 24-mo CR, but it was not a determinant of the change in brain, spleen, or kidney size, after adjustment for age, sex, race, and baseline weight.

Liver fat change ($−0.4\% ± 0.2\%$) quantified by proton magnetic resonance spectroscopy, as previously described (6), did not significantly correlate with liver volume change ($r = 0.29$, $P = 0.16$).

Discussion

Two years of 13.7% CR in nonobese participants triggered the expected beneficial impact on body composition with preferential loss of adipose tissue (SAT, VAT, IMAT) over skeletal muscle mass and organ volume (brain, liver, spleen, and kidneys) (i.e., adipose tissue comprised 34.9% of total tissue volume at baseline; the loss of adipose tissue comprised 78.0% of total tissue loss).
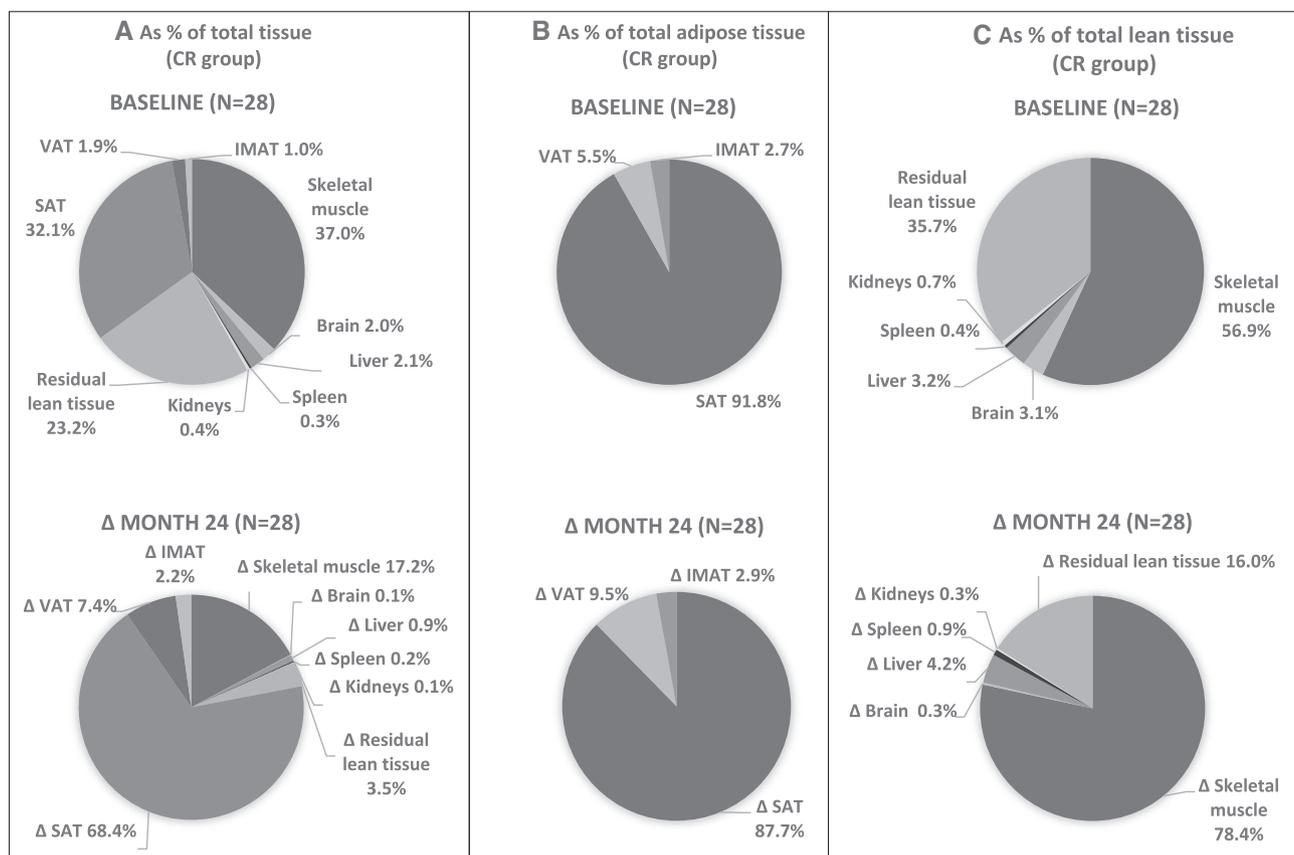
FIGURE 2 (A) Comparison of each tissue component (mean) as a percentage of total tissue volume (mean) at baseline and the change in each tissue component (mean) as a percentage of change in total tissue volume (mean) over 24 mo. (B) Comparison of each adipose tissue component (mean) as a percentage of total adipose tissue volume (mean) at baseline and the change in each adipose tissue component (mean) as a percentage of change in total adipose tissue volume (mean) over 24 mo. (C) Comparison of each lean tissue component (mean) as a percentage of total lean tissue volume (mean) at baseline and the change in each lean tissue component (mean) as a percentage of change in total lean tissue volume (mean) over 24 mo. Organs in the present study include brain, liver, spleen, and kidneys. CR, calorie restriction; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

This ancillary study is consistent with earlier reports in the entire CALERIE cohort that 2 y of CR had favorable effects on body composition determined by DXA (3, 6).

The observed relative preservation of organ mass and muscle mass, and the preferential loss of adipose tissue, especially VAT, contribute to the evidence supporting that CR has the capacity to mediate the attenuation of both primary and secondary aging in young, nonobese individuals without adverse effects on body composition (1). Prior to CALERIE, the majority of weight-loss trials have been conducted in individuals with obesity and have been limited to interventions spanning 3–12 mo. The present study distinguishes itself from this prior research by investigating the effects of CR in nonobese adults over 24 mo.

Adipose tissue distribution change during long-term CR

As illustrated in Figure 2A, 24-mo loss of adipose tissue comprised most of the loss of total tissue. In addition, the loss of total tissue as VAT is ∼4 times the total tissue as VAT at baseline (i.e., 1.9% vs. 7.4%). Therefore, the loss of adipose tissue, especially VAT, is likely to be one of the underlying mechanisms explaining the beneficial effect of CR attenuating secondary aging by reduction in cardiovascular disease and diabetes risk

factors and potentially extension of the health span (6). Men lost more VAT, whereas women lost more SAT after adjustment for baseline VAT and SAT. It would be interesting for future larger-scale studies to investigate if long-term CR reduces VAT-related morbidities more significantly in men than in women (4).

Lean tissue loss and muscle loss during long-term CR

CR is often blamed for its potential detrimental effect on lean mass, especially in the elderly (34, 35). In this relatively young healthy cohort, baseline lean tissue made up 65.1% of total tissue volume, whereas the loss of lean tissue only amounted to 17.5% of total tissue loss (Figure 2A). The observation that lean tissue loss was lower than adipose tissue loss during the 24-mo CR is consistent with many reports that lean tissue loss accounts for approximately one-quarter of the total weight loss (36).

Muscle loss made up most of the lean tissue loss during the 24-mo CR (Figure 2C). However, in the parent study, muscle strength was preserved despite the reduction in muscle mass. On the other hand, beyond the sixth decade of life, low muscle mass and low strength more than doubles the risk for all-cause mortality (37). Whether muscle loss in young age translates into deterioration of strength and mortality in later years needs future investigation.

TABLE 3 Standardized regression equation with changes in whole-body MRI-measured body composition as dependent variables in the CR group[1]

Dependent variable	Independent variables		
	% CR	Female	R^2
Δ SAT, L	0.10[2] (0.05)	−0.89[3] (0.38)	0.28
Δ VAT,[4] L	0.43[3] (0.15)	1.12[3] (0.32)	0.47
Δ IMAT,[4] L	0.40[3] (0.19)	0.05[5] (0.41)	0.15
Δ Skeletal muscle, L	0.55[3] (0.17)	0.03[5] (0.38)	0.29
Δ Brain, L	0.14[5] (0.20)	0.17[5] (0.43)	0.03
Δ Liver, L	0.45[3] (0.18)	0.31[5] (0.39)	0.22
Δ Spleen, L	0.23[5] (0.20)	0.02[5] (0.43)	0.05
Δ Kidneys, L	0.35[2] (0.19)	0.21[5] (0.41)	0.13
Δ Residual lean tissue, L	0.12[5] (0.20)	−0.51[5] (0.43)	0.07

[1] Values are estimates of standardized regression coefficients (SEEs). $n = 28$. Covariates tested in each model: race, age, and baseline weight were tested as covariates in each model but did not significantly enter any model. CR, caloric restriction; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; Δ, change.
[2] $P = 0.05$ to 0.09.
[3] $P < 0.05$.
[4] Either log or Box-Cox transformed.
[5] Covariate did not enter the model significantly.

The previously reported higher FFM loss in men than in women in the entire CALERIE cohort (men vs. women: -3.0 ± 0.32 kg vs. -1.6 ± 0.17 kg, $P < 0.001$) (3) contradicts the subset of men and women in this ancillary, who lost a similar amount of muscle, organ volume, and residual lean tissue. This could not be explained by % CR, which was similar between the parent study and the subset (3). MRI and DXA methodology differences and the sample size difference might partially explain the different role of sex in lean tissue loss in the parent study and in the subset. As sex affects age-related changes in body composition (38, 39), future larger and longer-term studies need to clarify the interactions among sex, aging, and CR.

Organ volume change during long-term CR

The significant reduction in liver size is unlikely to be explained by the reduction in glycogen and its binding water (40), since, by the end of the 24-mo study, the participants were likely no longer in a negative energy balance. Although liver fat loss explained liver mass loss in the weight loss of obese adults (11), the small reduction in liver fat (i.e., $0.4\% \pm 0.2\%$) during CR in our healthy, nonobese individuals does not account for much of the liver-size reduction ($r = 0.29$, $P = 0.16$) (6). Future studies need to clarify if metabolically active liver cell mass reduction occurs during CR. If so, significant liver-size reduction during 24-mo CR could attenuate primary aging through reducing resting metabolic rate (RMR), as RMR of the liver is ~15 times higher than in muscle (9). Reduction in RMR increases energy efficiency and decreases oxidative damage to tissues and cells (41). Future studies are needed to clarify the role of specific organ mass reduction and functional body-composition mass (42) in metabolic adaptation in nonobese individuals during long-term CR, as well as how these body-composition changes relate to physiological function (43, 44)

Previous weight-loss studies reported mixed results on brain loss in obese individuals, with the majority of the studies reporting no brain mass loss (11, 12, 14, 45). The ~0.40% and

~0.48% nonsignificant brain volume loss in the AL and CR groups can be explained by a decrease in brain volume of 0.23%/y reported (46).

Factors contributing to lean and adipose tissue loss

Our results suggested that % CR, rather than baseline weight, was the major factor determining body tissue loss including the loss of VAT, IMAT, skeletal muscle, and liver volume. Race and age do not seem to impact the loss of VAT, IMAT, skeletal muscle, and liver volume in young participants between 25 and 50 y old. Future studies need to investigate how CR interventions, designed to decrease obesity-related mortality in the elderly, affect muscle or organ tissue loss.

Strengths and limitations

One major strength of the present study is the utilization of whole-body MRI to quantify longitudinal body composition in the 24-mo CALERIE trial, which tested the effects of an intended 25% CR (13.7% as calculated) on biomarkers of aging and the health span. Compared with single-slice or multi-slice abdominal MRI studies, whole-body MRI has the advantage of quantifying whole-body muscle and organ mass in addition to total-body adipose tissue including VAT, IMAT, and SAT (26, 47).

One limitation of the study is, that due to expense, whole-body MRI scans were only collected in a subset of participants and only at 1 site. In addition, heart mass data was not available. Nonetheless, the organs in the present study included the most vital organs.

Conclusions

During 2-y 25% CR in nonobese participants, although SAT, VAT, and IMAT comprised a smaller proportion of total tissue volume than muscle and organs at baseline, the loss of SAT, VAT, and IMAT comprised a higher proportion of total tissue

loss during 24 mo of CR. VAT loss is most prominent among all adipose tissue depots. Men lost more VAT, whereas women lost more SAT during the 24-mo CR. There was a small decrease in the volume of liver, but not the volume of brain, spleen, and kidneys. Percentage CR, rather than race, age, or baseline weight, contributes to both the loss of lean tissue components and adipose tissue components.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending the CALERIE study committee and the Principal Investigators' (i.e., ER and WS) approval.

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