Title: Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men: a secondary analysis of energy expenditure and physical activity

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Abbreviations: BD, high-carbohydrate mixed macronutrient baseline diet; EE, energy expenditure; $EE_{chamber}$, total daily energy expenditure measured in metabolic chambers; EE_{DLW}, average energy expenditure measured by doubly labeled water; $EE_{nonchamber}$, average energy expenditure on days subjects were living on the ward outside metabolic chambers measured by doubly labeled water; KD, low-carbohydrate/high-fat ketogenic diet; OSF, Open Science Framework; SEE, sleeping energy expenditure.

Clinical Trial Registry: Data used in this study were derived from the clinical trial registered at www.clinicaltrials.gov as NCT01967563.

ABSTRACT

Background: Hall et al. (1) tested competing models for the etiology of obesity by measuring the change in energy expenditure (EE) of overweight and obese subjects after being switched from a baseline high-carbohydrate diet (BD) to an isocaloric low-carbohydrate ketogenic diet (KD). EE measured using metabolic chambers increased transiently and by what was considered a relatively small extent after the switch to the KD, whereas EE measured using doubly labeled water (EE_{DLW}) after the response in the chambers had waned increased more substantially.

Objective: Using the publicly available Hall et al. dataset, we examined the effect of housing conditions on the magnitude of the increase in EE_{DLW} after the switch to the KD and the role of physical activity in that response.

Design: The 14-day EE_{DLW} measurement period included 4 days when subjects were confined to chambers instead of living on wards. To determine the effect on EE_{DLW} only for the days subjects were living on the wards, we used a formula in Hall et al. to calculate non-chamber EE ($EE_{nonchamber}$). To assess the role of physical activity in the response to the KD, we analyzed accelerometer data from the BD and KD EE_{DLW} measurement periods.

Results: In comparison with the increase in 14-day EE_{DLW} of 151 kcal/d $\pm$ 63 ($P = 0.03$) after the switch to the KD, $EE_{nonchamber}$ increased by 203 $\pm$ 89 kcal/d ($P = 0.04$) or, with inclusion of a questionable outlier, by 283 $\pm$ 116 kcal/d ($P = 0.03$). Hip accelerometer counts decreased significantly ($P = 0.01$) after the switch to the KD, whereas wrist accelerometer counts did not change.

Conclusion: **S**witching to the KD increased EE_{DLW} only on non-chamber days to an extent substantially greater than that originally reported. Increased physical activity as measured by accelerometry does not appear to account for this effect.

Keywords: obesity, energy expenditure, physical activity, ketogenic diet, metabolic chamber, doubly labeled water, accelerometry

INTRODUCTION

Debate over the role of diet in the etiology of obesity often centers on the relative importance of dietary macronutrient composition versus total energy consumption. According to one view of obesity (2-4), the primary cause of fat accumulation involves a shift in the partitioning of metabolic fuels away from pathways of oxidation toward those of fat synthesis and storage. In this case, diet composition can be an important contributing factor; diets rich in carbohydrate, in particular those containing substantial amounts of refined grains and sugars, promote obesity because stimulation of insulin secretion by these nutrients drives metabolic fuels toward the synthesis and storage of fat (5). The more conventional model sees obesity as driven by an energy balance disorder in which energy intake exceeds energy expenditure (6,7). According to this "energy balance" hypothesis, this excessive total energy intake, regardless of the macronutrient source of the energy, is the primary cause of obesity.

The "carbohydrate-insulin" and energy balance hypotheses make distinctly different predictions about the effects of reducing dietary carbohydrate content on energy expenditure (EE) under conditions in which calories and protein remain constant. The carbohydrate-insulin hypothesis predicts that lowering the proportion of carbohydrate to fat, even while maintaining energy and protein intake, would minimize circulating insulin concentration and thereby promote lipolysis and oxidation of stored and ingested fat, and, as a result, increase EE. On the other hand, the energy balance hypothesis, which assumes "a calorie is a calorie," predicts that exchanging fat calories for carbohydrate calories would have no appreciable effect on energy expenditure (1,8).

To test these competing predictions, Hall et al. (1) measured EE in overweight and obese subjects housed on metabolic wards before and after they were switched from a high-

carbohydrate baseline diet to an isocaloric ketogenic diet containing equivalent protein and little carbohydrate. EE was measured two ways: using metabolic chambers for two consecutive days each week throughout the study and using doubly labeled water during the last 2 weeks of each 4-week diet period. EE measured in metabolic chambers increased significantly after the switch to the ketogenic diet, but this change was transient, lasting only two weeks, and was considered to be relatively small by Hall et al., which led them to conclude that the results did not support the carbohydrate-insulin model. However, in contrast to the results using metabolic chambers, EE, measured using doubly labeled water (EE_{DLW}) after the response in the chambers had waned, increased significantly to a greater extent (151 kcal/d vs. 57 kcal/day) after the switch to the ketogenic diet. This response was attributed (1) to greater energy expenditure from physical activity when subjects were outside the chambers living on the ward.

The carbohydrate-insulin and energy balance hypotheses have distinctly different implications for understanding the etiology of obesity and devising effective strategies for preventing and treating it. Consequently, it would be useful to reconcile the discrepant findings from measurements of EE using metabolic chambers and doubly labeled water. Hall et al. made the data from their study publicly available on the Open Science Framework (OSF) website (9). In this paper, we report results of additional analyses of this dataset to differentiate the effect of housing subjects in a metabolic chamber versus on a metabolic ward on the magnitude of the increase in EE_{DLW} after the switch to the KD and to assess the role of physical activity in this effect.

METHODS

Overview of the Hall et al. study

Details of the design and methods of the study can be found in the Hall et al. paper (1), including the online supplemental data (10), and in the published IRB-approved protocol (11). Briefly, focusing on methods relevant to the analyses described here, 17 overweight or obese males were admitted as inpatients to metabolic wards and fed a baseline diet (BD; 15:50:35 percent of calories from protein:carbohydrate:fat) for 4 weeks followed by an isocaloric ketogenic diet (KD; 15:5:80 percent of calories from protein:carbohydrate:fat) for another 4 weeks. Subjects were housed in a metabolic chamber for two consecutive days each week throughout the study to measure daily EE, sleeping EE, and respiratory quotient. During the last 2 weeks of each diet period, average daily EE was measured using doubly labeled water (EE_{DLW}). Physical activity level was monitored throughout the study using accelerometers; each subject wore an accelerometer on a hip, wrist and ankle, and accelerometer counts and the length of time wearing the devices were logged for each device location.

Rationale and Statistical Analyses

The sources and handling of data from the Hall et al. dataset for the analyses below are described in detail in the **Supporting Material**.

Replication of calorimetry data. To confirm the replicability of the data used in the secondary analyses of EE_{DLW} described below, we first reanalyzed the calorimetry results reported in Table 2 of the Hall et al. paper (1) using the dataset and code published on the OSF website (9) and SAS v9.4 (SAS Institute, Inc.).

Non-chamber EE_{DLW}. In the Hall et al. study, the 14-day period for measuring EE_{DLW} included 4 days when subjects were confined to a metabolic chamber and 10 days when subjects lived on the ward. Hall et al. reported EE_{DLW} as a daily average across the 14-day measurement period and did not differentiate the effect of diet during the non-chamber days, when subjects were housed on the ward, from the chamber days, when EE is relatively lower (12,13) and the effect of diet much reduced (1).

To determine average daily EE_{DLW} for only those days in which subjects were housed on the ward, we used a term in Hall et al.'s Equation 6 for calculating non-chamber EE ($EE_{nonchamber}$). In essence, the resulting equation (Equation 1 below) separates average daily EE for days subjects were housed on the wards from days they were confined to metabolic chambers by subtracting total EE measured during the 4 chamber days ($EE_{chamber}$) within the EE_{DLW} measurement period from the total 14-day EE_{DLW} and averaging the resulting value over the 10 non-chamber days.

$$EE_{nonchamber} = \frac{7}{5} EE_{DLW} - \frac{2}{5} EE_{chamber} \qquad (1)$$

Differences in $EE_{DLW,}$ $EE_{nonchamber}$ and $EE_{chamber}$ between the two diet conditions were evaluated using paired t-tests. Data from Subject 04-012 was not included in these analyses (see below) in keeping with Hall et al. A *P* value of < 0.05 (two-sided tests) was considered statistically significant for this and all other analyses below.

EE_{DLW} outlier. Hall et al. excluded one subject's (#04-012) data from the analysis of energy expenditure measured using doubly-labeled water. This subject showed the largest increase in EE_{DLW} after the switch from the BD to the KD, which was identified statistically as an outlier value using Cook's distance. Because this subject's relatively extreme change in EE_{DLW} was not apparently due to a documented error in, for example, sampling, recording, computation or

coding, best practices (14) indicate that the EE_{DLW} data should be reported with and without the outlier data. To that end, we compared EE_{DLW} and $EE_{nonchamber}$ during the two diet periods, as above, except that data from Subject 04-012 was included in the analysis.

Hall et al. also justified exclusion of Subject 04-012's data on the basis that he gained 0.2 kg during the KD period despite the marked increase in EE_{DLW} after the switch to the KD and an EE_{DLW} during the KD period that substantially exceeded his energy intake. The reported 0.2 kg weight gain occurred over two body composition assessments performed 13 days apart during the latter part of the KD period (similar assessments were also performed during the BD period). To evaluate this rationale for exclusion, we examined body weight data collected during the body composition assessments from the subject in question as well as the entire group of subjects both within the KD period and from the last body composition assessment near the end of the BD period to last assessment near the end of the KD period. In addition, we evaluated the change in body weight during the EE_{DLW} measurement period using daily body weight data collected on the ward. Although the rationale for exclusion was based on the magnitude of the increase in the subject's EE_{DLW} from the BD to KD periods, it is not clear how such a difference between the diet periods would affect body weight across the two body composition assessments during just the KD period. Therefore to examine the relationship between EE_{DLW} and changes in body weight in the study subjects, correlation coefficients were calculated between body weight change (taken from the body composition assessments) and EE_{DLW}, and between weight change and the difference between energy intake and EE_{DLW}, both during the KD EE_{DLW} measurement period.

Accelerometer data. Hall et al. evaluated whether greater physical activity accounted for the increase in EE_{DLW} during the KD period by calculating energy expenditure from physical activity

in and out of the metabolic chambers (i.e, $PAE_{chamber}$ and $PAE_{nonchamber}$ as per their Table 2). Physical activity energy expenditure outside the chambers was higher during the KD period compared to the BD phase, but the effect was not statistically significant. Physical activity level was measured throughout the study using accelerometers; however, only hip count data were reported as a percentage difference between chamber and non-chamber days during the entire BD period. Here, we used the accelerometer data in the OSF dataset to determine more directly whether differences in physical activity can account for the increase in EE_{DLW} after the switch to the KD .

To confirm replicability and help validate our use of the accelerometer data, we first reanalyzed the fractional difference between hip accelerometer counts from chamber and non-chamber days during the baseline period of the study using the published code. We next analyzed daily hip, wrist and ankle accelerometer counts during the BD and KD EE_{DLW} measurement periods with respect to whether subjects were confined to chambers or were housed on the ward (i.e., chamber and non-chamber days). Average accelerometer wear times varied little with respect to device location, diet and housing status. In keeping with Hall et al.'s calculation and analysis of physical activity energy expenditure, accelerometer data were analyzed excluding data from Subject 04-012. In a separate analysis, this subject's data was included. Accelerometer counts for each location with respect to chamber status and diet were compared using ANOVA with corresponding *F*-tests to determine statistical significance.

To further examine the relationship between physical activity and energy expenditure, we calculated coefficients of correlation between hip accelerometer counts and EE_{DLW} and $EE_{nonchamber}$ during the EE_{DLW} measurement period for each diet condition.

Sleeping energy expenditure. Hall et al. reported that sleeping energy expenditure (SEE; kcal/d) measured in the metabolic chambers increased in the first week after subjects were switched from the BD to the KD, and then declined during the subsequent three weeks. To determine whether this increase in SEE persisted during the end of the KD period and may have contributed to the increase in EE_{DLW} observed at that time, we compared SEE during the BD and KD EE_{DLW} measurement periods. Comparison of SEE for all subjects during the EE_{DLW} measurement periods in the BD and KD phases of the study were made using a paired t-test.

RESULTS

Replication of calorimetry data. Reanalysis of the calorimetry data provided in Table 2 in the Hall et al. paper using the OSF dataset and code fully replicated the reported results, including mean, standard error and probability values associated with statistical comparisons of diet periods.

Non-chamber EE_{DLW}. The increase in average daily EE after the switch to the KD, measured by doubly labeled water, was greater when calculated only for days when subjects were housed on the wards outside of the chambers than it was when calculated over the entire EE_{DLW} measurement period that included both days in and out of the chambers. Reanalysis of EE_{DLW} data from the Hall et al. dataset replicated the average 151 kcal/d increase in EE_{DLW} after subjects were switched to the KD (**Table 1**). Using Equation 1 above, energy expenditures for days when subjects were out of the chambers ($EE_{nonchamber}$) increased on average by 203 kcal/d after subjects were switch from the BD to the KD. Energy expenditure measured in the chambers ($EE_{chamber}$) during the EE_{DLW} measurement periods did not differ as a function of diet.

EE_{DLW} outlier. The effect of switching from the BD to the KD on EE measured using doubly labeled water was greater when data from the outlier was included in the analysis. As shown in Table 1, the increase in EE_{DLW} after the switch to the KD was greater (209 $\pm$ 83 kcal/d) when Subject 04-012's data were included in the analysis than when they were not (151 $\pm$ 63 kcal/d). When this subject's data were included in the calculation of $EE_{nonchamber}$, the effect of switching to the KD was greater (283 $\pm$ 116 kcal/d) than when his data were excluded (203 $\pm$ 89 kcal/d; see Table 1).

The weight gain of Subject 04-012 across the interval between two body composition assessments in the KD period reported by Hall et al., which underlies their rationale for exclusion of his data from analysis of the effect of diet on EE_{DLW}, was not representative of his change in body weight over the entire KD period or the KD EE_{DLW} measurement period. We confirmed that Subject 04-012 gained 0.2 kg between the two body composition assessments during the KD period; however, this subject lost 1.7 kg from the last body composition assessment 2 days before the end of the BD period to the last assessment during the KD period. Similarly, inspection of daily body weights from the dataset showed that he lost 0.5 kg over the 14 day EE_{DLW} measurement period while consuming the KD.

Subject 04-012's EE_{DLW} and weight gain, as reported by Hall et al., were well within the variability of the group as a whole and the relationship between his EE_{DLW} and weight gain was not inconsistent with that of the group. Although the subject's change in EE_{DLW} after the diet switch was an outlier, his EE_{DLW} during the KD period (3612 kcal/d), which would be the relevant measure to determine any discrepancy regarding changes in body weight during the KD period, was not, being well within one standard deviation from the mean of the group (3173 $\pm$ 583 kcal/day, mean $\pm$ SD). Variability in weight change between the two body composition

assessments during the KD period was substantial; however, the 0.2 kg weight gain of Subject 04-012 was within one standard deviation of the average weight change of all subjects (-0.6 kg ± 0.8, mean ± SD). Two other subjects gained more weight during the KD period (0.6 and 1.3 kg) accompanied by smaller, but substantial, increases in EE_{DLW} relative to the BD period (527 and 358 kcal/d, respectively) and, respectively, EE_{DLW} relative to energy intakes in these subjects was both greater (1751 kcal/d) and smaller (465 kcal/d). There was no significant correlation between the change in body weight and EE_{DLW} (r = -0.06) or the change in weight and the difference between energy intake and EE_{DLW} (r = 0.26) during the KD EE_{DLW} measurement period.

Accelerometer data. Reanalysis of hip accelerometer counts during the full BD period using the Hall et al. code replicated their finding that counts were 21 ± 4% greater on non-chamber days than they were on days when subjects were confined to metabolic chambers.

Although hip and wrist accelerometer counts were significantly greater when subjects were housed on the ward than when they were confined to metabolic chambers, counts either decreased (hip) or did not change (wrist) after the switch from the BD to the KD (**Table 2**). Ankle accelerometer counts were similar under both chamber and non-chamber conditions and did not vary as a function of diet. Ankle counts were used by Hall et al. to monitor stationary cycling exercise. The lack of an effect of chamber status or diet on ankle counts would be expected given that subjects were prescribed a set amount of such exercise daily throughout the study. Inclusion of data from Subject 04-012 did not materially affect accelerometer counts or the outcomes of the statistical analyses.

Correlation coefficients between hip accelerometer counts and EE_{DLW} or $EE_{nonchamber}$ during the BD or KD periods ranged from 0.04 to 0.11 and were not statistically significant.

Sleeping energy expenditure. SEE measured in the chambers during EE_{DLW} measurement periods was significantly greater after the switch from the BD to the KD (BD, 1576 $\pm$ 68 kcal/d and KD 1620 $\pm$ 56 kcal/d; t (16) = 2.60, P < 0.02). SEE values and the results of the analysis were nearly identical if data from Subject 04-012 were excluded.

DISCUSSION

Hall et al. measured energy expenditure using doubly labeled water over a 14-day period that included 4 days during which subjects were confined to a metabolic chamber and 10 days when they were housed on the ward. Because people expend less energy in a metabolic chamber than under more free-living conditions (12,13) and chamber measurement of EE showed little difference between the diet periods in the Hall et al. study (1), we quantified expenditures for non-chamber days ($EE_{nonchamber}$) separately from in-chamber days ($EE_{chamber}$). The results showed that the switch from the BD to the KD diet was accompanied by an increase in EE_{DLW} that is 34% larger than that originally reported (1). $EE_{chamber}$ during the doubly labeled water measurement periods did not differ significantly as a function of diet, further indicating that the increase in EE_{DLW} after the switch to the KD was limited to days when subjects were housed on the ward.

In keeping with best practices for handling outliers (14), we calculated EE_{DLW} and $EE_{nonchamber}$ with and without data from the subject considered an outlier by Hall et al. Relative to the increase in EE_{DLW} after the switch to the KD as originally reported, including this data increased the effect on EE_{DLW} and $EE_{nonchamber}$ by, respectively, 38% and 87%. The relatively small weight gain of 0.2 kg in this subject during the chosen sampling period between the two body composition assessments did not reflect the more substantial weight loss over the nearly entire KD period or just during the 14-day EE_{DLW} measurement period. Although the change in

the subject's EE_{DLW} after the diet switch was identified statistically as an outlier, his EE_{DLW} during the KD period, which is most relevant to any concomitant change in body weight, was not. The variability in the weight changes among subjects during the KD period was substantial and was reflected in the lack of significant correlations between weight change and EE_{DLW} or the difference between EE_{DLW} and energy intake. This suggests that, for this study, a lack of a relationship between these variables for any one subject cannot serve as an adequate basis for excluding that subject's data. Taken together with the relatively small number of subjects in this experiment, and the lack of previous research to help determine the range of response to KD diets, we do not find a compelling case for the exclusion of Subject 04-012. It will take additional research to determine whether this subject's data is invalid or reflects a relatively extreme response seen in a small proportion of the population.

According to Hall et al., the carbohydrate-insulin model predicts that consuming a KD would increase energy expenditures by 300-600 kcal/d. The higher value was based on a theoretical estimate (15) of a 400-600 kcal/d expenditure to support gluconeogenesis under conditions very different than those in the Hall et al. study, specifically, prior to adaptation to a low-carbohydrate diet with only endogenous, not dietary, protein as the substrate. The lower value was based on the results of a study by Ebbeling et al. (16) in which EE_{DLW} in free-living subjects was, depending on how expenditures were calculated, ~250-325 kcal/d greater when subjects ate a low-carbohydrate/high-fat diet compared with a high-carbohydrate/low-fat diet. The increase of ~200-280 kcal/d in $EE_{nonchamber}$ in the Hall et al. study is consistent with this earlier finding.

Estimates of the increase in $EE_{nonchamber}$ from the current analysis likely represent a minimal range for the effect size. The continuing weight loss throughout the study due to

unintentional underfeeding of the subjects, as described by Hall et al., would be expected to have suppressed EE (13), thereby mitigating any increase in energy expenditure after switching to the KD. Lower circulating concentrations of leptin and triiodothyronine during the KD versus the BD period reported by Hall et al. are consistent with such a reduction in EE. Inclusion of the losses of ketone bodies in urine and, possibly, fat in feces would also magnify expenditures during the KD period (17). On the other side of the ledger, the difference in EE_{DLW} may have been magnified if higher rates of *de novo* lipogenesis during consumption of the high-carbohydrate BD sequestered deuterium in fat, which would lower the estimate of CO_2 production and consequently reduce measured EE_{DLW} relative to that during consumption of the very low-carbohydrate KD (18). While possible, such deuterium trapping has been documented only in rapidly fattening piglets with no significant consequence for estimates of EE_{DLW} predicted for weight stable animals (18). Given this, it seems unlikely that differences in *de novo* lipogenesis during the diet periods would account for those in EE_{DLW} in subjects in the Hall et al. study who were continually losing weight. Clearly, a more comprehensive analysis, including direct measurements of relevant biochemical and physiological processes in weight-stable subjects, is needed to better characterize the magnitude of the effect of a ketogenic diet on EE_{DLW}.

The order in which subjects in the Hall et al. study were fed the BD and the KD was not counterbalanced or otherwise controlled for, a trial design limitation noted by the authors that limited causal inference about the effect of the KD. In contrast, subjects in the Ebbeling et al. study (16) were fed the different experimental diets in a randomized order. The similarity in the responses to a low carbohydrate diet in the Ebbeling et al. study and, with respect to $EE_{nonchamber}$,

to the KD in the Hall et al. study lends credence to the conclusion that consumption of the KD caused the increase in EE_{DLW} in the Hall et al. study.

Accelerometer counts, a direct measure of physical activity, did not increase and, in the case of hip counts, decreased during the EE_{DLW} measurement period after the switch from the BD to the KD. These findings do not support the suggestion in Hall et al. that the lack of a significant increase in non-chamber energy expenditure from physical activity during the KD period was due to limitations on activity imposed by the metabolic ward environment. The dissociation between changes in physical activity and EE_{DLW}, is consistent with earlier findings (16) that free-living subjects eating a very low-carbohydrate, high-fat diet show elevated EE_{DLW} but no change in physical activity measured using accelerometers. Accelerometry in this earlier study and the Hall et al. study may not have captured components of physical activity (19) that contributed to the increase in energy expenditures during the KD period, although the hip and wrist accelerometers counts likely reflected walking, which is a major component of non-exercise activity thermogenesis (19).

Sleeping energy expenditure, derived from sleeping metabolic rate, was greater during the EE_{DLW} measurement period after the switch to the KD. Because sleeping metabolic rate approximates as much as 80-100% of basal metabolic rate (20), the difference in sleeping energy expenditure suggests that the KD may have increased basal energy expenditure by ~50 kcal/d, accounting for ~18-25% of the increase in daily energy expenditures depending on the range of estimates for the effect of the KD. Such an estimate must be tempered, however, given that physical activity during sleep will increase metabolic rate and that the difference in sleeping metabolic rate as a function of diet measured in chambers may differ from that when subjects were sleeping on the ward.

After allowing for an elevation in basal metabolic rate and some contribution from physical activity not monitored by accelerometers, much of the increase in EE_{DLW} during the KD period appears left to be explained. Given that the effect of switching to the KD is limited to days subjects were housed on the ward, the increase in $EE_{nonchamber}$ may have been associated with the increase in physical activity outside the chambers but not directly caused by it. About one-third of the increase in thermogenesis in overfed subjects housed on a metabolic ward has been attributed to lower skeletal muscle work efficiency (21), suggesting that nutritional factors can affect energy expenditures by increasing the energetic cost of physical activity. Consumption of a very low carbohydrate, ketogenic diet could have similar effects; indeed, as early as 1920, Krogh and Lindhard (22) described the "waste" of energy from fat in exercising humans maintained on a largely fat, as compared with a primarily carbohydrate, diet. On the other hand, as reported by Hall et al., EE associated with the prescribed cycling exercise in the chambers was similar in the two diet periods. This observation may argue against a change in muscle work efficiency in the KD period; however, EE due to exercise in the chambers may not be reflective of exercise-related expenditures on the ward against a background of greater overall activity, and the type of muscular work involved in such exercise may not be affected by a ketogenic diet. Aside from changes in muscle efficiency, the increase in physical activity outside the chambers may also have increased EE during the KD period by creating a demand for glucose, some of which under the condition of severe dietary carbohydrate restriction would be met through the energetically expensive process of hepatic gluconeogenesis. Identification of the mechanisms underlying the increase in EE_{DLW} in response to a ketogenic diet will depend on additional research.

A discrepancy between chamber and doubly labeled water measures of EE in response to a nutritional manipulation was reported previously by Rosenbaum et al. (13) who found substantial changes in total daily EE in response to over- and under-feeding when measured using doubly labeled water or by caloric titration in subjects housed on a ward, but not when measured in the same subjects using chamber respirometry. These investigators attributed the different outcomes between methodologies to limitations on physical activity imposed by the metabolic chamber. The present analyses of the Hall et al. data similarly suggest that the magnitude of the effect of a KD on EE depends on conditions in which physical activity is not restricted by the confines of a metabolic chamber. Although more direct methodological comparisons are needed, the preferred approach for studies of the effect of nutritional status or dietary composition on energy balance would appear to entail at least the opportunity for physical activity afforded by a metabolic ward along with appropriate methods for measurement of EE that do not require restricted confinement.

On the basis of the transient and what was considered a relatively small increase in EE measured in the metabolic chambers after the switch to the KD, Hall et al. concluded that the results of their study did not support the prediction of the carbohydrate-insulin hypothesis of obesity that such an isocaloric change in diet would increase EE. This finding has been cited as evidence refuting the carbohydrate-insulin hypothesis, offering further support for the energy balance hypothesis of obesity that emphasizes a calorie-is-a-calorie perspective (23, 24). Such a conclusion seems premature given the robust increase in EE associated with consumption of the KD as measured using doubly-labeled water in Hall et al. Indeed, on the basis of the increase in EE_{DLW} and, more specifically, in $EE_{nonchamber}$, the results are entirely consistent with the carbohydrate-insulin hypothesis, which predicts an increase in EE with reduction of carbohydrate

intake. The metabolic basis for this increase in EE is not explained by factors assessed here or in the Hall et al. study (e.g., physical activity by accelerometry, circulating concentrations of leptin or triiodothyronine) and deserves further exploration.

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Authors' contributions. MF designed the analysis plan; SA performed the statistical analyses; MF and SA wrote the paper; MF had primary responsibility for final content.

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TABLE 1

Energy expenditure during the BD and KD doubly labeled water measurement periods[1]

	BD	KD	Difference	P^2
EE_{DLW}	2995 ± 45	3146 ± 45	151 ± 63	0.03
$EE_{nonchamber}$	3142 ± 45	3344 ± 45	203 ± 89	0.04
$EE_{chamber}$	2628 ± 110	2649 ± 95	21 ± 32	NS
EE_{DLW} (*included*)	2964 ± 59	3173 ± 59	209 ± 83	0.02
$EE_{nonchamber}$ (*included*)	3100 ± 82	3382 + 82	283 + 116	0.03

[1]Data are least squares mean ± SEs. Values are based on n = 16 except for those designated as "*included*" (n = 17), which include an outlier removed from the other analyses as described in the text. Difference values are the change from the BD to KD period. $EE_{chamber}$, daily energy expenditure measured for 4 days in metabolic chambers during the 14-day doubly labeled water measurements; EE_{DLW}, energy expenditure over 14 days measured using doubly labeled water; $EE_{nonchamber}$, energy expenditure on 10 days outside metabolic chambers.

[2]Values refer to the difference between diet periods by paired t-test.

TABLE 2

Accelerometer counts during the BD and KD doubly labeled water measurement periods as a function of housing condition[1]

	Hip		Wrist		Ankle	
	In Chamber	On Ward	In Chamber	On Ward	In Chamber	On Ward
BD	369,350 ± 26,021	480,607 ± 13,764	1,387,343 ± 61,421	1,719,534 ± 38,508	1,901,904 ± 88,940	1,975,959 ± 48,299
KD	301,807 ± 20,535	436,865 ± 13,784	1,341,069 ± 71,521	1,749,591 ± 40,614	1,795,941 ± 86,194	1,916,927 ± 45,994
Chamber	$F(1,363) = 27.7$; $P < .0001$		$F(1,376) = 30.0$; $P < .0001$		$F(1,363) = 1.43$; NS	
Diet	$F(1,363)=7.65$; $P = .006$		$F(1,376) = 0.13$; NS		$F(1,363) = 1.25$; NS	

[1]Data are least squares mean ± SEs based on n = 16 and represent total counts during the 14-day double labeled water measurement periods.

Supporting Material

Abbreviations

$EE_{chamber}$, total daily energy expenditure measured in metabolic chambers; EE_{DLW}, average energy expenditure measured by doubly labeled water; $EE_{nonchamber}$, average energy expenditure on days subjects were living on the ward outside metabolic chambers measured by doubly labeled water; KD, low-carbohydrate/high-fat ketogenic diet; SEE, sleeping energy expenditure.

Data Sources (see Flowchart below for additional information)

Replication of calorimetry data. Data from Hall et al.'s Table 2 (1) for re-calculation and analysis of were extracted from the "Intake," "chamber," "BC" (body composition) and "DLW" (doubly labeled water) tabs of the Hall et al. dataset published on the Open Science Framework website (2).

Non-chamber EE_{DLW}. Data for this and all other analyses of EE_{DLW}, $EE_{nonchamber}$, and $EE_{chamber}$ were taken from the "DLW" tab in the Hall et al. dataset. EE_{DLW} values in Equation 1 correspond to the "TEE DLWChamber unadjusted" values in the "DLW" tab, which were derived using respiratory quotient measured in the chambers during the EE_{DLW} measurement periods. $EE_{chamber}$ values in the "DLW" tab correspond to the "EE binned" values in the "Chamber" tab of the dataset averaged over the chamber days during the 14-day EE_{DLW} measurement periods.

EE_{DLW} outlier. For Subject 04-012, the first of the two body weight measurements associated with body composition assessments during the KD period was performed the day before the "Dose Date" for doubly labeled water (as indicated in the dataset "DLW" tab). The second was performed 12 days later, two days before the end of the EE_{DLW} measurement period. Body weights taken during body composition assessment ("BodyMass_kg") were extracted from the "BC" (body composition) tab of the dataset and, for the KD EE_{DLW} measurement periods, from "DailyBW" tab of the dataset. Energy intake data were taken from the "EI" tab in the dataset.

Accelerometer data. Accelerometer counts were extracted from the "Accelerometer" tab in the dataset using the "Dose Date" for doubly labeled water in the dataset "DLW" tab as the first day of the EE_{DLW} measurement periods. We included data only from those days during which accelerometer wear time exceeded 720 minutes (12 hours) as specified in the Hall et al. code for analysis of the fractional difference in counts during chamber and non-chamber days.

Sleeping energy expenditure. SEE data were extracted as "SMR Chamber unadjusted" values from the "DLW" tab of the Hall et al. dataset. These values correspond to the "SMR binned" values in the "Chamber" tab of the dataset averaged over the chamber days during the EE_{DLW} measurement periods described above. As in Hall et al. (1), SEE (as kcal/d) was extrapolated from sleeping metabolic rate (as kcal/min).

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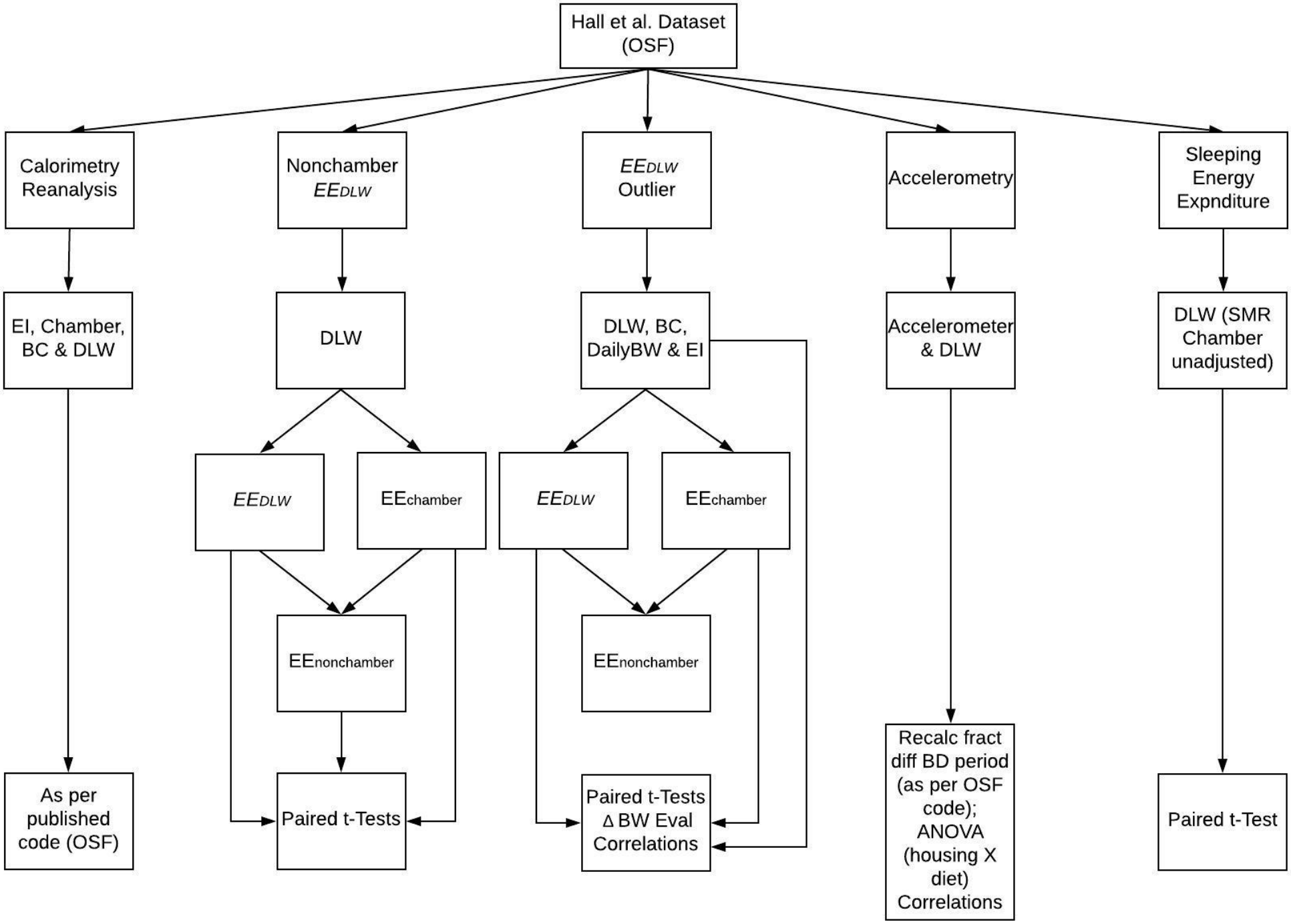
Hall et al. Dataset (OSF)
Calorimetry Reanalysis
Nonchamber EE_{DLW}
EE_{DLW} Outlier
Accelerometry
Sleeping Energy Expnditure
EI, Chamber, BC & DLW
DLW
DLW, BC, DailyBW & EI
Accelerometer & DLW
DLW (SMR Chamber unadjusted)
EE_{DLW}
EEchamber
EE_{DLW}
EEchamber
EEnonchamber
EEnonchamber
As per published code (OSF)
Paired t-Tests
Paired t-Tests
Δ BW Eval
Correlations
Recalc fract diff BD period (as per OSF code); ANOVA (housing X diet) Correlations
Paired t-Test