# Experimental Gerontology

## Challenges in defining successful adherence to calorie restriction goals in humans: Results from CALERIE™ 2

--Manuscript Draft--

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| Abstract: | Background: The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIETM) phase 2 trial tested the effects of two years of 25% calorie restriction (CR) on aging in humans. CALERIE 2 was one of the first studies to use a graph of predicted weight loss to: 1) provide a proxy of dietary adherence, and 2) promote dietary adherence. Assuming 25% CR, each participant's weight over time was predicted, with upper and lower bounds around predicted weights. Thus, the resulting weight graph included a zone or range of body weights that reflected adherence to 25% CR, and this was named the zone of adherence. Participants were considered adherent if their weight was in this zone. It is unlikely, however, that the entire zone reflects 25% CR.<br>Objectives: To determine the level of CR associated with the zone of adherence and if the level of CR achieved by participants was within the zone.<br>Methods: Percent CR associated with the upper and lower bounds of the zone were determined via the Body Weight Planner (https://www.niddk.nih.gov/bwp) for participants in the CALERIE 2 CR group (N=143). Percent CR achieved by participants was estimated with the intake-balance method.<br>Results: At month 24, the zone of adherence ranged from 10.4(0.0)% to 19.4(0.0)% CR [Mean(SEM)], and participants achieved 11.9(0.7)% CR and were in the zone.<br>Conclusion: The results highlight the challenges of: 1) setting a single CR goal vs. a range of acceptable values, and 2) obtaining real-time and valid measures of CR adherence to facilitate adherence. |
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## Abstract

**Background**: The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE™) phase 2 trial tested the effects of two years of 25% calorie restriction (CR) on aging in humans. CALERIE 2 was one of the first studies to use a graph of predicted weight loss to: 1) provide a proxy of dietary adherence, and 2) promote dietary adherence. Assuming 25% CR, each participant's weight over time was predicted, with upper and lower bounds around predicted weights. Thus, the resulting weight graph included a zone or range of body weights that reflected adherence to 25% CR, and this was named the zone of adherence. Participants were considered adherent if their weight was in this zone. It is unlikely, however, that the entire zone reflects 25% CR.

**Objectives**: To determine the level of CR associated with the zone of adherence and if the level of CR achieved by participants was within the zone.

**Methods**: Percent CR associated with the upper and lower bounds of the zone were determined via the Body Weight Planner (https://www.niddk.nih.gov/bwp) for participants in the CALERIE 2 CR group (N=143). Percent CR achieved by participants was estimated with the intake-balance method.

**Results**: At month 24, the zone of adherence ranged from 10.4(0.0)% to 19.4(0.0)% CR [Mean(SEM)], and participants achieved 11.9(0.7)% CR and were in the zone.

**Conclusion**: The results highlight the challenges of: 1) setting a single CR goal vs. a range of acceptable values, and 2) obtaining real-time and valid measures of CR adherence to facilitate adherence.

**Keywords:** calorie restriction, adherence, weight graph, weight loss, lifestyle change, intensive lifestyle intervention, dietary adherence, energy intake

**Abbreviations:** AL, ad libitum; CALERIE$^{TM}$, Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy; CTS, Computer Tracking System; CR, calorie restriction; DLW, doubly labeled water; DXA, dual energy X-ray absorptiometry; IBM SPSS, International Business Machines Statistical Package for the Social Sciences; kJ, kilojoules; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; PAL, Physical Activity Level; RMR, resting metabolic rate; SEM, standard error of the mean; SD, standard deviation; TDEE, total daily energy expenditure.

# 1. Introduction

## 1.1. Few methods exist to accurately quantify dietary adherence in real-time, particularly over the long-term

Promoting adherence to calorie-restricted diets has been very difficult due to the challenges of accurately quantifying energy intake. Traditional self-report methods to assess energy intake (e.g., food records, dietary recall) are commonly used, though the accuracy of these methods has been questioned [1-4] and it is difficult for participants to use them over the long term. The doubly labeled water (DLW) method can be used to estimate energy intake accurately over the short term (e.g., two weeks) and long-term when changes in body composition are measured.[5] This approach requires repeated DLW and body composition measurements, as well as isotope analyses; therefore, it is not practical for many studies and cannot provide real-time estimates of calorie restriction (CR) to inform intervention delivery.

## 1.2. Estimating dietary adherence in real-time by using body weight as a proxy for dietary adherence

An alternative method for estimating adherence to a calorie-restricted diet is to calculate expected body weight for study participants based on the prescribed level of CR. This allows the participant's actual weights to be compared to expected weights over time and, if the participant's actual body weight reflects the expected weight, adherence to the CR goal can be inferred. If the participant's actual body weight deviates from the expected weight, then it can be inferred that the participant is not adhering to the CR goal. One challenge to developing and deploying this approach is the erroneous assumption that human participants can control their body weight precisely enough to closely mirror a single body weight at any point in time. A second challenge is inherent error in calculations of expected body weight. One way to address these limitations is to provide participants with a range of acceptable body weights that reflects CR adherence.

To develop and facilitate this approach, a mathematical model was developed by Pieper et al. [6] that predicted the distribution of percent weight change over 12 months assuming 25% CR. The output from the model was used to create weight graphs for participants that reflect the goal weight, which is represented by the green line in Figure 1 (the green line reflects the 50$^{th}$ percentile of expected weight change from the model). Upper and lower bounds around this goal weight are represented by the yellow and light blue lines in Figure 1 (the yellow and light blue lines reflect the 80$^{th}$ and 10$^{th}$ percentiles of weight change from the model, respectively). The result is a weight graph that includes a zone or range of body weights that reflects adherence to 25% CR. Hence, this zone of acceptable weights is called the zone of adherence. A participant's measured body weights are plotted over time on the weight graph and the participant is considered adherent to 25% CR if his/her weight is within the zone. Because the Pieper et al. [6] model was not designed to predict body weight beyond 12 months, the zone of adherence is flat between months 12 and 24, as depicted Figure 1.

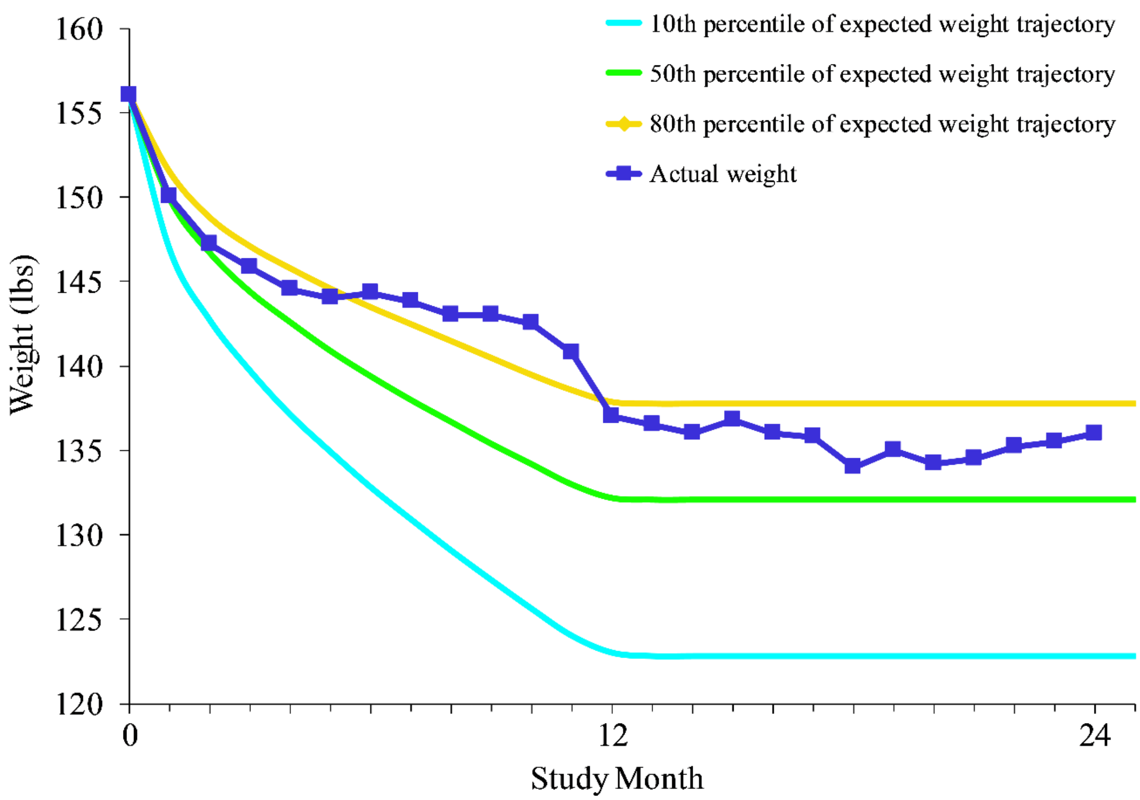


**Figure 1.** A sample weight graph is displayed for a hypothetical participant in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE™) phase 2 trial (CALERIE 2), which tested the effects of two years of CR on biomarkers of aging in humans. The light blue, green, and yellow lines correspond to the 10th, 50th, and 80th percentiles of expected weight trajectories, respectively. The dark blue line depicts the hypothetical participant's measured weight trajectory. The participant's starting weight was 70.7 in kilograms. From months 12 to 24, the yellow, green, and light blue lines represent 62.5, 60, and 55.7 kg, respectively. Reprinted from Contemporary Clinical Trials, Vol 32, Issue 6; Amy D. Rickman, Donald A. Williamson, Corby K. Martin, Cheryl H. Gilhooly, Richard I. Stein, Connie W. Bales, Susan Roberts, and Sai Krupa Das; The CALERIE Study: Design and methods of an

innovative 25% caloric restriction intervention; Page No. 880, 2011, with permission from Elsevier.

### 1.3. Using a weight graph and zone of adherence to personalize intervention delivery and promote CR adherence

The model and weight graphs from Pieper et al. [6] were integrated into the intervention [7] for the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE$^{TM}$) phase 2 trial (CALERIE 2), which tested the effects of two years of CR on biomarkers of aging in humans. As detailed by Rickman et al., [7] the model and weight graph were central in directing the delivery of the intervention by assessing if a participant was adherent or nonadherent in real time and adjusting treatment delivery accordingly. Participants were weighed at each intervention session and their weights were plotted onto their weight graph. Participants were considered adherent to the 25% CR goal if their weight was within the zone of adherence. When weight was above the zone, participants were considered nonadherent to 25% CR and intervention strategies were deployed to help participants better restrict energy intake. Conversely, a weight below the zone indicated that the participant had been too restrictive and efforts were needed to increase energy intake.

### 1.4. Current objectives

The use of the model and weight graphs to foster adherence during CALERIE 2 was novel and provided a much-needed real time metric of adherence. The approach also provided a framework to personalize intervention delivery and to guide deployment of treatment strategies. [7] Nonetheless, the utility of the weight graphs and the success of the CALERIE 2 intervention require further analysis. To that end, the objectives of this analysis were twofold. First, determine the level of CR associated with the zone of adherence by utilizing a validated weight loss calculator that was not used during CALERIE 2.[8,9] Second, determine if participants' actual level of CR was within the zone by using the intake-balance method, which is considered accurate,[10] but cannot provide data in real time and thus necessitates a post-hoc analysis. It was hypothesized that the upper bound of the zone at months 12 and 24 would be less than 25% CR. It was further hypothesized that the level of CR achieved by participants would be within the zone at months 12 and 24.

## 2. Methods

The CALERIE™ phase 2 randomized controlled trial was a multi-site study conducted at Pennington Biomedical, (Baton Rouge, LA, USA), Washington University School of Medicine (St. Louis, MO, USA), and Tufts University (Boston, MA, USA). The coordinating center was Duke Clinical Research Institute (Durham, NC, USA). The clinicaltrials.gov registration number is NCT00427193. All sites received Institutional Review Board approval and all participants provided written informed consent. The CALERIE 2 study aimed to test the effects of two years of 25% CR on aging biomarkers in comparison to an ad libitum (AL) control group. The study design,[11] screening and recruitment procedures,[12] and intervention[6,7] have been described extensively.

### 2.1. Participants and randomization

CALERIE 2 recruited participants who were 20-50 years old (men) or 20-47 years old (women) and had body mass index ≥ 22.0 and < 28.0 kg/m$^2$. Exclusion criteria included significant medical conditions (e.g., cardiovascular disease, diabetes, hypertension), psychological disorders, high levels of physical activity (≥ 30 mins ≥ 5 days/week), and women who were pregnant or planning to become pregnant during the trial.

Participants were randomized into the CR or AL group in a 2:1 ratio favoring CR. A permuted block randomization approach was used to stratify by study site, sex, and BMI category (normal weight: BMI 22.0 - 24.9 kg/m$^2$ and overweight: BMI 25.0 - 27.9 kg/m$^2$). 145 participants were randomized to the CR group and 75 participants were randomized to the AL group. The AL group was asked to continue eating their habitual diet and did not receive any intervention; they are not included in the analyses reported herein.

**2.2. Description of the CR intervention**

The goal of the CR intervention was to promote 25% CR for two years. As described in Rickman et al., [7] the CR participants received an intensive lifestyle intervention to foster adherence, including individual sessions with an interventionist once per week for the first month, twice per month from month 2 through 12, and once per month from month 13 through 24. Additional sessions were scheduled as needed. Finally, participants attended group sessions twice per month from month 1 through 6, and once per month from month 7 through 24.

As noted earlier, the model and weight graphs developed by Pieper et al. [6] were central to guiding intervention delivery throughout the two-year intervention, which was deployed via an Internet-based Computer Tracking System (CTS) that was created for the project . [7] Briefly, the CTS facilitated intervention fidelity and provided structure to how the intervention was deployed over time, across interventionists, and across participants. A central feature of the CTS was tracking weight as a proxy of CR adherence. Participants' demographic information was entered into the CTS, as well as their starting body weight and their energy intake target, which reflected 25% CR. A personalized weight graph was then generated for each participant based on the Pieper et al. [6] model. Participants were weighed at each session and the interventionist entered the measured body weight into the CTS, which plotted the participant's weight onto his/her graph. Adherence was considered acceptable if the participant's weight was within the zone. A sample weight graph is provided in Figure 1 and illustrates that this hypothetical participant was in the zone and adherent in the early period of the intervention. The participant's weight was above the zone, however, from around month 6 to month 11, indicating non-adherence to the CR prescription. During this period, the CTS would automatically suggest toolbox options or specific intervention strategies (e.g., use of portion-controlled foods) to support the participant in achieving their prescribed energy intake level and re-entering the zone. This also helped standardize the delivery of treatment options among participants when they presented with similar challenges (e.g., difficulty adhering their prescribed energy intake level, weight being above or below the zone, poor attendance to sessions, etc.). As indicated in Figure 1, this hypothetical participant re-entered the zone around month 12 and maintained adherence throughout the rest of the trial.

### 2.3. Percent CR calculations

The purpose of the analyses reported in this paper were to: 1) determine the level of CR associated with the zone of adherence in CALERIE 2, and 2) examine the level of CR achieved by participants in relation to the percent CR values from the zone of adherence.

**2.3.1. Percent CR associated with the zone of adherence**. To calculate the percent CR associated with the zone of adherence, a model was needed that was both valid and different from the model that was used in CALERIE 2 (i.e., the Pieper et al. [6] model). The NIDDK Body Weight Planner [8,9] was selected since the models used in the planner have been validated [8,9] and the models were found to accurately quantify change in energy intake over two years in the CALERIE 2 study when compared to the intake-balance method. [13] Additionally, the NIDDK Body Weight Planner provides the ability to adjust each participant's physical activity level

(PAL) to match participants' baseline energy requirements with the energy requirement measured in CALERIE 2. Thus, the NIDDK Body Weight Planner provided: 1) a valid method to quantify the percent CR associated with the zone of adherence, 2) a model that was not used to generate the zone of adherence during CALERIE 2, and 3) the ability to adapt PAL such that energy requirements were most accurate for each individual participant.

The percent CR associated with the zone of adherence, specifically, the upper bound of the zone (80th percentile), the lower bound of the zone (10th percentile), and the 50th percentile line, was calculated with the NIDDK Body Weight Planner. To do so, the following procedures were followed, and the example provided is to determine the percent CR associated with the 80th percentile or the upper bound of the zone at month 12. First, each participant's weight, sex, age, height, and baseline weight from CALERIE 2 were entered into the planner. The physical activity level (PAL) was adjusted in the planner until each participant's baseline energy requirement in the planner matched the energy requirement measured in CALERIE 2 (each participant's PAL was also measured during CALERIE 2, and agreement between this measure and the value entered into the planner was evaluated). Second, each participant's predicted weight at the 80th percentile from the weight graph at month 12 was entered into the planner as the goal weight, and the duration to achieve the goal was set to 12 months. Third, the planner then produced the energy intake value needed to achieve this goal. Fourth, this energy intake value was used in conjunction with the baseline energy requirements to calculate the percent CR reflective of the 80th percentile at month 12. This process was repeated for the 80th percentile at month 24, and for the 10th and 50th percentiles at months 12 and 24.

Once percent CR was calculated for the zone of adherence for each participant, the mean (and standard error of the mean or SEM) percent CR values for the zone of adherence were calculated across all of the CR participants.

**2.3.2. Percent CR achieved by participants in CALERIE 2**. The second purpose of the analyses reported herein was to examine the level of CR achieved by participants in relation to the percent CR values from the zone of adherence. This process determined if the participants were adherent to the CR goal, as defined by the zone of adherence, even if the level of CR that they achieved failed to reach 25%.

The previous section outlined the methods to calculate the percent CR associated with the zone of adherence, and these calculations relied on the NIDDK Body Weight Planner. As detailed in the following paragraphs, determining each participant's percent CR required different methods, namely, the intake-balance method, [10] which relied on state-of-the art measures that were collected during CALERIE 2.

The intake-balance method [10] relies on measures of total daily energy expenditure (TDEE) and, if weight is not stable during the TDEE assessment, a measure of change in body energy stores, which can be determined by measuring change in body composition during the TDEE assessment. During energy balance or weight stability, energy intake is equal to TDEE. Hence, measured TDEE is equal to energy intake during weight stability. If weight is not stable, then TDEE is not equal to energy intake. In this case, TDEE must be corrected for the energy cost of the change in body composition during the period of TDEE assessment. Hence, energy intake is calculated as the difference between energy expenditure (TDEE) and the energy cost of changes in body composition.

During CALERIE 2, TDEE and body composition were assessed at several time points, allowing our team to calculate the percent CR that each participant achieved for different intervals in the study (e.g., from baseline to month 12, and baseline to month 24). Specifically, participants' TDEE was measured with doubly labeled water over four weeks at baseline to establish baseline energy requirements. Doubly labeled water was also used to measure TDEE for two weeks at months 6, 12, 18, and 24. To quantify change in body composition during the TDEE assessments, fat mass and fat-free mass were measured with dual energy X-ray absorptiometry (DXA; Hologic QDR 4500A; Hologic, Bedford, MA) before and after each TDEE assessment.

The TDEE and body composition measures outlined above allowed the mean energy intake of each participant to be estimated with the intake-balance method. Specifically, each participant's mean daily energy intake from baseline to month 12, and baseline to month 24, was calculated. The calculation for energy intake was: mean TDEE for each interval plus changes in body energy stores. Change in energy stores was calculated assuming 9,300 kcal/kg (38,893 kJ/kg) for the energy content of fat mass change and 1,100 kcal/kg (4,602 kJ/kg) for fat-free mass change. [5] The mean daily energy intake values were then used to calculate percent CR in relation to each participant's baseline energy requirements.

Once percent CR at months 12 and 24 were calculated for each participant, mean (and SEM) percent CR at months 12 and 24 were calculated across all CR participants.

### 2.4. Physical activity level (PAL)

Physical activity level was calculated as TDEE from DLW divided by resting metabolic rate (RMR). RMR was measured via indirect calorimetry using a Vista-MX metabolic measurement system (Vacumed, Ventura, CA).

### 2.4. Data Analytic Plan

Measured PAL from CALERIE 2 and the PAL used in the Body Weight Planner were compared with Pearson correlation coefficients. As noted earlier, mean percent CR across all CR participants was calculated for the 80th, 50th, and 10th percentiles at months 12 and 24. These values were graphed and participants' actual percent CR was plotted in relation to these values. Independent sample t-tests and analysis of variance (ANOVA) were used to determine if participants' actual percent CR, and the percent CR values for the 80th, 50th, and 10th percentiles at months 12 and 24, differed by sex, BMI category, or race. Alpha was set at 0.05. All analyses were conducted using IBM SPSS, Version 27 (Armonk, NY, IBL Corp).

## 3. Results

### 3.1. CALERIE 2 results

The CALERIE 2 results have been reported extensively but, in brief, indicated that two years of CR was safe, resulted in significantly improved aging and longevity biomarkers, and reduced risk factors for age-related diseases. [10,14-17] Additionally, CR was found to have no detrimental, and some positive effects, on health-related quality of life. [18]

### 3.2. Participant characteristics

The descriptive characteristics of the sample are provided in Table 1. The sample was predominantly female (69.2%) with a slightly higher proportion of participants in the overweight (52.4%) vs. normal weight (47.6%) BMI stratum. The sample was comprised of 143 CR participants who started the intervention, as reflected in Table 1, though data were available for 128 participants at month 12, the first time point of interest for this analysis. Table 2 includes the sample sizes at each time point in total and by grouping variable (i.e., sex, race, and BMI stratum).

**Table 1.** Baseline characteristics of participants in the calorie restriction group (N=143).

| | | |
|---|---|---|
| Sex, n (%) | | |
| Male | 44 | (30.8) |
| Female | 99 | (69.2) |
| Race, n (%) | | |
| White | 111 | (77.6) |
| African American | 15 | (10.5) |
| Asian | 11 | (7.7) |
| Other | 6 | (4.2) |
| Age (years), mean (SD) | 38.2 | (7.3) |
| Weight (kg), mean (SD) | 73.7 | (9.9) |
| BMI ($kg/m^2$), mean (SD) | 25.8 | (1.9) |
| BMI Category, n (%) | | |
| Normal weight (22.0 – 24.9 $kg/m^2$) | 68 | (47.6) |
| Overweight (25.0 – 27.9 $kg/m^2$) | 75 | (52.4) |

Abbreviations: BMI, body mass index; kg, kilogram; SD, standard deviation.

**Table 2.** Percent CR at the 80th, 50th, and 10th percentile, and the percent CR achieved by participants at months 12 and 24 during CALERIE 2.

| | All participants * | |
|---|---|---|
| | **Mean** | **(SEM)** |
| **% CR at 80th percentile** | | |
| **M12** | 13.7 | (0.1) |
| **M24** | 10.4 | (0.0) |
| **% CR at 50th percentile** | | |
| **M12** | 17.8 | (0.1) |
| **M24** | 13.6 | (0.0) |
| **% CR at 10th percentile** | | |
| **M12** | 24.9 | (0.1) |
| **M24** | 19.4 | (0.0) |
| **Actual % CR** | | |
| **M12** | 15.2 | (0.7) |
| **M24** | 11.9 | (0.7) |

| | Men † | | Women † | | | | |
|---|---|---|---|---|---|---|---|
| | **Mean** | **(SEM)** | **Mean** | **(SEM)** | ***t*** | **df** | ***p*** |
| **% CR at 80th percentile** | | | | | | | |
| **M12** | 13.3 | (0.1) | 13.8 | (0.1) | −2.9 | 126 | **0.004** |
| **M24** | 10.3 | (0.0) | 10.5 | (0.0) | −3.1 | 116 | **0.002** |
| **% CR at 50th percentile** | | | | | | | |
| **M12** | 17.4 | (0.2) | 18.0 | (0.1) | −3.2 | 126 | **0.002** |
| **M24** | 13.4 | (0.0) | 13.6 | (0.0) | −2.9 | 116 | **0.004** |
| **% CR at 10th percentile** | | | | | | | |
| **M12** | 24.3 | (0.2) | 25.2 | (0.1) | −3.5 | 126 | **<0.001** |
| **M24** | 19.3 | (0.1) | 19.4 | (0.1) | −1.4 | 116 | 0.156 |
| **Actual % CR** | | | | | | | |
| **M12** | 15.4 | (1.2) | 15.2 | (0.8) | 0.1 | 123 | 0.904 |
| **M24** | 11.7 | (1.2) | 12.0 | (0.8) | −0.2 | 113 | 0.845 |

| | Normal Weight ‡ | | Overweight ‡ | | | | |
|---|---|---|---|---|---|---|---|
| | **Mean** | **(SEM)** | **Mean** | **(SEM)** | ***t*** | **df** | ***p*** |
| **% CR at 80$^{th}$ percentile** | | | | | | | |
| **M12** | 13.3 | (0.1) | 14.0 | (0.1) | −4.3 | 126 | **<0.001** |
| **M24** | 10.3 | (0.0) | 10.5 | (0.0) | −2.7 | 116 | **0.008** |
| **% CR at 50$^{th}$ percentile** | | | | | | | |
| **M12** | 17.4 | (0.1) | 18.1 | (0.1) | −4.2 | 126 | **<0.001** |
| **M24** | 13.5 | (0.0) | 13.6 | (0.0) | −2.7 | 116 | **0.009** |
| **% CR at 10$^{th}$ percentile** | | | | | | | |
| **M12** | 24.4 | (0.2) | 25.4 | (0.2) | −4.2 | 126 | **<0.001** |
| **M24** | 19.3 | (0.1) | 19.5 | (0.1) | −2.7 | 116 | **0.008** |
| **Actual % CR** | | | | | | | |
| **M12** | 13.8 | (1.0) | 16.5 | (0.8) | −2.1 | 123 | **0.036** |
| **M24** | 10.5 | (1.0) | 13.1 | (0.9) | −1.9 | 113 | 0.056 |

| | White § | | African American § | | Asian § | | Other § | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **(SEM)** | **Mean** | **(SEM)** | **Mean** | **(SEM)** | **Mean** | **(SEM)** | ***F*** | **df** | ***p*** |
| **% CR at 80$^{th}$ percentile** | | | | | | | | | | | |
| **M12** | 13.7 | (0.1) [a] | 14.2 | (0.4) [b] | 13.1 | (0.2) [a] | 13.3 | (0.3) [a,b] | 3.5 | 3 | **0.018** |
| **M24** | 10.4 | (0.0) | 10.4 | (0.1) | 10.3 | (0.1) | 10.4 | (0.1) | 1.2 | 3 | 0.315 |
| **% CR at 50$^{th}$ percentile** | | | | | | | | | | | |
| **M12** | 17.8 | (0.1) [a] | 18.3 | (0.3) [a] | 17.0 | (0.3) [b] | 17.3 | (0.3) [a,b] | 3.4 | 3 | **0.020** |
| **M24** | 13.6 | (0.0) | 13.5 | (0.1) | 13.3 | (0.1) | 13.5 | (0.1) | 1.7 | 3 | 0.167 |
| **% CR at 10$^{th}$ percentile** | | | | | | | | | | | |
| **M12** | 25.0 | (0.1) [a] | 25.5 | (0.4) [a] | 24.0 | (0.4) [b] | 24.4 | (0.4) [a,b] | 2.7 | 3 | **0.047** |
| **M24** | 19.4 | (0.1) | 19.3 | (0.2) | 19.1 | (0.2) | 19.3 | (0.2) | 1.4 | 3 | 0.238 |
| **Actual % CR** | | | | | | | | | | | |
| **M12** | 15.7 | (0.7) | 16.0 | (2.1) | 11.8 | (2.3) | 10.6 | (2.3) | 1.6 | 3 | 0.186 |
| **M24** | 12.5 | (0.8) | 12.7 | (1.9) | 6.3 | (1.9) | 6.8 | (2.7) | 2.6 | 3 | 0.057 |

Data are mean (SEM). Superscripts that differ from each other within a row indicate significant differences between subgroups ($P$<0.05)

* Percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentiles are available for 128 (M12) and 118 (M24) participants. Actual percent CR is available for 125 (M12) and 115 (M24) participants.

† For men, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile are available for 39 (M12) and 35 (M24) participants, and actual percent CR is available for 38 (M12) and 35 (M24) participants. For women, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile is available for 89 (M12) and 83 (M24) participants, and actual percent CR is available for 87 (M12) and 80 (M24) participants.

‡ For the low BMI category, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile is available for 61 (M12) and 57 (M24) participants, and actual percent CR is available for 57 (M12) and 54 (M24) participants. For the high BMI category, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile is available for 67 (M12) and 61 (M24) participants, and actual percent CR is available for 68 (M12) and 61 (M24) participants.

§ For Whites, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile is available for 99 (M12) and 92 (M24) participants, and actual percent CR is available for 97 (M12) and 90 (M24) participants. For African Americans, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile is available for 14 (M12 and M24) participants, and actual percent CR is available for 13 (M12 and M24) participants. For Asians, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile and actual percent CR are available for 10 (M12) and 7 (M24) participants. For other races, percent CR at the $80^{th}$, $50^{th}$, and $10^{th}$ percentile and actual percent CR are available for 5 (M12 and M24) participants.

Abbreviations: BMI, body mass index; CR, calorie restriction; df, degrees of freedom; *F*, F-value; M, month; SEM, standard error of mean; *t*, t-value

### 3.3. Physical Activity Level (PAL)

Overall, PAL entered into the Body Weight Planner [1.66 (0.02)] correlated significantly with measured PAL [1.75 (0.02)] from CALERIE 2 (n = 127, r = 0.60, $p < 0.001$).

### 3.4. Percent CR associated with the zone of adherence

As hypothesized, the upper bound of the zone of adherence (the 80$^{th}$ percentile) reflected less than 25% CR (Figure 2, Table 2). At months 12 and 24, the mean CR levels for the upper bound of the zones were approximately half (13.7% CR) and less than half (10.4% CR) of the 25% CR goal, respectively. The lower bound of the zone (the 10$^{th}$ percentile) essentially reflected 25% CR (24.9% CR) at month 12 only, with the CR value decreasing to 19.4% at month 24. Moreover, the 50$^{th}$ percentile, which many participants considered their body weight target, reflected 17.8% and 13.6% CR at months 12 and 24, respectively.

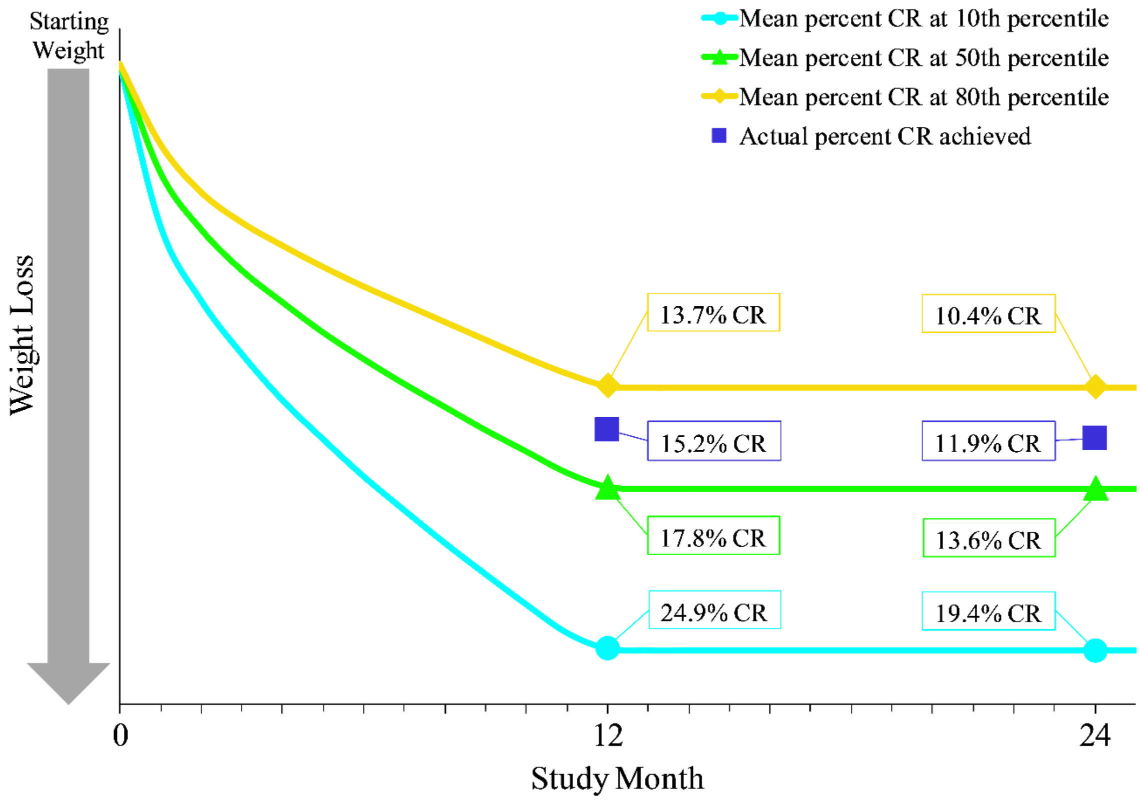


**Figure 2.** Percent CR, determined by the Body Weight Planner, at months 12 and 24 for the upper (80th percentile; yellow line) and lower (10th percentile; blue line) bounds of the adherence zone, as well as the 50th percentile (green line). Actual percent CR achieved by participants at months 12 and 24 is depicted by the dark blue squares and was measured with the intake-balance method.

The percent CR associated with the zone of adherence was greater for women and participants in the overweight BMI stratum; only the sex effect for the 10$^{th}$ percentile at month 24 was non-significant (Table 2 includes the sex and BMI effects; Figure 3 illustrates the BMI effect). Race effects for percent CR associated with the zone of adherence were present at month 12 only, with African Americans have greater percent CR than Whites and Asians at the 80$^{th}$

percentile (Table 2). African Americans and Whites had greater percent CR values than Asians at the 50th and 10th percentiles at month 12.

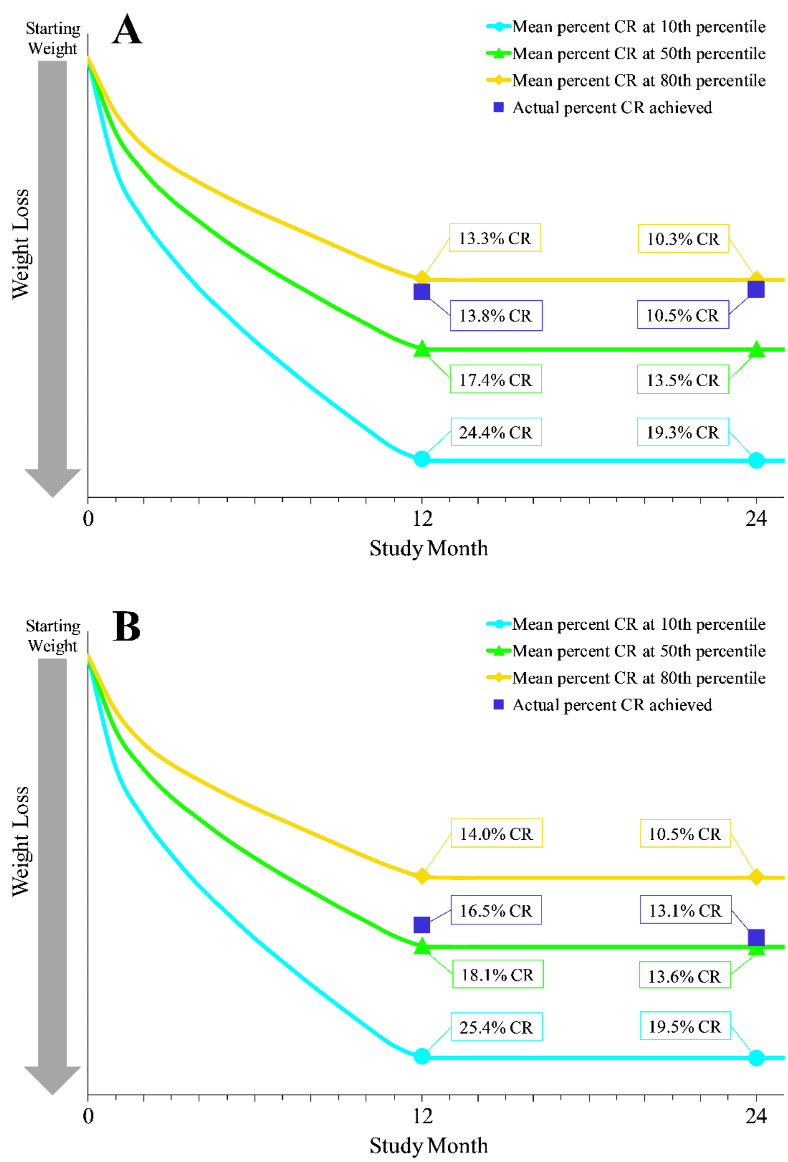


**Figure 3.** Percent CR, determined by the Body Weight Planner, at months 12 and 24 for the normal weight BMI category (22.0 – 24.9 kg/m2, **Panel A**) and the overweight BMI category (25.0 – 27.9 kg/m2, **Panel B**). The yellow line depicts the upper (80th percentile) and the blue

line the lower (10th percentile) bounds of the adherence zone. The green line depicts the 50th percentile. Actual percent CR achieved by participants at months 12 and 24 is depicted by the dark blue squares and was measured with the intake-balance method.

### 3.5. Percent CR achieved by participants

As hypothesized, the actual level of CR achieved by participants, assessed with the intake-balance method, was within the zone of adherence at both month 12 (15.2% CR) and month 24 (11.9%) (Table 2 and Figure 2). Percent CR did not differ by sex or race at month 12 or 24, though the race effect at month 24 had a p-value of 0.057. Inspection of the means suggests that participants who identified as Asian and Other had lower percent CR, although the sample sizes in these cells are small (Table 2). Participants in the overweight BMI stratum achieved higher percent CR at month 12; this effect was not statically significant at month 24 ($p = 0.056$) (Table 2).

## 4. Discussion

The hypotheses of the study were supported. First, the upper bound of the zone of adherence reflected a percent CR that was well below the 25% CR goal at months 12 and 24. Second, the average level of CR achieved by participants was within the zone at months 12 and 24. The lower bound of the zone nearly reflected 25% CR only at month 12 and, by month 24, the lower bound of the zone reflected ~19% CR. This highlights a problem that CALERIE 2 faced when using a model designed to predict weight loss over 12 months in a 24-month trial. The predicted weight loss trajectory was flat between months 12 and 24 because the model was not designed to predict weight loss past 12 months. This is problematic since different levels of CR are required to produce the same amount of weight loss over two different periods of time and body weight was used as a proxy measure of CR. Specifically, more severe CR is necessary to produce the same level of weight loss over a shorter duration, resulting in different levels of CR for the same goal weights at months 12 and 24.

Based on the weight graph and the definition of adherence used during the CALERIE 2 trial to inform intervention delivery, participants were, on average, adherent. Moreover, participants would need to have achieved a weight loss below the lower bound of the zone to achieve 25% CR between months 12 and 24. While it cannot be confirmed if 25% CR is feasible for most participants, the interpretation that the 25% CR intervention was a relative failure, and that participants could only achieve 12% CR on average over the two years, is confounded by the accuracy of the tool used to guide participants toward the prescribed goals. Indeed, the present analyses uncovered a discrepancy between the adherence metric that was obtained in real-time to guide intervention delivery with adherence calculations computed post hoc from state-of-the-art techniques, such as the intake-balance method. This highlights challenges with quantifying the success of a study or an intervention. When the intake-balance method is used to estimate participants' percent CR, it is noted that the level of achieved CR is below the 25% CR target; hence, the CALERIE 2 intervention is interpreted as failing to achieve its goal. Conversely, when a zone of adherence is used to determine adherence, as it was during delivery of the CALERIE 2 intervention, participants were considered adherent, on average, by virtue of their weights being in the zone of adherence. This discrepancy is noteworthy since a measure of percent CR from the intake-balance method cannot be obtained in real time to modify intervention delivery. Moreover, determining adherence with the intake-balance method creates a conundrum since any deviation from 25% CR technically reflects non-adherence, unless there is an a priori decision to consider a range of percent CR (e.g., 22% to 28% CR) as adherent.

The results of the study also indicate that the percent CR associated with the zone of adherence varied by sex, BMI stratum, and race. Specifically, the zone of adherence resulted in greater percent CR for women and for participants in the overweight BMI stratum. Nonetheless, due to the low variability of these measures, relatively small differences in percent CR were significant. The percent CR achieved did not differ by sex, but it did differ by BMI stratum. Specifically, the participants in the overweight BMI stratum achieved higher percent CR compared to those in the normal weight BMI stratum at month 12. Finally, Asians had lower percent CR associated with the zone of adherence compared to African Americans and sometimes Whites, though the percent CR achieved did not vary by race. These results highlight the need to: 1) build and validate models on representative samples of participants, and 2) build and validate models that better model the effects of sex and body mass on energy balance, which has been the focus of recent efforts. [8,19] The effects of race likely require further investigation, as body composition [20] and metabolism may differ among races, [21] even after adjusting for fat-free mass. Lastly, the results indicate that different groups of participants inadvertently may be held to different standards of adherence, which will affect the delivery of their intervention. This is an important area of study, particularly given the challenges of applying models and techniques to individual participants when they were validated at the group level.

The primary aim of the CALERIE 2 trial was to determine if CR favorably slowed biomarkers of aging, as it does in animal models, among human participants without obesity, including normal weight participants. A lower BMI limit of 22.0 kg/m$^2$ was established to allow investigation of the anti-aging effects of CR among participants of normal weight, specifically avoiding a study of obesity treatment, which has been the focus of many prior studies. [11] A rigorous safety plan was established that included monitoring bone mineral density and BMI, and

CR was discontinued temporarily or permanently if participants' values went below predefined limits (e.g., BMI<18.5 kg/m$^2$). [16] The level of CR achieved in CALERIE 2 was found to be generally safe and well-tolerated, with no significant differences in adverse events between the CR and control (ad libitum) group. [16] Within the CR group, however, participants of normal weight had a significantly higher incidence of nervous system, musculoskeletal, and reproductive system adverse events compare to the CR participants in the overweight BMI stratum at baseline. [16] Bone mineral density decreases with weight loss, and the CR group experienced expected levels of decreased bone mineral density, though the increase in predicted osteoporotic fracture risk over 10 years was minimal (0.2%). Loss of fat free mass also occurs during weight loss and, as expected, this was the case in CALERIE 2. Nonetheless, the CR participants, compared to the ad lib control, experienced an increase in the percent of body weight that was fat free mass, and a decrease in the percent of body weight that was fat mass, [22] and CR did not negatively affect aerobic capacity. [23]

The findings from CALERIE 2 indicate that CR is feasible and generally safe in adults without obesity. The lower level of CR achieved by participants in the normal weight BMI stratum compared to those in the overweight stratum at baseline suggests that leaner individuals may have experienced more difficulty adhering to CR, though this conclusion is confounded by the fact that the zone of adherence resulted in a greater percent CR for participants in the overweight BMI stratum. Further research is needed to evaluate the influence of weight status and BMI on adherence to a CR regimen.

This study has many strengths, including frequent TDEE and body composition assessments, which were necessary to estimate percent CR using the intake-balance method. An additional strength was the use of individualized weight graphs and a mathematical model to guide intervention delivery by estimating adherence to the 25% CR goal in real time throughout the two-year trial. The study also has limitations, including the inherent limitations in estimating percent CR with both the intake-balance method and a mathematical model and weight graph. Regarding estimation of percent CR with the intake-balance method, the method requires an accurate estimate of TDEE and changes in energy stores throughout the period of interest. It is not possible to obtain accurate estimates of TDEE throughout the intervention without frequent DLW measurements, which is impractical in most trials. Rather, mean TDEE was determined from DLW assessments at baseline and months 6, 12, 18, and 24, with the assumption that changes in TDEE were linear over time. Linear change in TDEE is unlikely since change in body weight, which is tightly associated with TDEE, is curvilinear, and changes in physical activity between DLW assessments will not be detected. Similarly, change in body composition requires repeated assessments with DXA or other techniques, and these measurements include inherent error, in addition to the error associated with the estimated energy costs of changes in fat mass and fat-free mass [5]. A final limitation is the application of mathematical models of energy balance, as well as other techniques, to individual participants since the models are typically validated at the group level. Importantly, the mathematical model used in this study [8,9] provides valid estimates of energy intake [13] and was different from the model used to direct intervention delivery in CALERIE 2. [6]

## Conclusions

The mathematical model and zone of adherence used in CALERIE 2 were novel and represent a pragmatic approach for estimating and promoting adherence to CR goals in real time. The clinical significance of the approach is exemplified by its integration into adaptive interventions that can be deployed remotely via mobile devices, such as smartphones and tablets.

[24] Such interventions have been found to promote clinically significant weight loss of 9.4% among healthy adults when delivered remotely [25] and to decrease the proportion of pregnant women who exceed gestational weight gain guidelines. [26] The zone of adherence in CALERIE 2, however, considered CR far less than the 25% goal as being adherent. This must be considered in designing CR interventions and strategies to promote adherence. For example, by structuring adherence zones that are lower, which would result in higher levels of CR being achieved when participants' weights were in the zone of adherence. The results also demonstrate the need to better understand the effects of sex, BMI, and race on zones of adherence, as well as intervention delivery. Specifically, research is needed to determine if the widths of adherence zones are sufficient to account for error in the models and to not hold some participants to a more stringent (or lenient) adherence metric. Finally, the way in which intervention success is evaluated after a trial requires further exploration since even state-of-the-art techniques, including the intake-balance method, have limitations and will not always align with measures of adherence used during intervention delivery.

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## Figure Legends

**Figure 1.** A sample weight graph is displayed for a hypothetical participant in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE$^{TM}$) phase 2 trial (CALERIE 2), which tested the effects of two years of CR on biomarkers of aging in humans. The light blue, green, and yellow lines correspond to the 10th, 50th, and 80th percentiles of expected weight trajectories, respectively. The dark blue line depicts the hypothetical participant's measured weight trajectory. The participant's starting weight was 70.7 in kilograms. From months 12 to 24, the yellow, green, and light blue lines represent 62.5, 60, and 55.7 kg, respectively. Reprinted from Contemporary Clinical Trials, Vol 32, Issue 6; Amy D. Rickman, Donald A. Williamson, Corby K. Martin, Cheryl H. Gilhooly, Richard I. Stein, Connie W. Bales, Susan Roberts, and Sai Krupa Das; The CALERIE Study: Design and methods of an innovative 25% caloric restriction intervention; Page No. 880, 2011, with permission from Elsevier.

**Figure 2.** Percent CR, determined by the Body Weight Planner, at months 12 and 24 for the upper (80th percentile; yellow line) and lower (10th percentile; blue line) bounds of the adherence zone, as well as the 50th percentile (green line). Actual percent CR achieved by participants at months 12 and 24 is depicted by the dark blue squares and was measured with the intake-balance method.

**Figure 3.** Percent CR, determined by the Body Weight Planner, at months 12 and 24 for the normal weight BMI category (22.0 – 24.9 kg/m2, **Panel A**) and the overweight BMI category (25.0 – 27.9 kg/m2, **Panel B**). The yellow line depicts the upper (80th percentile) and the blue line the lower (10th percentile) bounds of the adherence zone. The green line depicts the 50th

percentile. Actual percent CR achieved by participants at months 12 and 24 is depicted by the dark blue squares and was measured with the intake-balance method.

## Appendix

## Investigators and Staff Participating in CALERIE (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy)

The following is a list of the principal investigators (PIs), Coinvestigators (CIs), site intervention leaders (SILs), intervention counselors (ICs), study managers (SMs), project leaders (PLs), study coordinators (SCs), and other staff (OS) participating in the CALERIE study.

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# Challenges in defining successful adherence to calorie restriction goals in humans: Results from CALERIE™ 2

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