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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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ABSTRACT

Background

18   A previously published pilot study assessed energy expenditure (EE) of participants with

19   overweight and obesity after they were switched from a baseline high-carbohydrate diet (BD) to

20   an isocaloric low-carbohydrate ketogenic diet (KD). EE measured using metabolic chambers

21   increased transiently by what was considered a relatively small extent after the switch to the KD,

22   whereas EE measured using doubly labeled water (EE_{DLW}) increased to a greater degree after the

23   response in the chambers had waned. Using a publicly available dataset, we examined the effect

24   of housing conditions on the magnitude of the increase in EE_{DLW} after the switch to the KD and

25   the role of physical activity in that response.

Methods

27   The 14-day EE_{DLW} measurement period included 4 days when subjects were confined to

28   chambers instead of living in wards. To determine the effect on EE_{DLW} only for the days subjects

29   were living in the wards, we calculated non-chamber EE ($EE_{nonchamber}$). To assess the role of

30   physical activity in the response to the KD, we analyzed chamber and non-chamber

31   accelerometer data for the BD and KD EE_{DLW} measurement periods.

Results

33   In comparison with the increase in average 14-day EE_{DLW} of 151 kcal/d $\pm$ 63 ($P = 0.03$) after the

34   switch to the KD, $EE_{nonchamber}$ increased by 203 $\pm$ 89 kcal/d ($P = 0.04$) or 283 $\pm$ 116 kcal/d ($P =$

35   0.03) depending on the analytical approach. Hip accelerometer counts decreased significantly (P

36   $= 0.01$) after the switch to the KD, whereas wrist and ankle accelerometer counts did not change.

Conclusions

38   Switching from the BD to the KD substantially increased EE_{DLW}, but apparently only on days

39   subjects were living in the ward outside the metabolic chamber. Increased physical activity as

40   measured by accelerometry did not appear to account for this effect.

Introduction

42   Debate over the role of diet in the etiology of obesity often centers on the relative

43   importance of dietary macronutrient composition versus total energy consumption. According to

44   one view of obesity [1-3], the primary cause of fat accumulation involves a shift in the

45   partitioning of metabolic fuels away from pathways of oxidation toward those of fat synthesis

46   and storage. In this case, diet composition can be an important contributing factor; diets rich in

47   carbohydrate, in particular those containing substantial amounts of refined grains and sugars,

48   promote obesity because stimulation of insulin secretion by these nutrients drives metabolic fuels

49   toward the synthesis and storage of fat [4]. This is known as the "carbohydrate-insulin"

50   hypothesis. The more conventional model sees obesity as caused by an energy balance disorder

51   in which energy intake exceeds energy expenditure [5,6]. According to this "energy balance"

52   hypothesis, this excessive total energy intake, regardless of the macronutrient source of the

53   energy, is the primary cause of obesity.

54   The carbohydrate-insulin and energy balance hypotheses make distinctly different

55   predictions about the effects of reducing dietary carbohydrate content on energy expenditure

56   (EE) under conditions in which calories and protein remain constant. The carbohydrate-insulin

57   hypothesis predicts that lowering the proportion of carbohydrate to fat, even while maintaining

58   energy and protein intake, would minimize circulating insulin concentration and thereby promote

59   lipolysis and oxidation of stored and ingested fat, and, as a result, increase EE. On the other

60   hand, the energy balance hypothesis, which assumes "a calorie is a calorie," predicts that

61   exchanging fat calories for carbohydrate calories would have no appreciable effect on energy

62   expenditure [7].

63   In preparation for an anticipated full-scale trial to test these competing predictions, Hall

64   et al. conducted a pilot study [8,9] in which they measured EE in participants with overweight

65   and obesity who were housed in metabolic wards before and after they were switched from a

66   high-carbohydrate baseline diet to an isocaloric ketogenic diet containing equivalent protein and

67   little carbohydrate. EE was measured two ways: using metabolic chambers for two consecutive

68   days each week throughout the study, and using doubly labeled water during the last 2 weeks of

69   each 4-week diet period. EE measured in metabolic chambers increased significantly after the

70   switch to the ketogenic diet, but this change was transient, lasting only two weeks, and was

71   considered to be relatively small by Hall et al., which led them to conclude that the results did

72   not support the carbohydrate-insulin model. However, in contrast to the results using metabolic

73   chambers, EE, measured using doubly labeled water (EE_{DLW}) after the response in the chambers

74   had waned, increased more substantially after the switch to the ketogenic diet (151 kcal/d vs. 57

75   kcal/day). This response was attributed [8] to greater energy expenditure from increased

76   physical activity when subjects ate the ketogenic diet and were outside the chambers living in the

77   ward.

78   The carbohydrate-insulin and energy balance hypotheses have distinctly different

79   implications for understanding the etiology of obesity and devising effective strategies for

80   preventing and treating it. Consequently, it would be useful to reconcile the discrepant findings

81   from measurements of EE using metabolic chambers and doubly labeled water. Hall et al. made

82   the data from their study publicly available on the Open Science Framework (OSF) website [10].

83   In this paper, we report results of additional analyses of this dataset to differentiate the effect of

84   housing subjects in a metabolic chamber versus in a metabolic ward on the magnitude of the

85   increase in EE_{DLW} after the switch to the ketogenic diet and to assess the role of physical activity

86   in this effect.

Methods

Overview of the Hall et al. study

89   Details of the design and methods of the study can be found in the Hall et al. paper [8],

90   including the online supplementary data [11], and in the published IRB-approved protocol [9].

91   Briefly, focusing on methods relevant to the analyses described here, 17 males with overweight

92   or obesity were admitted as inpatients to metabolic wards and fed a baseline diet (BD; 15:50:35

93   percent of calories from protein:carbohydrate:fat) for 4 weeks followed by an isocaloric

94   ketogenic diet (KD; 15:5:80 percent of calories from protein:carbohydrate:fat) for another 4

95   weeks. Subjects were housed in a metabolic chamber for two consecutive days each week

96   throughout the study to measure daily EE, sleeping EE, and respiratory quotient (considered

97   primary endpoints of the study). During the last 2 weeks of each diet period, average daily EE

98   was measured using doubly labeled water (EE_{DLW}; considered an exploratory endpoint of the

99   study). Physical activity level was monitored throughout the study using accelerometers; each

100   subject wore an accelerometer (GT3XE+; Actigraph Corporation) on a hip, wrist and ankle, and

101   accelerometer counts and the length of time wearing the devices were logged for each device

102   location. The study was registered at www.clinicaltrials.gov as NCT01967563.

Reproducing calorimetry results

104   To confirm the replicability of the data used in the secondary analyses of EE_{DLW}

105   described below, we first reanalyzed the calorimetry results reported in Table 2 of the Hall et al.

106   paper [8] using the dataset and code published on the OSF website [10] and SAS v9.4 (SAS

107   Institute, Inc.).

108   Details regarding the sources and handling of data from the Hall et al. dataset for the

109   secondary analyses described below are provided in the Supporting Information (S1 File) along

110   with the SAS code used for these secondary analyses (S2 File). All endpoint values reported

111   herein were calculated using individual data from the Hall et al. dataset.

Non-chamber EE$_{DLW}$

113   A primary purpose of the Hall et al. pilot study was to determine the magnitude and

114   variability of changes in EE after subjects were switched from the BD to KD in preparation for

115   an anticipated larger study. The 14-day period for measuring EE$_{DLW}$ included 4 days when

116   subjects were confined to a metabolic chamber and 10 days when subjects lived in the ward. Hall

117   et al. reported EE$_{DLW}$ as a daily average across the 14-day measurement period and did not

118   differentiate EE during the non-chamber days, when subjects were housed in the ward, from the

119   chamber days, when EE is relatively lower [12,13] and the effect of diet was much reduced [8].

120   To determine average daily EE$_{DLW}$ for only those days in which subjects were housed in

121   the ward, we used a term in Hall et al.'s Equation 6 for calculating non-chamber EE

122   (EE$_{nonchamber}$) [8]. In essence, the resulting equation (Equation 1 below) separates average daily

123   EE for days subjects were housed in the wards from days they were confined to metabolic

124   chambers by subtracting total EE measured during the 4 chamber days (EE$_{chamber}$) within the

125   EE$_{DLW}$ measurement period from total 14-day EE$_{DLW}$ and averaging the resulting value over the

126   10 non-chamber days.

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

127   Differences in EE_{DLW}, $EE_{nonchamber}$ and $EE_{chamber}$ between the two diet conditions were

128   evaluated using a repeated linear mixed model. Data from Subject 04-012 was not included in

129   these analyses (see below) in keeping with Hall et al. [8]. A *P* value of < 0.05 (two-sided tests)

130   was considered statistically significant for this and all other analyses below.

131   As a check on Equation 1, we also calculated $EE_{nonchamber}$ by subtracting total CO_2

132   production measured during the four chamber days within the EE_{DLW} measurement periods from

133   the total 14-day CO_2 production measured using doubly labeled water, and dividing the result by

134   the 10 non-chamber days. The resulting rCO_2 values for the BD and KD conditions were

135   converted to kcal/d using equations described in Hall et al. (8). Details of these calculations are

136   provided in the Supporting Information (S1 file).

EE_{DLW} outlier

138   Hall et al. excluded one subject's (#04-012) data from the analysis of energy expenditure

139   measured using doubly-labeled water. This subject showed the largest increase in EE_{DLW} after

140   the switch from the BD to the KD (1136 kcal/d), which was identified statistically as an outlier

141   value using Cook's distance. Because this subject's relatively extreme change in EE_{DLW} was not

142   apparently due to a documented error in, for example, data collection, recording, computation or

143   coding, best practices [14] indicate that the EE_{DLW} analysis should be reported with and without

144   the outlier data. To that end, we compared EE_{DLW} and $EE_{nonchamber}$ during the two diet periods, as

145   above, except that data from Subject 04-012 were included in the analysis.

146   Hall et al. justified exclusion of Subject 04-012's data on the basis that he gained 0.2 kg

147   during the KD period despite the marked increase in EE_{DLW} after the switch to the KD and an

148   EE_{DLW} during the KD period that substantially exceeded his energy intake. To determine

149   whether the extreme change in Subject 04-012's EE_{DLW} was reflected in other parameters related

150   to his energy balance in the KD period, we examined his change in body weight, absolute EE_{DLW}

151   and energy intake, and the difference between EE_{DLW} and intake relative to the entire group of

152   subjects during the KD EE_{DLW} measurement period.

153   The reported 0.2 kg weight gain occurred over two body composition assessments

154   performed during the latter part of the KD period. As a check on the weight change based on

155   body weights collected during the body composition assessments, we evaluated the change in

156   body weight during the EE_{DLW} measurement period using daily body weight data from the

157   dataset.

Accelerometer data

159   Hall et al. evaluated whether greater physical activity accounted for the increase in

160   EE_{DLW} during the KD period by calculating energy expenditure from physical activity in and out

161   of the metabolic chambers (i.e, $PAE_{chamber}$ and $PAE_{nonchamber}$ as per their Table 2). Physical

162   activity energy expenditure outside the chambers was higher during the KD period compared to

163   the BD phase, but the effect was not statistically significant. Physical activity level was

164   measured directly throughout the study using accelerometers; however, only hip count data were

165   reported and only as a percentage difference between chamber and non-chamber days during the

166   entire BD period. Here, we used the accelerometer data in the OSF dataset to determine more

167   directly whether differences in physical activity can account for the increase in EE_{DLW} after the

168   switch to the KD.

169   To confirm reproducibility and help validate our use of the accelerometer data, we first

170   reanalyzed the fractional difference between hip accelerometer counts from chamber and non-

171   chamber days during the baseline period of the study using the published dataset and code. We

172   next analyzed daily hip, wrist and ankle accelerometer counts during the BD and KD EE_{DLW}

173   measurement periods with respect to whether subjects were confined to chambers or were

174   housed in the ward (i.e., chamber and non-chamber days) using generalized linear mixed models.

175   Average accelerometer wear times varied little with respect to device location, diet and housing

176   status. In keeping with Hall et al.'s calculation and analysis of physical activity energy

177   expenditure, accelerometer data were analyzed excluding data from Subject 04-012. In a

178   separate analysis, this subject's data was included. Accelerometer counts for each location with

179   respect to chamber status and diet were compared in the generalized linear mixed model by t-test

180   to determine statistical significance.

Sleeping energy expenditure

182   Hall et al. reported that sleeping energy expenditure (SEE; kcal/d) measured in the

183   metabolic chambers increased in the first week after subjects were switched from the BD to the

184   KD, and then declined during the subsequent three weeks. To determine whether this increase in

185   SEE persisted during the end of the KD period and may have contributed to the increase in

186   EE_{DLW} observed at that time, we compared SEE during the BD and KD EE_{DLW} measurement

187   periods. Comparison of SEE for all subjects during the EE_{DLW} measurement periods in the BD

188   and KD phases of the study were made using a paired t-test.

RESULTS

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Reproducing calorimetry results

192   Reanalysis of the calorimetry data in Table 2 in the Hall et al. paper [8] using the OSF

193   dataset and code fully reproduced the reported results, including mean, standard error and

194   probability values associated with statistical comparisons of diet periods.

Non-chamber EE_{DLW}

196   The increase in EE_{DLW} after the switch to the KD was greater when calculated only for

197   days when subjects were housed in the wards outside of the chambers than it was when

198   calculated over the entire EE_{DLW} measurement period that included both days in and out of the

199   chambers. Reanalysis of EE_{DLW} data from the Hall et al. dataset reproduced the average 151

200   kcal/d increase in EE_{DLW} after subjects were switched to the KD (Table 1). Using Equation 1

201   above, energy expenditures for days when subjects were out of the chambers ($EE_{nonchamber}$)

202   **Table1. Energy expenditure during the BD and KD doubly labeled**

203   **water measurement periods[a]**

	BD	**KD**	**Difference**	***P*[b]**
EE_{DLW}	2995 ± 45	3146 ± 45	151 ± 63	0.03
$EE_{nonchamber}$	3142 ± 45	3344 ± 45	203 ± 89	0.04
$EE_{chamber}$	2628 ± 22	2649 ± 22	21 ± 32	0.52
EE_{DLW} (*included*)	2964 ± 59	3173 ± 59	209 ± 83	0.02
$EE_{nonchamber}$ (*included*)	3100 ± 82	$3382 + 82$	$283 + 116$	0.03

204   [a]Data are least squares mean $\pm$ SEs from a linear mixed model. Values are

205   based on n = 16 except for those designated as "*included*" (n = 17), which

206   include an outlier removed from the other analyses as described in the text.

207   Difference values are the change from the BD to KD period. $EE_{chamber}$, daily

208   energy expenditure measured for 4 days in metabolic chambers during the

209   14-day doubly labeled water measurements; EE_{DLW}, average energy

210   expenditure over 14 days measured using doubly labeled water; $EE_{nonchamber}$,

211   average energy expenditure over 10 days outside metabolic chambers.

212   [b]Values refer to the difference between diet periods by t-test using modeled

213   standard errors.

214   increased on average by 203 kcal/d after subjects were switch from the BD to the KD. Energy

215   expenditure measured in the chambers ($EE_{chamber}$) during the EE_{DLW} measurement periods did not

216   differ as a function of diet.

217   Calculation of $EE_{nonchamber}$ based on the difference between CO_2 production measured in

218   the chambers and by doubly labeled water produced results very similar to those using Equation

219   1. With Subject #04-012 removed from the analysis, $EE_{nonchamber}$ during the BD and KD periods

220   were, respectively, 3140 ± 146 kcal/d and 3355 ± 189 kcal/d with a significant difference of 215

221   ± 87 kcal/d ($P = 0.03$ by paired t-test).

222   ## EE_{DLW} outlier

223   The effect of switching from the BD to the KD on EE measured using doubly labeled

224   water was greater when data from the outlier was included in the analysis. As shown in Table 1,

225   the increase in EE_{DLW} after the switch to the KD was greater when Subject 04-012's data were

226   included in the analysis than when they were not (209 ± 83 kcal/d vs. 151 ± 63 kcal/d).   When

227   this subject's data were included in the calculation of $EE_{nonchamber}$, the effect of switching to the

228   KD was greater (283 ± 116 kcal/d) than when his data were excluded (203 ± 89 kcal/d; see Table

229   1). With all subjects included in the analysis, $EE_{nonchamber}$ during the BD and KD periods, as

230   calculated using CO_2, were, respectively, 3100 ± 142 kcal/d and 3394 ± 182 kcal/d with a

231   significant difference of 295 ± 113 kcal/d ($P = 0.02$ by paired t-test).

232   Subject 04-012's weight gain, absolute EE_{DLW} and energy intake, and the difference

233   between his EE_{DLW} and intake during the KD EE_{DLW} measurement period as reported by Hall et

234   al., were each within the variability of the group as a whole. The 0.2 kg weight gain of Subject

235 04-012 was within one standard deviation of the average weight change of all subjects (-0.6 kg $\pm$

236 0.8, mean $\pm$ SD). His EE_{DLW} (3612 kcal/d) and energy intake (2794 kcal/d), and difference

237 between them (818 kcal/day) were well within one standard deviation from the means of the

238 group (3173 $\pm$ 583 kcal/day, 2736 $\pm$ 428 kcal/day, and 437 $\pm$ 481 kcal/day, respectively; mean $\pm$

239 SD).

240   Inspection of the dataset revealed that other subjects showed changes in body weight that

241 appeared anomalous relative to the difference between their EE_{DLW} and energy intakes. Two of

242 these subjects gained weight during the KD period (0.6 and 1.3 kg) despite a difference in

243 expenditure and intake of, respectively, 1751 and 465 kcal/d. Two participants lost weight (1.9

244 and 0.2 kg) despite an excess of energy intake relative to EE_{DLW} of (291 and 250 kcal/d,

245 respectively.

246   The weight gain of Subject 04-012 across the interval between two body composition

247 assessments in the KD period reported by Hall et al. underlies their rationale for exclusion of his

248 data from analysis of the effect of diet on EE_{DLW}. We confirmed that Subject 04-012 gained 0.2

249 kg between the two body composition assessments during the KD period; however, inspection of

250 the dataset also revealed that the interval between the two body composition assessments and the

251 EE_{DLW} measurement period were not concurrent. Consequently, we referred to daily body weight

252 data from the dataset, which showed that Subject 04-012 lost 0.5 kg over the EE_{DLW}

253 measurement period.

Accelerometer data

254

255   Reanalysis of hip accelerometer counts during the full BD period using the Hall et al.

256 code reproduced their finding that counts were 21 $\pm$ 4% greater on non-chamber days than they

257 were on days when subjects were confined to metabolic chambers.

258   Although during the DLW measurement periods hip, wrist, and ankle accelerometer

259 counts were significantly greater when subjects were housed in the ward than when they were

260 confined to metabolic chambers (P's < 0.001), counts either decreased (hip; $P = 0.006$) or did not

261 change significantly (wrist and ankle) after the switch from the BD to the KD (Table 2).

262 Inclusion of data from Subject 04-012 did not materially affect accelerometer counts or the

263 outcomes of the statistical analyses.

264 **Table 2. Accelerometer counts during the BD and KD doubly labeled water measurement**

265 **periods as a function of housing condition[a]**

	Hip		Wrist		Ankle	
	Chamber	Ward	Chamber	Ward	Chamber	Ward
BD	365,235	473,936	1,412,429	1,709,423	1,826,763	1,993,827
	$\pm$ 37,907	$\pm$ 34,098	$\pm$ 110,453	$\pm$ 98,602	$\pm$ 144,176	$\pm$ 138,876
KD	304,945	437,871	1,329,377	1,763,157	1,855,863	1,960,542
	$\pm$ 38,064	$\pm$ 34,037	$\pm$ 110,484	$\pm$ 98,644	$\pm$ 144,191	$\pm$ 138,895
Chamber	$t(347) = 5.37; P < .0001$		$t(360) = 4.93 ; P < .0001$		$t(360) = 3.57; P = .0004$	
Diet	$t(347) = 3.09; P = .0022$		$t(360) = 0.79; P = .4304$		$t(363) = 1.26; P = .2074$	

266 [a]Data are least squares mean $\pm$ SEs based on n = 16, and represent estimated total counts during the

267 14-day doubly labeled water measurement periods. t(degrees of freedom) denotes the value of the t

268 statistic used to test the significance of each predictor in the generalized linear mixed model.

269   Interactions between chamber status and diet were not significant ($P = 0.40$, 0.20, and 0.35 for hip,

270   wrist, and ankle, respectively).

Sleeping energy expenditure

272   SEE measured in the chambers during EE_{DLW} measurement periods was significantly

273   greater after the switch from the BD to the KD (BD, 1576 ± 68 kcal/d and KD 1620 ± 56 kcal/d;

274   difference $= 44 \pm 18$ kcal/d, $P < 0.02$). SEE values and the results of the analysis were nearly

275   identical if data from Subject 04-012 were excluded.

Discussion

Non-chamber energy expenditure – effect size

278   Hall et al. measured average daily energy expenditure using doubly labeled water over a

279   14-day period that included 4 days during which subjects were confined to a metabolic chamber

280   and 10 days when they were housed in the ward. Because people expend less energy in a

281   metabolic chamber than under more free-living conditions [12,13] and chamber measurement of

282   EE showed little difference between the diet periods in the Hall et al. study [8], we quantified

283   expenditures for non-chamber days ($EE_{nonchamber}$) separately from in-chamber days ($EE_{chamber}$).

284   The results showed that the switch from the BD to the KD diet was accompanied by an increase

285   in EE_{DLW} that was 34% greater than that originally reported [8]. $EE_{chamber}$ during the doubly

286   labeled water measurement periods did not differ significantly as a function of diet, further

287   indicating that the increase in EE_{DLW} after the switch to the KD was limited to days when

288   subjects were housed in the ward.

289   In keeping with best practices for handling outliers [14], we calculated EE_{DLW} and

290    $EE_{nonchamber}$ with and without data from Subject 04-012, considered an outlier by Hall et al. [8].

291   Relative to the increase in EE_{DLW} after the switch to the KD as originally reported, including

292   these data increased the effect on EE_{DLW} and $EE_{nonchamber}$ by, respectively, 38% and 87%.

293   Although, as discussed below, exclusion of this subject's data seems unwarranted based on

294   changes in his body weight relative to expenditures, his outlier status regarding the increase in

295   EE_{DLW} from the BD to KD period is clear on a statistical basis. However, because there is no

296   documented error in, for example, data collection, recording or computation, the cause of this

297   apparently exaggerated response is unknown. Taken together with the relatively small number

298   of subjects in this pilot study, and the lack of previous research to help determine the range of

299   response to KD diets under these controlled conditions, additional research will be required to

300   determine whether this subject's data are invalid or reflect a relatively extreme response seen in a

301   small proportion of the population.

302   According to Hall et al., the carbohydrate-insulin model predicts that consuming a KD

303   would increase energy expenditures by 300-600 kcal/d. The higher value is suspect, having been

304   based on a theoretical estimate [15] of a 400-600 kcal/d expenditure to support gluconeogenesis

305   under conditions very different than those in the Hall et al. study, specifically prior to adaptation

306   to a low-carbohydrate diet with only endogenous, not dietary, protein as the substrate. In

307   contrast, Bistrian [16] recently estimated the energy cost of gluconeogenesis associated with

308   consumption of a ketogenic diet at 110 kcal/day (not allowing for tissue glucose demands) under

309   conditions very similar to those employed in Hall et al. (i.e., eucaloric after an adaptation

310   period). The lower value cited by Hall et al.'s (300 kcal/d) was based on the results of a

311   randomized cross-over study by Ebbeling et al. [17] in which EE_{DLW} in free-living weight-

312   reduced subjects was, depending on how expenditures were calculated, ~250-325 kcal/d greater

313   when subjects ate a low-carbohydrate/high-fat diet compared with a high-carbohydrate/low-fat

314   diet. More recently, Ebbeling et al. [18] reported results from a larger and longer duration

315   randomized group trial in which EE_{DLW} in free-living weight-reduced subjects eating a low-

316   carbohydrate diet was, depending on whether data were analyzed by an intention-to-treat or per

317   protocol analysis, ~200-280 kcal/d greater than that of subjects consuming a high-carbohydrate

318   diet. Whereas these effects of diet on EE_{DLW} in these two studies were observed under very

319   different conditions than those in the Hall et al. study, including during maintenance of a 10-15%

320   weight loss, the magnitude of the effects was similar to the increase of ~200-280 kcal/d in

321     $EE_{nonchamber}$ after the diet switch in the Hall et al. study.

322   The order in which subjects in the Hall et al. study were fed the BD and the KD was not

323   counterbalanced or otherwise controlled for, a trial design limitation noted by the authors that

324   precluded causal inference about the effect of the KD. In contrast, the Ebbeling et al. studies

325   described above randomized the order in which subjects ate the experimental diets [17] or

326   randomized the diets to which they were assigned [18]. The similarity in the responses to a low

327   carbohydrate diet in the Ebbeling et al. studies and, with respect to $EE_{nonchamber}$, to the KD in the

328   Hall et al. study, lends credence to the conclusion that consumption of the KD caused the

329   increase in EE_{DLW} in the Hall et al. study.

330   Estimates of the increase in $EE_{nonchamber}$ from the current analysis likely represent a

331   minimal range for the effect size. The continuing weight loss throughout the study due to

332   unintentional underfeeding of the subjects, as described by Hall et al., would be expected to have

333   suppressed EE [13], thereby mitigating any increase in energy expenditure after switching to the

334   KD. Lower circulating concentrations of leptin and triiodothyronine during the KD versus the

335   BD period reported by Hall et al. are consistent with such a reduction in EE. Accounting for the

336   excretion of fat in feces might also magnify energy losses during the KD period [].

Methodological considerations

338   Hall et al. [20] recently argued that the increase in EE_{DLW} after the switch to the KD in

339   their earlier pilot study [8] was partially due to methodological issues associated with the doubly

340   labeled water technique. Based on hypothetical relationships between diet composition, energy

341   balance and measured RQ, they suggested that their earlier calculation of EE_{DLW} overestimated

342   the effect of switching to the KD. Adjusting for these factors, they found that the increase in

343   EE_{DLW} after the diet switch was diminished to statistically nonsignificant levels, an effect that

344   was due primarily to an increase in estimated EE_{DLW} in the BD period as opposed to a change in

345   the KD period. Hall et al. [20] identified two participants as outliers (Subjects A and B; Subject

346   B is Subject #04-012 referred to above). Excluding these subjects' data from the new analysis

347   largely eliminated the effect of switching the diet on EE_{DLW}. Hall et al. [8, 20] did not pre-

348   specify criteria or methods for identifying and handling outliers. Subjects were identified as

349   outliers *post hoc*, particularly on the basis of observations indicating that the difference between

350   their EE_{DLW} and energy intake was not commensurate with changes in body weight during the

351   KD EE_{DLW} measurement period.

352   The two outliers identified by Hall et al. [20] showed large discrepancies between EE_{DLW}

353   and energy intake. However, as reported above in relation to analysis of Subject 04-012's

354   (Subject B's) designation as an outlier and as described in the Supporting Information (S3 File,

355   Table 1S), other subjects showed differences between expenditure and intake that, while not as

356   large, were substantial and were also incommensurate with changes in weight. Which of the

357   outlier data is chosen for exclusion in data analysis markedly affects estimates of energy

358   expenditure (Supporting Information File S3, Figure 2S). In the case of Subjects A and B

359   together, it reduced effect size to nonsignificant levels. In other cases, it reduced the effect size

360   less while retaining statistical significance, and, importantly, in some cases the choice of outliers

361   increased the effect size. These finding suggest that the selection of outliers by Hall et al. [20]

362   was too restrictive and thereby may have overly circumscribed the interpretation of the results of

363   their reanalysis.

364   The changes in weight that were part of the assessment of outliers in the original and

365   recent Hall et al. studies [8, 20] were derived from body weight measurements taken over the

366   interval between two body composition assessments in the latter part of each diet period. Hall et

367   al. [20] claimed that assessments were coincident with the EE_{DLW} measurement periods. As

368   discussed above, this was not the case for Subject 04-012 (Subject B), nor, for Subject A

369   (Supporting Information File S3; Table 2S). Hall et al. reported that Subjects A and B gained

370   weight during the EE_{DLW} measurement period based on the body composition assessment

371   measures (0.6 and 0.2 kg, respectively). In contrast, both subjects lost 0.5 kg of weight when

372   body weight change was measured over a period that was in fact concurrent with the EE_{DLW}

373   measurement period using daily body weight data from the Hall et al. [8] dataset. Although the

374   magnitude of body weight changes differed in all but one subject depending on whether it was

375   based on body composition assessment data or was synchronized with the EE_{DLW} measurement

376   period using daily body weight data, only Subjects A and B showed such a reversal. Differences

377   in measurement precision does not appear to account for the difference in body weight change

378   because weight changes across the interval for the body composition assessments measured

379   using daily body weight data were similar to those based on weights measured during the

380   assessments (File S3, Table 2S). Because a loss of body weight would be commensurate with an

381   excess of energy expenditure relative to energy intake, the outlier status of Subjects A and B

382   seems difficult to justify.

383   Calculation of energy balance based on changes in fat mass and fat free mass are central

384   to Hall et al.'s reanalysis. Hall et al. [20] acknowledged uncertainly regarding these calculated

385   values "because DXA has a limited ability to precisely and accurately detect small changes in

386   body energy stores." The body composition assessments were presumed to be coincident with

387   those of the EE_{DLW} measurement period, thus providing an accurate assessment of changes in

388   bodily energy stores associated with those in energy expenditure. However, as discussed above,

389   the timing of body composition assessments and EE_{DLW} measurement periods differed and a

390   difference of just a few days markedly affected estimates of changes in body weight. Such

391   asynchrony might add additional uncertainty to estimates of body composition and, in turn,

392   energy balance. To what extent the results of Hall et al.'s study [20] reflect the hypothetical

393   relationships between diet composition, energy balance and RQ upon which their reanalysis is

394   based or the imprecision of their measurements remains to be determined.

395   In their reconsideration of the original paper, Hall et al. [20] also suggested that higher

396   rates of *de novo* lipogenesis during consumption of the high-carbohydrate BD as compared with

397   the low-carbohydrate KD magnified the increase in EE_{DLW} after switching to the KD because

398   more deuterium would be sequestered in fat during the BD period and thereby lower estimated

399   CO_2 production. Such an effect of deuterium trapping has been documented only in rapidly

400   fattening piglets with no significant consequence for estimates of EE_{DLW} predicted for weight

401   stable animals [21]. Given this, it seems unlikely that differences in *de novo* lipogenesis during

402   the diet periods would account for much, if any, of the increase in EE_{DLW} in the original Hall et

403   al. [8] study since subjects lost weight throughout the trial. Hall et al.'s quantitative estimates of

404   the effects of *de novo* lipogenesis on EE_{DLW} also appear overestimated by at least two-fold. Their

405   estimates were based on previously published total triglyceride turnover values [22], but did not

406   take into account that *de novo* lipogenesis involves only nonessential fatty acids, which comprise

407   only half of stored fatty acids [22]. Also, from earlier work [23] they estimated that 10-20% of

408   the daily production of very low density lipoprotein triglycerides are derived from hepatic *de*

409   *novo* lipogenesis; however, this estimate was based on meal-to-meal changes in *de novo*

410   lipogenesis after two meals of a rapidly absorbed liquid diet [23] and did not take into account

411   that the contribution of hepatic *de novo* lipogenesis to triglyceride production falls to < 5%

412   during an overnight fast [24]. Clearly, a more definitive estimate of the magnitude of the effect

413   of a ketogenic diet on EE_{DLW} will require studies using weight-stable subjects along with direct

414   analysis of relevant biochemical and physiological processes.

415   A discrepancy between chamber and doubly labeled water measures of EE in response to

416   a nutritional manipulation was reported previously by Rosenbaum et al. [13] who found

417   substantial changes in total daily EE in response to over- and under-feeding when measured

418   using doubly labeled water or by the non-isotopic method of caloric titration in subjects housed

419   in a ward, but not when measured in the same subjects using chamber respirometry. These

420   investigators hypothesized that the different outcomes between methodologies to limitations on

421   physical activity imposed by the metabolic chamber. The present analyses of the Hall et al. data

422   similarly suggest that the magnitude of the effect of a KD on EE depends on conditions in which

423   physical activity is not restricted by the confines of a metabolic chamber. Although more direct

424   methodological comparisons are needed, the preferred approach for studies of the effect of

425   nutritional status or dietary composition on energy balance would appear to entail at least the

426   opportunity for physical activity afforded by a metabolic ward along with appropriate methods

427   for measurement of EE that do not require restricted confinement.

428   **Increased non-chamber energy expenditure – possible mechanisms**

429   Accelerometer counts, a direct measure of physical activity, did not increase and, in the

430   case of hip counts, decreased during the EE_{DLW} measurement period after the switch from the

431   BD to the KD. These findings do not support the suggestion in Hall et al. [8] that the lack of a

432   significant increase in non-chamber energy expenditure from physical activity during the KD

433   period was due to limitations on activity imposed by the metabolic ward environment. The

434   dissociation between changes in physical activity and EE_{DLW}, is consistent with other findings

435   [17,18] that free-living subjects eating a low-carbohydrate diet show elevated EE_{DLW} but little or

436   no change in physical activity measured using accelerometers. Accelerometry in these studies

437   and the Hall et al. study may not have captured all components of physical activity contributing

438   to nonexercise activity thermogenesis (NEAT) [25], although the hip and wrist accelerometers

439   counts likely reflected walking, which is a major component of NEAT [25].

440   Sleeping energy expenditure, derived from sleeping metabolic rate, was greater during

441   the EE_{DLW} measurement period after the switch to the KD. Because sleeping metabolic rate

442   approximates as much as 80-100% of basal metabolic rate [26], the difference in sleeping energy

443   expenditure suggests that the KD may have increased basal energy expenditure by ~50 kcal/d,

444   accounting for ~18-25% of the increase in daily energy expenditures depending on the range of

445   estimates for the effect of the KD. Such an estimate must be tempered, however, given that

446   physical activity during sleep will increase metabolic rate and that the difference in sleeping

447   metabolic rate as a function of diet measured in chambers may differ from that when subjects

448   were sleeping in the ward.

449   After allowing for an elevation in basal metabolic rate and some contribution from

450   physical activity not monitored by accelerometers, much of the increase in EE_{DLW} during the KD

451   period appears left to be explained. Given that the effect of switching to the KD is limited to

452   days subjects were housed in the ward, the increase in $EE_{nonchamber}$ may have been dependent on

453   the increase in physical activity outside the chambers but not directly caused by it. Much of the

454   increase in thermogenesis in overfed subjects housed on a metabolic ward is due to an increase in

455   non-resting energy expenditure and about one-third of this effect has been attributed to lower

456   skeletal muscle work efficiency [27]. This suggests that nutritional factors can affect energy

457   expenditures and may do so in part by increasing the energetic cost of physical activity.

458   Consumption of a very low carbohydrate, ketogenic diet could have similar effects; indeed, as

459   early as 1920, Krogh and Lindhard [28] described the "waste" of energy from fat in exercising

460   humans maintained on a largely fat, as compared with a primarily carbohydrate, diet. On the

461   other hand, in the Hall et al. study [8], EE measured during the prescribed cycling exercise in the

462   chambers was similar in the two diet periods. This observation argues against a change in muscle

463   work efficiency in the KD period; however, it is not known whether the efficiency of muscle

464   work associated with the increase in other forms physical activity in a ward setting may have

465   been affected. Greater physical activity outside the chambers may also have increased EE during

466   the KD period by creating a demand for glucose, some of which under the condition of severe

467   dietary carbohydrate restriction would be met through the energetically expensive process of

468   hepatic gluconeogenesis [see also 16]. The elevated plasma concentrations of glucagon and the

469   increase in protein catabolism (as evidenced by increased urinary nitrogen, urea and ammonia

470   excretion) during the KD period in the Hall et al. study are consistent with such a higher rate of

471   gluconeogenesis, which has been suggested as a contributing cause of the increased

472   thermogenesis associated with consumption of ketogenic diets [16].

473   **Conclusions**

474   On the basis of the transient and what was considered a relatively small increase in EE

475   measured in the metabolic chambers after the switch to the KD, Hall et al. [8] concluded that the

476   results of their pilot study did not support the prediction of the carbohydrate-insulin hypothesis

477   of obesity that such an isocaloric change in diet would increase EE. This finding has been cited

478   as evidence refuting the carbohydrate-insulin hypothesis, offering further support for the energy

479   balance hypothesis of obesity that emphasizes a calorie-is-a-calorie perspective [29-31]. Such a

480   conclusion may be premature given the robust increase in EE associated with consumption of the

481   KD as measured using doubly-labeled water in Hall et al. [8]. Indeed, on the basis of the

482   increase in EE_{DLW} and, especially, in $EE_{nonchamber}$, the results are entirely consistent with the

483   carbohydrate-insulin hypothesis, which predicts an increase in EE with restriction of

484   carbohydrate intake and the resulting decrease in insulin secretion. Overnight insulin withdrawal

485   in patients with type 1 diabetes increases basal (resting) energy expenditure, a response that has

486   been attributed to hyperglucagonemia and is associated with increased protein catabolism [see 32

487   for a review]. Although the chronic reduction in insulin secretion during the KD period in the

488   Hall et al. study [8] was not as great, it was also associated with increases in basal metabolic rate

489   (as estimated from sleeping energy expenditures), plasma glucagon concentrations, and protein

490   catabolism (as indicated from urinary nitrogen, urea and ammonia excretion). These factors,

491   along with a possible increase in gluconeogenesis, deserve further exploration in future studies of

492   the effect of ketogenic diets on energy expenditure.

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497   water and respirometry data.

Authors' contributions

506   MF designed the analysis plan; SA performed the statistical analyses; MF and SA wrote the

507   paper; MF had primary responsibility for final content.

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582   **Supporting information**

583   **S1 File. Data sources and handling (S1_file.pdf).**

584   **S2 File. SAS code for secondary analysis (SI_file.txt).**

585   **S3 File. Hall et al. 2019: Evaluation of Outlier Selection S3_file.pdf).**

Supporting Information File S1

Data Sources and Handling

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 and Handling (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]. In keeping with the original analyses, P values were not corrected for multiplicity in this replication or in the analyses below, which may limit inferences when multiple comparisons are made. Similarly, due to the small sample size, no multivariable model was applied to adjust for potential confounders.

Non-chamber EE_{DLW}

Data to calculate Equation (1) 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 four chamber days during the 14-day EE_{DLW} measurement periods.

$EE_{nonchamber}$ for each participant was also calculated using the difference between CO_2 production rates measured in the chambers and by doubly labeled water ($rCO_{2nonchamber}$) according to the following equation:

$$rCO_{2nonchamber}(L) = \frac{(rCO_2 \times 22.4 \times 14) - (TCO_2 \times .001)}{10}$$

Where rCO_2 is the daily production rate of CO_2 (mol/d), corresponding to "rCO_2Redo values in the "DLW" tab of the Hall et al. dataset, which is multiplied by the molar volume of gas (22.4) and the number of days in the DLW measurement period. TCO_2 is the total CO_2 production (in ml) measured by respirometry during the four days participants were housed in chambers, which were multiplied by 0.001 to convert to liters. These CO_2 values were derived from "TVCO2"

data in the "chamber" tab of the data set. The product of these calculations was divided by 10 to determine the daily volume of rCO_2 (in L) produced during the 10 non-chamber days.

To calculate $EE_{nonchamber}$ (kcal/d) during the BD period we used Equation 4 from Hall et al. [1] substituting $rCO_{2nonchamber}$ for rCO_2 as below:

$EE_{nonchamber} = \left[\frac{3.85}{RQ} + 1.07\right] \times rCO_{2nonchamber} (L)$ We used Hall et al.'s [1] Equation 5, which corrects for ketone body excretion (K_{excr} taken from "ketone_excr" values in the "DLW" tab of the dataset), to calculate $EE_{nonchamber}$ during the KD period:

$$EE_{nonchamber} = \left[\frac{3.85}{RQ} + 1.07\right] \times rCO_{2nonchamber} (L) - (3.85 \times 0.32 + 1.39) \times K_{excr} (g)$$

RQ is respiratory quotient and for both equations corresponds to the "RQ_Chamber" values in the "DLW" tab of the dataset.

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.
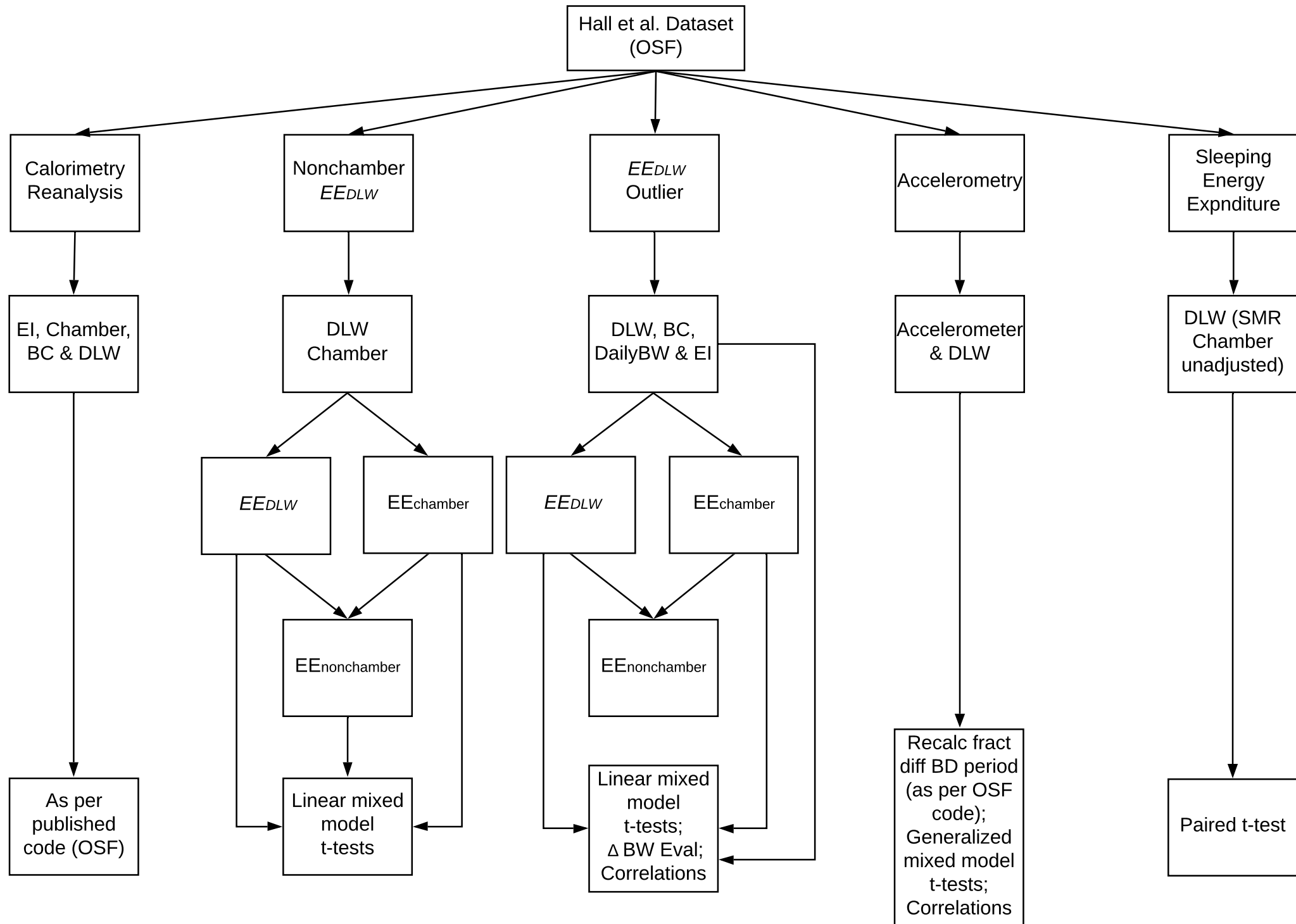
Sleeping energy expenditure

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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Supporting Information File S2

```
                    Documentation Friedman Paper_March2019
**  This SAS code is the statistical analyses for the 2018 study by Mark Friedman
and Scott Appel
    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   */

**  Excel Data from the Hall et al Study can be downloaded:    https://osf.io/6srfq/
    Hall et al SAS Code can be downloaded there as well;


PROC IMPORT OUT= Chamber
DATAFILE= "Z:\BAC_0541_Friedman\datasets\DataDocumentation.xlsx"
DBMS=xlsx REPLACE; SHEET="Chamber"; GETNAMES=YES; RUN;

PROC IMPORT OUT=DLW
DATAFILE="Z:\BAC_0541_Friedman\datasets\DataDocumentation.xlsx"
DBMS= xlsx REPLACE; SHEET='DLW'; RUN;

PROC IMPORT OUT=DLW
DATAFILE="Z:\BAC_0541_Friedman\datasets\DataDocumentation.xlsx"
DBMS= xlsx REPLACE; SHEET='DLW'; RUN;

PROC IMPORT OUT=accel
DATAFILE="Z:\BAC_0541_Friedman\datasets\DataDocumentation.xlsx"
DBMS= xlsx REPLACE; SHEET="Accelerometer"; RUN;

/* Table 1 */
/* N = 16 EEdlw */
proc mixed data=dlw;
class subject_ID stage;
model TEE_DLWChamber_unadjusted = stage subject_ID / s ;
repeated /subject=subject_ID type=CS;
lsmeans stage;
estimate 'stage 3 vs 2' stage -1 1;
Ods output lsmeans= lsm;
Ods output tests3= fixed;
where subject_ID not in ('04-012');
run;

/* N= 17 EEdlw */
proc mixed data=dlw;
class subject_ID stage;
model TEE_DLWChamber_unadjusted = stage subject_ID / s  ;
repeated /subject=subject_ID type=CS;
lsmeans stage;
estimate 'stage 3 vs 2' stage -1 1;
Ods output lsmeans= lsm;
Ods output tests3= fixed;
/*where subject_ID not in ('04-012'); */
run;

data DLW1; set DLW;
TEEDLWnonChamber= TEE_DLWChamber_unadjusted*7/5 - TEE_Chamber_unadjusted*2/5;
run;
/* N = 16 EEnonchamber */
proc mixed data=dlw1;
class subject_ID stage;
model TEEDLWnonChamber = stage subject_ID / s   /* influence(iter=10 estimates)
TDEE_DLW*/;
repeated /subject=subject_ID type=CS;
lsmeans stage;
estimate 'stage 3 vs 2' stage -1 1;
Ods output lsmeans= lsm;
```

```
Ods output tests3= fixed;
where subject_ID not in ('04-012');
run;
/* N = 17 EEnonchamber */
proc mixed data=dlw1;
class subject_ID stage;
model TEEDLWnonChamber = stage subject_ID / s   /* influence(iter=10 estimates)
TDEE_DLW*/;
repeated /subject=subject_ID type=CS;
lsmeans stage;
estimate 'stage 3 vs 2' stage -1 1;
Ods output lsmeans= lsm;
Ods output tests3= fixed;
run;
/* N = 16 EEchamber */
proc mixed data=dlw1;
class subject_ID stage;
model TEE_Chamber_unadjusted = stage subject_ID / s   /* influence(iter=10
estimates)   TDEE_DLW*/;
repeated /subject=subject_ID type=CS;
lsmeans stage;
estimate 'stage 3 vs 2' stage -1 1;
Ods output lsmeans= lsm;
Ods output tests3= fixed;
where subject_ID not in ('04-012');
run;


/* Table 2 -- Accelerometer */
proc means data = accel mean stderr;
    class stage chamber_day location;
    var total_3D_Counts;
    where stage in (2,3) and wear_time__min_ ge 720 and last2weeks = 1 and
chamber_day in (0,1) and subID ne 'KEE 04012'; ;
run;

/* mixed models for accelerometer data
Separate models for Hip/Ankle/Wrist
with 'the outlier' [04-012] removed */

proc univariate plot data = accel normaltest; class location; var total_3d_counts;
where stage in (2,3) and wear_time__min_ ge 720 and last2weeks = 1 and chamber_day
in (0,1); run; /* test outcome var for normality */

/* due to non-normality, running mixed model that doesn't require outcome
variable be normal */
proc glimmix data= accel;
    class stage (ref="2") chamber_day (ref="0") subID;
    model total_3d_counts = stage chamber_day  stage*chamber_day / SOLUTION CL ;
    random subID;
    LSMEANS stage*chamber_day;
    where stage in (2,3) and wear_time__min_ ge 720 and last2weeks = 1 and
chamber_day in (0,1) and location = 'hip' and subID ne 'KEE 04012';
run;

proc glimmix data= accel;
    class stage (ref="2") chamber_day (ref="0") subID;
    model total_3d_counts = stage chamber_day  stage*chamber_day / SOLUTION CL ;
    random subID;
    LSMEANS stage*chamber_day;
    where stage in (2,3) and wear_time__min_ ge 720 and last2weeks = 1 and
chamber_day in (0,1) and location = 'wrist' and subID ne 'KEE 04012';
run;
```

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```
proc glimmix data= accel;
    class stage (ref="2") chamber_day (ref="0") subID;
    model total_3d_counts = stage chamber_day  stage*chamber_day  / SOLUTION CL ;
    random subID;
    LSMEANS stage*chamber_day;
    where stage in (2,3) and wear_time__min_ ge 720 and last2weeks = 1 and
chamber_day in (0,1) and location = 'ankle' and subID ne 'KEE 04012';
run;

/* Sleeping energy expenditure */
/* Using data from DLW tab */
data dlw;
set dlw;
hourly_SEE = smr_chamber_unadjusted * 60;
daily_SEE = hourly_SEE * 24;
run;

proc means data = dlw n mean stderr;
class stage;
var smr_chamber_unadjusted daily_SEE ;
**where subject_id ne "04-012";
run;

/* one sample ttest (on the differences KD-BD) */
proc transpose data = dlw out = flipped_dlw;
by subject_id ;
id  stage;
var daily_SEE;
run;

data flipped_dlw;
set flipped_dlw;
diff_SEE = _3 - _2;
run;

proc ttest data = flipped_dlw; var diff_SEE; run;

/* EEDLW and hip accelerometer correlations */
data hip;
set accel;
subID_rebuild = substr(subID,5,2)||"-"||substr(subID,7,3);
drop subID;
if location ne 'hip' then delete;
run;

data hip2;
set hip;
rename subid_rebuild = subid;
run;

data hip_accel_teedlw;
merge hip2 (in=in1)
      chamber (in=in2);
by subid stage date;
if in2;
run;

proc corr data = hip_accel_teedlw;
var total_3D_counts  EE_binned;
where stage = 2;
run;
```

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```
proc corr data = hip_accel_teedlw;
var total_3D_counts  EE_binned;
where stage = 3;
run;
```

Supporting Information File S3

Hall et al. 2019: Evaluation of Outlier Selection

Restricted Selection

The protocol for the original Hall et al. study [1], which is the subject of their recent analysis [2], did not specify criteria or methods for identification and handling of outliers. In both studies, subjects were considered outliers, and their energy expenditure data excluded from analysis, based on *post hoc* observations indicating that their energy expenditure measured using doubly labeled water (EE_{DLW}) was not commensurate with other parameters of energy balance. In their recent reanalysis of the original study, two outliers so identified were reported to have gained weight during the ketogenic diet (KD) period despite EE_{DLW} in excess of energy intake (EI). One of the two participants ("Subject A") also gained weight during the BD period although EE_{DLW} exceeded energy intake and showed a "sleight" gain in fat mass during both periods. The other participant ("Subject B") was also identified statistically as an outlier with respect to the magnitude of his increase in EE_{DLW} after the switch from the BD to KD diet. We examined the database for other participants who showed changes in body weight that were discrepant with respect to the difference between their EE_{DLW} and EI because this was a criterion for outlier status that was met by both Subject A and B.

Subjects A and B (Subject ID #'s 04-006 and 04-012, respectively; Group 1 in Table 1S) showed the greatest increase in EE_{DLW} over EI associated with weight gain of all 17 study participants.

Table 1S. Putative outliers based on discrepancies between body weight change and the difference in energy expenditure and energy intake.

| Group	ID#	EE_{DLW} (kcal/d)	EI (kcal/d)	EE-EI (kcal/d)	ΔBW (kg)
1	04-006[a]	4448	2697	1751	0.6
	04-012[b]	3612	2794	818	0.2
2	02-004	2858	2393	465	1.3
	03-009	2395	2645	-250	-0.2
	04-007	3859	4150	-291	-1.9
3	03-002	2390	2445	-55	-0.4
	03-008	2691	2745	-54	-0.2

[a] and [b] = Subjects A and B, respectively. All measures are during the ketogenic diet (KD) period. EE_{DLW} and EE, energy expenditure by doubly labeled water; EI, energy intake; ΔBW, change in body weight during body composition assessments.

However, five other participants had changes in body weights during the KD period that were incommensurate with the difference between their EE_{DLW} and EI. Three participants (Group 2;

Table 1S) showed more moderate, but substantial, differences between EE_{DLW} and EI. In two of these, body weight decreased despite an EE_{DLW} that was less than their EI, whereas the third gained weight although EE_{DLW} exceeded EI. Two additional participants (Group 3; Table 1S), exhibited small, negative differences between EE_{DLW} and EI that were associated with a decrease in body weight.

Subjects A and B showed the two greatest increases in EE_{DLW} and nonchamber energy expenditure ($EE_{nonchamber}$) after the switch from the BD to KD of all 17 participants. Therefore, exclusion of their expenditure data would be expected to reduce any increase in average expenditures after the switch whether adjusted for energy balance or not. Figure 1S shows the effect of excluding these two participants (Group 1) on average $EE_{nonchamber}$, a primary outcome
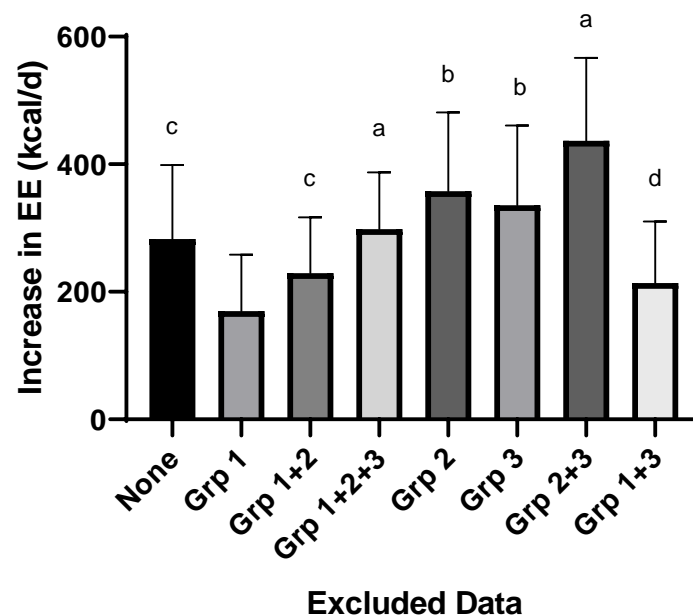
Figure 1S. Effect of excluding putative outlier data on the increase in non-chamber energy expenditure by doubly labeled water ($EE_{nonchamber}$) after switching from a basal to a ketogenic diet. Values are mean $\pm$ SE. a, b, c, and d = $P < 0.01$, 0.02, 0.03, and 0.05, respectively, by paired t-test. For Group 1, $P = 0.076$.

of our analysis, along with the effect of excluding other outliers (Groups 2 and 3) listed in Table 1S either separately or in combination with those in Group 1.

Although $EE_{nonchamber}$ increased significantly after the switch to the KD when all 17 participants are included in the analysis, the increase was not statistically significant when data from Subjects A and B (Group 1) were excluded from the analysis. In contrast, the increase of $EE_{nonchamber}$ was significant despite removal of other groups of putative outliers either alone or in combination

with other groups. The effect of excluding Group 2 alone or in combination with Group 1 is especially notable because the energy imbalances reflected by the differences between EE_{DLW} and EI, while less than that in Group 1, were substantial. As reported in this paper (Table 1), the increase in $EE_{nonchamber}$ after the diet switch was statistically significant after exclusion of Subject B's (ID# 04-012) data from the analysis. When only Subject A's data was removed from the analysis, the increase in $EE_{nonchamber}$ after the diet switch (257 ± 116 kcal/d) was also statistically significant ($P = 0.49$ by paired t-test).

Asynchronous Measurement Intervals

The reported gain of body weights during the KD period of Subjects A and B was based on body weight measurements taken during two body composition assessments in the latter part of the diet period. Hall et al. [2] claimed that the interval for body composition assessments was coincident with the EE_{DLW} measurement period. However, inspection of dates in the original study's dataset for DLW dosing and body composition assessments shows this was not the case; body composition and EE_{DLW} measurements periods were coincident in only 6 and 4 out of 17 participants in the BD and KD periods, respectively.

Because the dataset includes dates and daily body weight measurements for all subjects throughout the study, it is possible to determine the change in body weight over the EE_{DLW} measurement period independently from the body weight measures taken during body composition assessments.

As discussed in this paper, Hall et al. [1] reported that Subject B gained 0.2 kg during the KD EE_{DLW} measurement period based on body composition assessments (Table 2S), but daily body weight measurements show a body weight loss of 0.5 kg during the actual EE_{DLW} measurement period (Table 2S). Similarly, according to the database, Subject A gained 0.6 kg of weight as per

Table 2S. Changes in body weight in putative outliers as a function of measurement interval during the ketogenic diet period.

		BC Period	BWBC (kg)	BWCD (kg)	DLW period	BWDD (kg)
Subject A	Start	26-Mar-14	63.9	64.5	28-Mar-14	64.6
	End	9-Apr-14	64.5	65.0	10-Apr-14	64.1
	diff	--	**0.6**	**0.5**	--	**-0.5**
Subject B	Start	18-Jun-14	88.6	89.1	19-Jun-14	89.0
	End	30-Jun-14	88.8	89.1	2-Jul-14	88.5
	diff	--	**0.2**	**0.0**	--	**-0.5**

BC, body composition assessment dates; **DLW**, doubly labeled water measurement dates; **BWBC**, body weight for body composition measurement dates using body composition assessment dates; **BWCD**, body weight for body composition measurement dates using daily body weight measurements; **BWDD**, body weight for doubly labeled water measurement dates using daily body weight measurements; **diff**, change in body weight from start to end.

body composition assessments, but according to daily body weight measurements, lost 0.5 kg during the EE_{DLW} measurement period.

No other participants showed such a reversal of body weight change from gain to loss (or vice versa) during the KD period when body weight measurements were synchronized with the EE_{DLW} measurement period, although one participant did so during the BD period. The changes in body weight of Subjects A and B over the EE_{DLW} measurement period measured using daily body weight data were well within one standard deviation of that for the group as a whole (-0.9 $\pm$ 0.6 kg, mean $\pm$ SD). The differences between body weight measurements from body composition assessments and recorded daily body weights do not appear to be due to differences in the precision of measurement under the two conditions because changes in body weight over the two body composition assessments as determined using daily body weight measurements were consistent with those measured during composition evaluations (Table S2).

References

1. Hall KD, Chen KY, Guo J, Lam YY, Leibel RL, Mayer LES, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. Am J Clin Nutr 2016;104: 324–33.

2. Hall KD, Guo J, Chen KY, Leibel RL, Reitman ML, Rosenbaum, M, et al. Methodologic considerations for measuring energy expenditure differences between diets varying in carbohydrate using the doubly labeled water method. Am J Clin Nutr 2019;109: 1328-34.