Challenges of Measuring Energy Expenditure Differences Between Diets Varying in Carbohydrate using Doubly Labeled Water

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Abstract

Objective: To examine the doubly labeled water (DLW) method for measuring energy expenditure differences between diets varying in carbohydrate.

Methods: DLW measurements were obtained during the final two weeks of month-long baseline (BD; 50% carbohydrate, 35% fat, 15% protein) and isocaloric ketogenic diets (KD; 5% carbohydrate, 80% fat, 15% protein) in 17 men with BMI 25-35 kg/m^2. Subjects resided 2d/week in respiratory chambers to measure energy expenditure ($EE_{chamber}$). DLW expenditure was calculated using chamber-determined respiratory quotients (RQ) either unadjusted (EE_{DLW}) or adjusted ($EE_{DLW\Delta RQ}$) for energy imbalance. Accelerometers measured physical activity. Body composition and energy intake measurements were used to calculate energy expenditure by balance (EE_{bal}).

Results: After transitioning from BD to KD, neither $EE_{chamber}$ nor EE_{bal} were significantly changed ($\Delta EE_{chamber}$=24±30 kcal/d; p=0.43 and ΔEE_{bal}=-141±118 kcal/d; p=0.25). Similarly, physical activity (-5.1±4.8%; p=0.3) and exercise efficiency (-1.6±2.4%; p=0.52) were unchanged. However, EE_{DLW} was 209±83 kcal/d higher during the KD (p=0.023) but $EE_{DLW\Delta RQ}$ was not significantly increased (139±89 kcal/d; p=0.14).

Conclusions: Increased EE_{DLW} during KD was incommensurate with other measurements and could not be explained by objective measures of physical activity or exercise efficiency. Our data highlight the challenges of the DLW method for measuring expenditure differences between diets varying in carbohydrate.

Keywords: Energy expenditure; doubly labeled water, diet composition

What is already known about this subject?

- The doubly labeled water (DLW) method has been successfully applied to measuring energy expenditure in humans since the 1980s and has been validated in comparison to respiratory chamber measurements in subjects consuming moderate carbohydrate diets.
- DLW has never been validated in humans consuming low-carbohydrate diets and there are theoretical reasons why the DLW method might result in systematic differences in calculated energy expenditure changes between diets widely varying in carbohydrate.

What this study adds:

- While not designed as a validation experiment, this two-month long inpatient isocaloric feeding study represents the most rigorously controlled investigation of the DLW method during a low-carbohydrate diet in humans.
- The DLW method calculated a significant increase in energy expenditure after transitioning from a moderate carbohydrate baseline diet to an isocaloric very low carbohydrate, ketogenic diet. However, the increased expenditure measured by the DLW method was inconsistent with both the coincident respiratory chamber measurements, changes in body energy stores, physical activity, and exercise efficiency measurements. Diet-specific adjustments of the DLW measurements for the prevailing state of energy imbalance substantially attenuated the DLW expenditure differences between the diets.
- Our data highlight the challenges of the DLW method for calculating energy expenditure differences between diets varying widely in the proportion of carbohydrate.

Introduction

The doubly labeled water (DLW) method was devised in 1955 by Lifson et al. for measuring daily energy expenditure averaged over periods of several days (1). While originally applied to small animals, the method has provided important insights regarding human energy metabolism since the 1980s (2, 3, 4, 5). Recently, the DLW method was used in a controlled feeding study in humans to conclude that low-carbohydrate diets result in substantially increased total energy expenditure compared to isocaloric diets with higher proportions of carbohydrate (6). However, such results appear to run counter to several controlled feeding studies that employed respiratory chambers to measure energy expenditure that found no such expenditure increases with isocaloric lower carbohydrate diets (7). Forthcoming studies rely on the DLW method for assessing the energy expenditure effects of diets varying widely in the proportion of dietary carbohydrate (8), but the DLW has never been validated in humans consuming low-carbohydrate diets.

The apparent simplicity of the DLW method, along with the elegance of the underlying theory, belies the fact that its practical application requires many assumptions. Depending on the choice of assumptions and corresponding parameters, the calculated average rate of CO_2 production can vary by as much as 15% (9). To inform the choice of equations, calibration and validation studies have been performed in humans where the mean CO_2 production rate measured using the DLW method has been compared to simultaneous respiratory chamber measurements in subjects consuming typical western diets with moderate proportions of carbohydrate (i.e., ~50% of energy) (4). However, the DLW method has never been validated in humans consuming a low-carbohydrate diet.

There are theoretical reasons why the DLW method can result in calculated energy expenditure differences between diets widely varying in carbohydrate that do not reflect true physiological differences. Specifically, the DLW method requires an estimate of the overall metabolic fuel utilization of the body as quantified by the average daily respiratory quotient (RQ). While it is widely known that RQ depends on the composition of the diet, RQ also depends on the overall state of energy balance in a way that also depends on dietary carbohydrate proportion (10). Furthermore, whereas the DLW method assumes that deuterium is lost only via body water, deuterium is also incorporated into other molecules (lipids, protein, glycogen, DNA, etc.) via processes at rates likely dependent on dietary carbohydrate and circulating hormones such as insulin (11, 12, 13). The Supplementary Materials describe how DLW calculations of energy expenditure differences between diets varying widely in carbohydrate content can be biased because of unaccounted variations in the state of energy balance as well as rates of de novo lipogenesis.

Here, we explore the challenges of using the DLW method for measuring energy expenditure differences using data from our previously published study (14) in which 17 men were transitioned from a one-month inpatient run-in period consuming a moderate-carbohydrate baseline diet (BD) (50% carbohydrate, 35% fat, 15% protein) directly to a second month-long inpatient period consuming an isocaloric ketogenic diet (KD) (5% carbohydrate, 80% fat, 15% protein).

Methods

Details of the study and methods were reported previously (14). We studied 17 men with BMI between 25-35 kg/m^2 who were admitted as inpatients to metabolic wards where they consumed a standard baseline diet (BD) composed of 50% energy from carbohydrate, 35% fat, and 15% protein for 4 weeks immediately followed by 4 weeks of an isocaloric very low-carbohydrate, ketogenic diet (KD) composed of 5% carbohydrate, 80% fat, 15% protein. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, with subjects wearing a hospital gown and undergarments and following an overnight fast. Body fat was measured using DXA scanners (Lunar iDXA, GE Healthcare, Madison, WI, USA).

Subjects spent two consecutive days each week residing in respiratory chambers to measure energy expenditure ($EE_{chamber}$). As described previously (14), during the BD period, the daily energy expenditure was calculated as follows:

$$EE_{chamber}\,(\text{kcal}) = 3.88 \times VO_2\,(\text{L}) + 1.08 \times VCO_2\,(\text{L}) - 1.57 \times N\,(\text{g})$$

where VO_2 and VCO_2 were the volumes of oxygen consumed and carbon dioxide produced, respectively, and N was the 24hr urinary nitrogen excretion measured by chemiluminescence (Antek MultiTek Analyzer, PAC, Houston, TX). During the KD period, the equations were adjusted to account for 24-hour urinary ketone excretion, K_{excr}:

$$EE_{chamber}\,(\text{kcal}) = 3.88 \times \left[VO_2\,(\text{L}) - 0.32\ (\text{L/g}) \times K_{excr}\,(\text{g})\right] + 1.08 \times VCO_2\,(\text{L}) - 1.57 \times N\,(\text{g}) + 1.39 \times K_{excr}\,(\text{g})$$

Energy efficiency of physical activity was measured in the respiratory chamber with subjects exercising at a constant, self-selected, level of moderate-intensity cycle ergometry.

Energy expenditure was calculated by energy balance (EE_{bal}) using the daily metabolizable energy intake (EI) along with the measured rates of change of the body energy storage pools

determined from measurements of fat mass (FM) and fat-free mass (FFM) at the beginning and end of each two-week BD and KD period coincident with the DLW measurements. Using the calculated rates of change of FM and FFM, dFM/dt and dFFM/dt, EE_{bal} was calculated according to the following equation:

$$EE_{bal} = EI - \rho_{FFM}\frac{dFFM}{dt} - \rho_{FM}\frac{dFM}{dt}$$

where ρ_{FM} = 9300 kcal/kg is the energy density of body fat mass, ρ_{FFM} = 1100 kcal/kg is the energy density of fat-free mass.

Energy expenditure was measured by DLW during the final two weeks of the BD and KD periods to allow sufficient time for fluid shifts as subjects to adjusted to each diet. Subjects drank from a stock solution of $^{2}H_2O$ and $H_2{}^{18}O$ water in which 1 g of $^{2}H_2O$ (99.99% enrichment) was mixed with 19 g of $H_2{}^{18}O$ (10% enrichment). An aliquot of the stock solution was saved for dilution to be analyzed along with each set of urine samples. The water was weighed to the nearest 0.1 g into the dosing container. The prescribed dose was 1.0 g per kg body weight and the actual dose amounts were entered in the dose log. Spot urine samples were collected daily. Isotopic enrichments of urine samples were measured by isotope ratio mass spectrometry. The average CO_2 production rate (rCO_2), corrected for previously administered isotope doses, can be estimated from the rate constants describing the exponential disappearances of the labeled ^{18}O and deuterated water isotopes (k_O and k_D) in repeated spot urine samples collected over several days. We used the parameters of Racette et al. (15) with the weighted dilution space calculation, R_{dil}, proposed by Speakman (16):

$$rCO_2 = (N/2.078)(1.007k_O - 1.007R_{dil}k_D) - 0.0246r_{GF}$$
$$r_{GF} = 1.05(1.007k_O - 1.007R_{dil}k_D)$$
$$R_{dil} = \left[(N_D/N_O)_{ave} \times n + 1.034 \times 255\right] / (n + 255)$$

where r_{GF} accounts for the fractionation of the isotopes and $(N_D / N_O)_{ave}$ is the mean of the N_D / N_O values from the n=17 subjects.

As described in our previous report (14), we used the 24hr respiratory quotient, RQ, during the respiratory chamber stays to calculate energy expenditure (EE_{DLW}) during the baseline period was calculated as:

$$EE_{DLW}\,(\text{kcal}) = \left[\frac{3.85}{RQ} + 1.07\right] \times rCO_2\,(\text{L})$$

During the KD period, EE_{DLW} was calculated as:

$$EE_{DLW}\,(\text{kcal}) = \left[\frac{3.85}{RQ} + 1.07\right] \times rCO_2\,(\text{L}) - [3.85 \times 0.32 + 1.39] \times K_{excr}\,(\text{g})$$

Because the effective RQ depends not only on the diet composition, but also the state of energy balance, the RQ values measured by the respiratory chambers were adjusted by ΔRQ to account for the relative energy imbalance (EB) by the following equation derived in the Supplementary Materials:

$$\Delta RQ = \lambda \times EB$$

where $\lambda = 5.28\times10^{-5}$ d/kcal for the BD and $\lambda = 9.17\times10^{-6}$ d/kcal for the KD.

Different values of λ result from diets varying in carbohydrate which thereby results in substantial changes in the energy equivalent of CO_2 for the same degree of energy imbalance.

Adjusting the chamber RQ measurements requires accounting for the difference between the overall rate of change in body energy stores and the energy imbalance during the chamber stays as follows:

$$EB = \rho_{FFM}\frac{dFFM}{dt} + \rho_{FM}\frac{dFM}{dt} - \left(EI - EE_{chamber}\right)$$

Thus, the ΔRQ adjustment for each individual subject's diet-specific state of energy imbalance results in the following calculation of DLW energy expenditure ($EE_{DLW\Delta RQ}$) during the baseline period:

$$EE_{DLW\Delta RQ}(\text{kcal}) = \left[\frac{3.85}{\left(RQ + \Delta RQ\right)} + 1.07\right] \times rCO_2(\text{L})$$

During the KD period, $EE_{DLW\Delta RQ}$ was calculated as:

$$EE_{DLW\Delta RQ}(\text{kcal}) = \left[\frac{3.85}{\left(RQ + \Delta RQ\right)} + 1.07\right] \times rCO_2(\text{L}) - \left[3.85 \times 0.32 + 1.39\right] \times K_{excr}(\text{g})$$

As opposed to our previous study (14) that reported results during the entire six-week period when EI was held constant (i.e. the last two weeks of the BD phase and the entirety of the KD phase), we now report results based upon data obtained only during the two-week DLW phase of both BD and KD periods. In addition to allowing direct comparison with the coincident DLW measures, focusing on the last two weeks of each diet allowed for dissipation of any transient changes in energy expenditure needed to adapt to new fuel sources, whether transitioning from the usual diet to the BD or transitioning from BD to the KD.

Statistical analysis was performed using a paired, two-sided t-test with significance declared at the $p<0.05$ threshold. The data are reported as mean±SE.

Results

During the final two weeks of the BD, EI was 2738±107 kcal/d which was significantly higher than $EE_{chamber}$ = 2626±104 kcal/d ($p<0.0001$). EE_{DLW} was 2964±126 kcal/d and significantly higher than EI ($p=0.011$). Adjusting for the energy imbalance, $EE_{DLW\Delta RQ}$ = 3045±135 kcal/d which was significantly greater than EE_{DLW} ($p=0.0003$). Energy expenditure calculated using EI and the rate of change in body energy stores was EE_{bal} = 3136±171 kcal/d and was not significantly different from EE_{DLW} ($p=0.23$) or $EE_{DLW\Delta RQ}$ ($p=0.47$). Compared to $EE_{chamber}$, EE_{DLW} was 338±77 kcal/d higher ($p=0.0005$), $EE_{DLW\Delta RQ}$ was 419±76 kcal/d higher ($p<0.0001$), and EEbal was 509±100 kcal/d higher ($p<0.0001$) indicating that subjects expended significantly more energy outside the chamber during the BD phase.

During the final two weeks of the KD phase, EI was 2730±110 kcal/d which (by design) was not significantly different from the BD phase ($p=0.16$). Whereas we previously reported a transient increase in $EE_{chamber}$ during the first two weeks after introducing the KD (14), neither $EE_{chamber}$ (2650±89 kcal/d; $p=0.43$) nor EE_{bal} (2995±160 kcal/d; $p=0.25$) were significantly different during the last two weeks of the KD compared to the last two weeks of the BD coincident with the DLW measurement periods (Figure 1A). Likewise, physical activity measured using an accelerometer mounted on the hip was not significantly different (KD relative to BD, -5.1±4.8%; $p=0.3$); and energy efficiency of physical activity measured in the respiratory chamber with subjects exercising at a constant level of moderate-intensity cycle ergometry was not significantly different (-1.6±2.4%; $p=0.52$) between the BD and KD phases.

Despite no significant differences in EI, $EE_{chamber}$, EE_{bal}, physical activity, or exercise efficiency between the KD and BD phases (Figure 1B), EE_{DLW} was 209±83 kcal/d higher during the KD phase (p=0.023). The transition from the BD to KD coincided with increases in EE_{DLW} that were in the opposite direction to EE_{bal} indicating that the DLW calculations during the KD were incommensurate with the changes in body weight and fat mass. After adjusting for the state of energy imbalance, $EE_{DLW\Delta RQ}$ was 139±89 kcal/d higher during the KD, but this difference was no longer significant (p=0.14) and was still in the opposite direction to the changes in body energy stores.

During the BD, $EE_{chamber}$ was highly correlated with EE_{bal} (Figure 2A, r=0.85; p<0.0001), as were the correlations between $EE_{chamber}$ and EE_{DLW} (Figure 2B, r=0.79; p=0.0002) and between EE_{DLW} and EE_{bal} (Figure 2C, r=0.62; p=0.008). The correlation between $EE_{chamber}$ and EE_{bal} remained high during the KD (Figure 2D, r=0.88; p<0.0001), whereas the correlation between $EE_{chamber}$ and EE_{DLW} was somewhat attenuated (Figure 2E, r=0.58, p=0.014), and EE_{DLW} was not significantly correlated with EE_{bal} (Figure 2F, r=0.40; p=0.11). Adjusting the DLW calculations for energy imbalance resulted in $EE_{DLW\Delta RQ}$ being even more highly correlated with both $EE_{chamber}$ (r=0.82; p<0.0001) and EE_{bal} (r=0.70; p=0.002) during the BD, but during the KD $EE_{DLW\Delta RQ}$ remained only modestly correlated with $EE_{chamber}$ (r=0.60; p=0.011) and was not significantly correlated with EE_{bal} (r=0.42; p=0.09) (not shown). In other words, whereas DLW calculated energy expenditure measurements were highly correlated with both $EE_{chamber}$ and EE_{bal} during the BD, and $EE_{chamber}$ remained highly correlated with EE_{bal} during the KD, the correlations with DLW measurements were diminished.

There were two clear DLW outliers. The first outlier, "subject A", had an EE_{DLW} that was 1220 kcal/d greater than EI during the BD, and was 1751 kcal/d greater than EI during the KD despite having slight gains in weight and body fat during these periods. In contrast, the $EE_{chamber}$ measurements for this subject were only 173 kcal/d less than EI during the BD and 65 kcal/d less than EI during the KD. The second outlier, "subject B", had an EE_{DLW} during the BD that was only 123 kcal/d higher than $EE_{chamber}$, but during the KD his EE_{DLW} increased by 1136 kcal/d which was ~3 standard deviations greater than the mean increase in $EE_{DLW,}$ suggesting severe negative energy balance despite the subject gaining weight during this period and $EE_{chamber}$ increasing by only 72 kcal/d. Supplementary Tables S1-S4 provide data on the energy expenditure comparisons between BD and KD phases with and without the exclusion of these subjects. After excluding these subjects, the increase in EE_{DLW} after the KD was 126±62 kcal/d (p=0.063) and $EE_{DLW\Delta RQ}$ increased by only 46±65 kcal/d (p=0.49), neither of which were significant (Supplementary Table 4)

Discussion

Our inpatient isocaloric feeding study represents the most rigorously controlled investigation of the DLW method during low-carbohydrate diets in humans. Four days of respiratory chamber measurements that were coincident with each DLW period did not detect significant changes in energy expenditure. The observed increase in EE_{DLW} after transitioning to the KD was substantially greater than could be accounted for by changes in body energy stores. Whereas individual measurements of $EE_{chamber}$ and EE_{bal} were highly correlated with each other during both diet periods, the DLW measurements were only highly correlated with these measurements

during the BD. Thus, the KD period appeared to result in DLW data that were inconsistent with the other expenditure measurements.

We previously reported that $EE_{chamber}$ transiently increased during the first two weeks of the KD but there was no significant difference by the end of the KD (14). The current study shows that $EE_{chamber}$ during the final two weeks of the KD was not significantly different from the final two weeks of the BD. These periods were coincident with the DLW measurements that indicated a significant increase in EE_{DLW}. We previously hypothesized that the discrepancy between the respiratory chamber and DLW measurements was due to increased physical activity outside the respiratory chamber during the KD (14). However, this potential explanation does not agree with the lack of increase in objectively measured physical activity. Also, the energy expended to perform the same low-intensity exercise in the respiratory chamber was not significantly changed by the KD, making it unlikely that the energy efficiency of skeletal muscle contraction had been altered after transitioning to the KD. Thus, the EE_{DLW} measurements were discordant with several independent measures all indicating that the KD did not result in significant energy expenditure changes.

Our results raise the possibility that our previously reported increase in energy expenditure calculated by the DLW method after transitioning to a very low-carbohydrate diet may have been due to methodological issues. An important and common source of error is the failure to employ diet-specific RQ adjustments in the DLW calculations to account for energy imbalance (10). We found that such RQ adjustments resulted in a substantial attenuation of the calculated increase in DLW energy expenditure during the KD such that the increase was no longer significant.

Another methodological concern is the theoretical possibility that CO_2 production rates calculated by the DLW method can be influenced by the fluxes through biosynthetic pathways that likely vary substantially depending on the carbohydrate content of the diet, especially the de novo lipogenesis pathway (11, 12, 13). However, the magnitude of this potential bias in humans is thought to be relatively small, amounting to an energy expenditure difference of only about 30-60 kcal/d (see Supplementary Materials). Interestingly, even this small systematic bias would have been sufficient to nullify the statistical significance of the observed increased EE_{DLW} in our study. Furthermore, we cannot rule out the possibility of a larger bias with a low carbohydrate diet without direct measurements of changes in de novo lipogenesis or other contributing metabolic fluxes. Individual variability of de novo lipogenesis changes after transitioning to the KD might explain the diminished correlations during the KD between the DLW measurements with $EE_{chamber}$ and EE_{bal}. To directly address this question, we need a validation study in humans with several days of simultaneous respiratory chamber and DLW measurements high- and low-carbohydrate diets, ideally with simultaneous measurements of de novo lipogenesis.

In support of the concept that the DLW method may overestimate CO_2 production differences between diets varying widely in carbohydrate content, a recent study in mice found that the DLW method overestimated CO_2 production during low-carbohydrate, high-fat diets, but agreed with respiratory chamber measurements during a high-carbohydrate diet (17). However, such results have been questioned based on criticisms of the DLW assumptions and model equations as well as potential problems with the mouse respiratory chamber data (18).

Similar to our study, Ebbeling et al. (6) reported significant differences in EE_{DLW} during the last two weeks in each of three consecutive month-long periods during which subjects consumed a low-carbohydrate diet (10% of energy) compared to isocaloric diets with moderate (40% of energy) or high (60% of energy) carbohydrate in random order. The RQ was estimated without adjustment for the subjects' state of energy balance. The lower-carbohydrate diets appeared to increase EE_{DLW} by 250-325 kcal/d compared to the high-carbohydrate diet. However, despite constant energy intake there were no significant weight differences among the three month-long diet periods. Objectively measured physical activity was not different among the diets, and the resting energy expenditure differences were much smaller (~29-67 kcal/d) than the EE_{DLW} differences. Compared to the high- and moderate-carbohydrate diets, the lowest carbohydrate diet had 50% more dietary protein – which is known to be more thermogenic than dietary carbohydrate and fat (19) – and likely contributed to a portion of the observed increase in EE_{DLW}. But it is unlikely that the thermic effect of protein alone could explain the discrepancy between the differences in resting energy expenditure and EE_{DLW}. Thus, the data by Ebbeling et al. could be interpreted as supporting the possibility that the EE_{DLW} calculations are systematically biased by dietary carbohydrate content such that isocaloric diets that are low in carbohydrate content result in higher estimates of energy expenditure.

In contrast, Bandini et al. (20) found that EE_{DLW} was lower during the low-carbohydrate diet (~7% of energy) as compared to a high-carbohydrate diet (~83% of energy) in an outpatient study, but this reduction was attributed to decreased physical activity because no significant differences in REE were found and the subjects reported nausea and lethargy on the low-carbohydrate diet. Stubbs et al. (21) found no significant difference between EE_{DLW} using a

narrower range of diets with 29-67% of energy as carbohydrate. But the diets were fed *ad libitum* and energy intake on the low-carbohydrate diet was greater than the moderate-carbohydrate diet which was greater than the high-carbohydrate diet. Thus, the variation in total carbohydrate content of the test diets was attenuated such that daily carbohydrate intake varied by only ~22% of the mean energy intake between diets which may not have been a sufficient range to observe a systematic bias of the DLW method. Furthermore, variation in EI between the diets led to different states of energy balance that offset the RQ differences between the diets.

It is important to emphasize that our study was not intended to be a DLW validation study and there were several limitations. The DLW measurements were not pre-specified as either primary or secondary endpoints of the study. Whereas respiratory chamber measurements have high-precision, with an intrasubject coefficient of variation of $EE_{chamber}$ ~2-3% (22), the DLW method is less precise with an intrasubject coefficient of variation of energy expenditure of ~ 8-15% (23). Therefore, the relatively large inherent variability of the DLW method may have led to an apparent increase in EE_{DLW} during the KD simply by chance (type-1 error). However, we cannot definitively exclude the possibility of a real increase in energy expenditure, especially at the modest effect size of ~50-140 kcal/d after excluding two likely DLW outliers or using diet-specific adjustments of the DLW calculations to account for the energy imbalance.

Another limitation of our study was that the subjects lost weight and body fat throughout the study, consistent with a state of inadvertent overall negative energy balance (14). The DLW method has been validated during 30% caloric restriction with a 55% carbohydrate diet (24) and agrees with our result that EE_{DLW} and $EE_{DLW\Delta RQ}$ were not significantly different from EE_{bal}

during the BD diet phase. Nevertheless, the calculated EE_{bal} values are somewhat uncertain because DXA has a limited ability to precisely and accurately detect small changes in body energy stores (25), and the calculated metabolizable energy intake was not adjusted using direct measures of fecal energy content. While accelerometer measurements did not detect significant differences in physical activity between the diets, these devices do not capture all forms of physical activity and we cannot rule out the possibility that the KD resulted in undetected increases in activity-related energy expenditure. Finally, the order of the diets was not randomized, and it is possible that the elevated EE_{DLW} occurred simply because the KD followed the BD. Indeed, other investigators have noted that repeated DLW measurements resulted in a mean expenditure increase of ~100 kcal/d for the second measurement, perhaps for methodological reasons related to the isotopes in the first DLW dose interfering with subsequent measures (Jim DeLany, personal communication).

In summary, our data illustrate the challenges of using the DLW method to estimate energy expenditure differences between diets varying widely in the proportion of carbohydrate. Therefore, we urge caution when interpreting DLW data comparing energy expenditure differences between diets varying widely in carbohydrate content, especially if the DLW calculations do not appropriately estimate and adjust RQ for energy imbalance and if the DLW results are not corroborated by quantitatively commensurate observations of energy intake and body composition change.

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Figure Legends

Figure 1. A) Neither energy expenditure by respiratory chamber ($EE_{chamber}$), nor energy expenditure by balance (EE_{bal}) were significantly different during the baseline diet (BD) versus the ketogenic diet (KD). However, energy expenditure by doubly labeled water (EE_{DLW}) was significantly greater during the KD, but not after adjustment of the respiratory quotient (RQ) to account for the differential diet effect of energy imbalance ($EE_{DLW\Delta RQ}$) . B) Differences in $EE_{chamber}$, EE_{bal}, EE_{DLW}, and $EE_{DLW\Delta RQ}$ between KD and BD phases. NS = not significant, $p > 0.05$.

Figure 2. Correlations between individual measurements of A) $EE_{chamber}$ and EE_{bal}, B) $EE_{chamber}$ and EE_{DLW}, and C) EE_{DLW} and EE_{bal} were high during the BD phase. During the KD phase, D) $EE_{chamber}$ and EE_{bal} remained highly correlated, whereas the correlation between E) $EE_{chamber}$ and EE_{DLW} was diminished and F) EE_{DLW} and EE_{bal} were no longer significantly correlated.

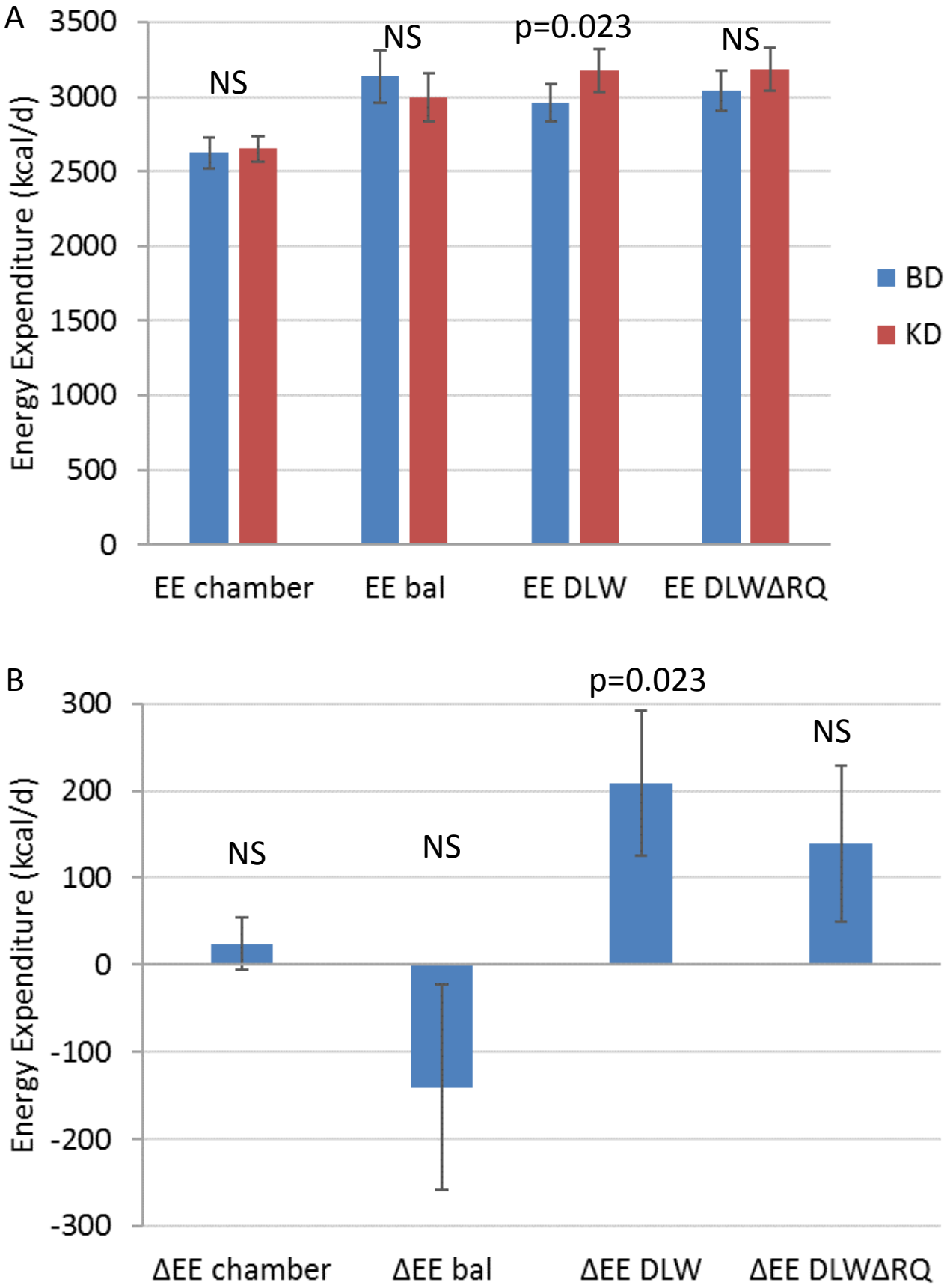
A
3500
3000
2500
2000
1500
1000
500
0
Energy Expenditure (kcal/d)
NS
NS
p=0.023
NS
BD
KD
EE chamber
EE bal
EE DLW
EE DLWΔRQ
B
300
200
100
0
-100
-200
-300
Energy Expenditure (kcal/d)
NS
NS
p=0.023
NS
ΔEE chamber
ΔEE bal
ΔEE DLW
ΔEE DLWΔRQ

Figure 1

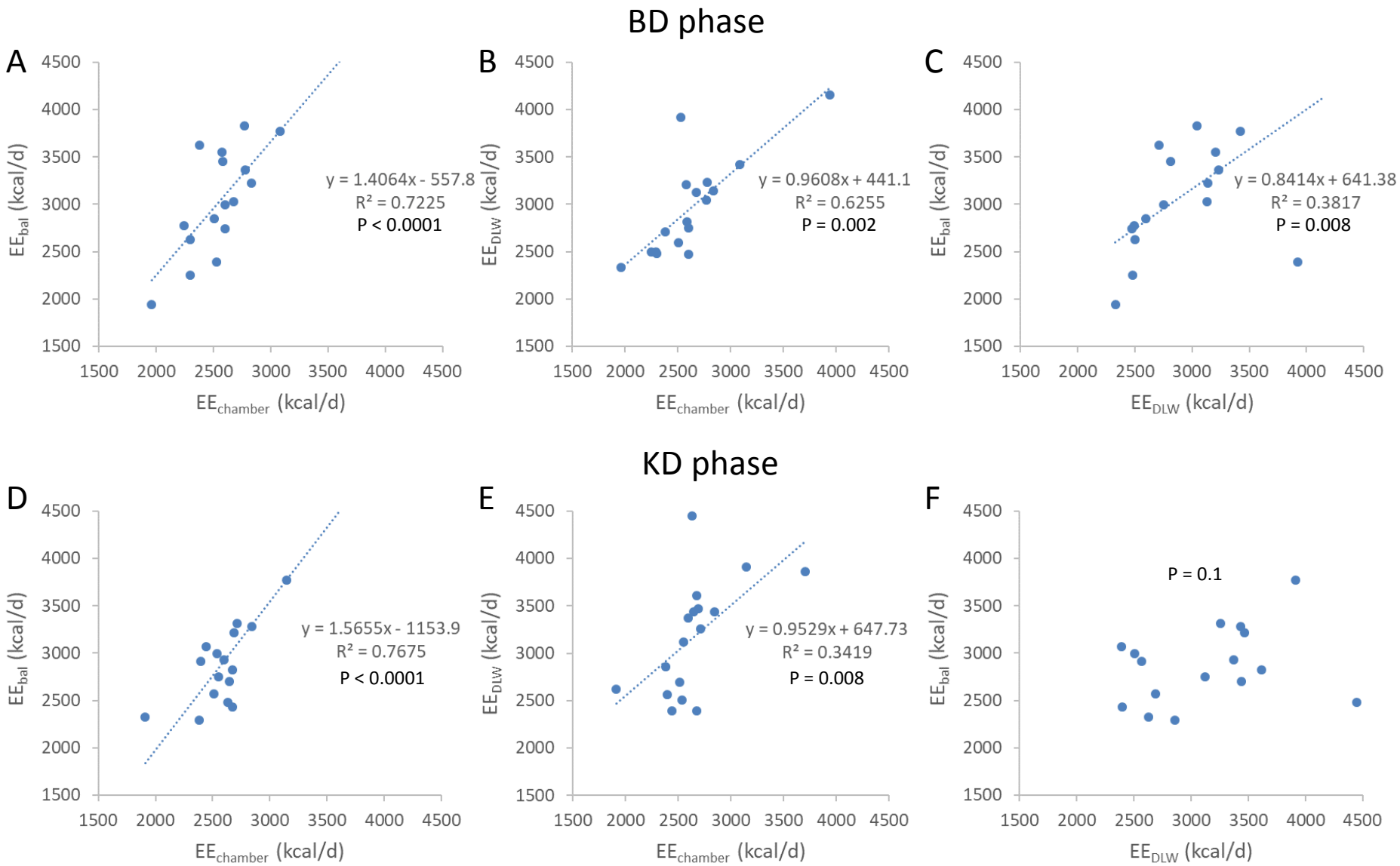
BD phase
A
B
C
KD phase
D
E
F
y = 1.4064x - 557.8
R² = 0.7225
P < 0.0001
y = 0.9608x + 441.1
R² = 0.6255
P = 0.002
y = 0.8414x + 641.38
R² = 0.3817
P = 0.008
y = 1.5655x - 1153.9
R² = 0.7675
P < 0.0001
y = 0.9529x + 647.73
R² = 0.3419
P = 0.008
P = 0.1
EEbal (kcal/d)
EEDLW (kcal/d)
EEchamber (kcal/d)
1500
2000
2500
3000
3500
4000
4500

Figure 2

Supplementary Materials

Diet-specific Adjustments of Respiratory Quotient to account for Energy Imbalance

Translating the measured rate of CO_2 production (rCO_2) to energy expenditure (EE) requires an estimate of the respiratory quotient (RQ) to calculate the energy equivalent of CO_2 [1]. The RQ depends on both the composition of the diet via the food quotient (FQ) as well as the overall state of energy balance. Using the substrate oxidation equations previously derived [2] and assuming a state of approximate carbohydrate balance after two weeks on each of the isocaloric baseline diet (BD) and ketogenic diet (KD) periods prior to doubly labeled water (DLW) administration in our study, the following equation can be derived for the diet-specific adjustment in RQ (ΔRQ):

$$\Delta RQ = \left(1 - \frac{EI}{EE}\right)\left[\frac{0.7\rho_{FM} + 0.83\rho_{FFM} \times C_{Forbes}/FM}{\rho_{FM} + \rho_{FFM} \times C_{Forbes}/FM} - FQ\right]$$

where EI is energy intake, EE is energy expenditure, ρ_{FM} = 9300 kcal/kg is the energy density of body fat mass, ρ_{FFM} = 1100 kcal/kg is the energy density of fat-free mass, C_{Forbes} = 10.4 kg is the Forbes constant, and FM is the average body fat mass which was ~25 kg for our subjects. The average baseline EI = 2740 kcal/d for both diets and the FQ was 0.87 and 0.73 for the BD and KD, respectively. Figure S1 depicts the ΔRQ results of varying EE in 100 kcal/d increments around the average baseline EI for each diet to result in a range of energy imbalances. The best-fit linear functions through the origin had slopes $\lambda = 5.28\times10^{-5}$ d/kcal for the BD and $\lambda = 9.17\times10^{-6}$ d/kcal for the KD. The energy equivalent of CO_2 is thereby adjusted according to:

$$Eeq_{CO_2}\left(\text{kcal/L}\right) = \frac{3.85}{\left(RQ_{chamber} + \Delta RQ\right)} + 1.07$$

where $\Delta RQ = \lambda \times (EI - EE)$.

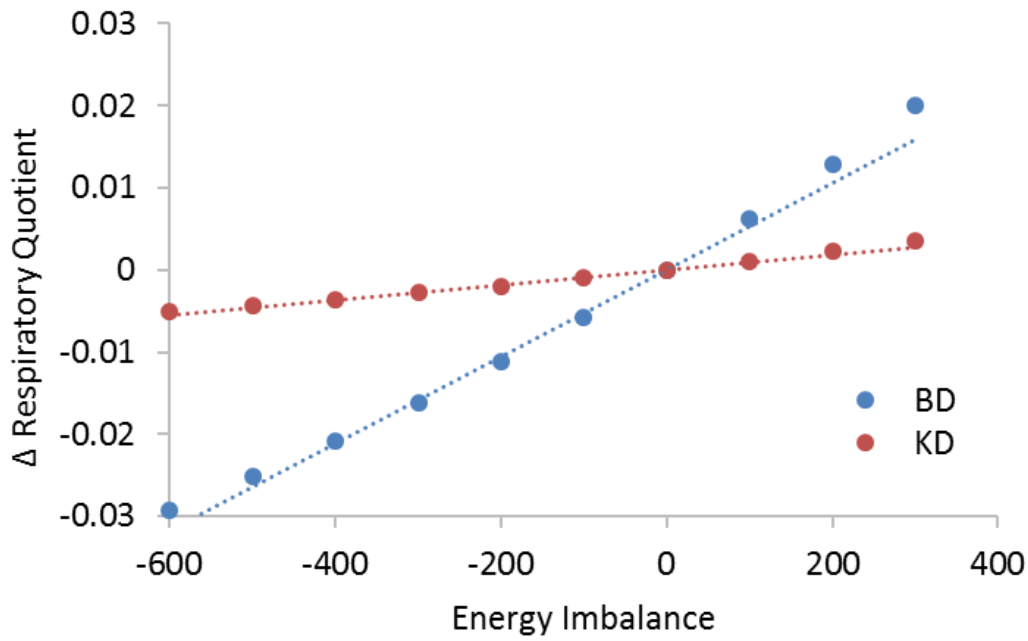

Figure S1. Theoretical diet-specific relationships between the state of energy imbalance and the respiratory quotient adjustments to the doubly labeled water energy expenditure calculations.

Potential Effect of De Novo Lipogenesis

We used the two-pool DLW equations of Speakman [3] to calculate the rCO_2 due to EE as follows:

$$r_{CO_2\,EE}\left(\text{mol/d}\right) = (N/2.078)\left[1.007\times k_o - 1.007\times 1.034\times k_d\right] - 0.0246\times r_{GF}$$

where N is the body water pool size (in moles), k_O and k_D are the rate constants (in d^{-1}) describing the exponential disappearances of the labeled ^{18}O and deuterated water isotopes in repeated spot urine samples collected over several days, and r_{GF} accounts for the fractionation of the isotopes.

It is known that deuterium is incorporated into body constituents other than water via reductive biosynthesis, and the fluxes through some of these biosynthetic pathways likely depends on diet composition [4-6]. For example, the incorporation of deuterium into newly synthesized fatty acids via de novo lipogenesis (DNL) results in an apparent sequestration of 0.0296 moles of deuterated water per gram of DNL [4] and the flux through the DNL pathway depends highly on dietary carbohydrate. Therefore, during ongoing DNL, the CO_2 production rate measured by DLW is correspondingly altered:

$$r_{CO_2\,meas}\left(\text{mol/d}\right) = (N / 2.078)\left[1.007 \times k_o - 1.007 \times 1.034 \times k_d\right] - 0.0246 \times r_{GF} - \frac{0.0296\left(\text{mol/g}\right)}{2.078} \times DNL\left(\text{g/d}\right)$$

$$r_{CO_2 meas}\left(\text{mol/d}\right) = r_{CO_2 EE}\left(\text{mol/d}\right) - \frac{0.0296\left(\text{mol/g}\right)}{2.078} \times DNL\left(\text{g/d}\right)$$

Therefore, when DNL is ongoing the rate of CO_2 production (and therefore EE) as measured by the DLW method underestimates the rate of CO_2 production. However, parameterization of the equations employed in the DLW method used data in subjects with ongoing DNL, since they were consuming moderate to high carbohydrate (HC) diets. The potential bias of the DLW method with HC diets was likely offset in the calibration/validation process of developing the DLW model equations. Thus, if such an offset were significant then transitioning to a lower carbohydrate diet (LC) would be expected to result in a decrease in DNL and an apparent increase in EE_{DLW} solely due to a methodological bias.

How much could DNL differences potentially contribute to the EE_{DLW} differences between LC and HC diets? In our study [7], we calculated the energy equivalent of CO_2 as:

$$Eeq_{CO_2}\left(\text{kcal/L}\right)=\frac{3.85}{RQ}+1.07$$

Assuming that the LC diet had negligible DNL (DNL=0) and there was no actual difference in EE between the diets: $Eeq_{CO_2HC}\times r_{CO_2EE_{HC}}=Eeq_{CO_2LC}\times r_{CO_2EE_{LC}}$, then the measured difference in EE_{DLW} between LC and HC due solely to DNL is:

$$\Delta EE_{meas}\left(\text{kcal/d}\right)=22.261\left(\text{L/mol}\right)\left[Eeq_{CO_2HC}\times r_{CO_2meas_{HC}}-Eeq_{CO_2LC}\times r_{CO_2meas_{LC}}\right]$$
$$=-22.261\left(\text{L/mol}\right)\times Eeq_{CO_2HC}\left(\text{kcal/L}\right)\times\frac{0.0296\left(\text{mol/g}\right)}{2.078}\times DNL\left(\text{g/d}\right)$$

Therefore, the increment in kcal/d for every g/d of DNL is:

$$\frac{\Delta EE_{meas}\left(\text{kcal/d}\right)}{DNL\left(\text{g/d}\right)}=-22.261\ (\text{L/mol})\times Eeq_{CO_2HC}(\text{kcal/L})\times\frac{0.0296\ (\text{mol/g})}{2.078}$$

Assuming that RQ is between 0.85 and 0.90 on the HC diet, then the increment in EE kcal/d per DNL g/d ranges between -1.78 to -1.70.

About 20% of subcutaneous adipose TG synthesis on a HC diet is derived from DNL and adipose TG turnover is in the range of 0.7 to 1.5 g/d per kg BW [8]. Hepatic VLDL TG production rate is about 0.7 g/d per kg BW [9] and about 10-20% of the VLDL TG produced daily may be derived from DNL with higher carbohydrate diets [10]. So, about 17-35 g/d is probably a reasonable estimate for whole body DNL for an 80 kg subject during a HC diet, with a likely large degree of individual variability. Therefore, DNL differences between HC and LC diets likely introduced an error in the EE by DLW to result in an apparent ΔEE_{meas} of about 30-60 kcal/d between LC and HC diets.

We cannot rule out the possibility of larger systematic errors without direct measurements of DNL. Furthermore, other biosynthetic pathways may also contribute to diet-dependent systematic errors in the DLW method in the same direction, although likely not to the same extent as DNL. For example, both glycogen and protein synthesis result in incorporation of deuterium [11] and the fluxes through these pathways would be expected to decrease with a LC diet. Overall, these methodological issues introduce potentially important systematic biases when considering the effect of diet differences on EE as measured by DLW.

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Supplementary Table 1

N=17	BD Mean ± SE	KD Mean ± SE	KD-BD Mean ± SE	P-value
EI (kcal/d)	2738±107	2730±110	-8±5	0.16
$EE_{chamber}$ (kcal/d)	2626±104	2650±89	24±30	0.43
EE_{bal} (kcal/d)	3136±171	2995±160	-141±118	0.25
EE_{DLW} (kcal/d)	2964±126	3173±146	209±83	0.023
$EE_{DLW\Delta RQ}$ (kcal/d)	3045±135	3184±147	139±89	0.14

Supplementary Table 2

N=16 (excluding subject A)	BD Mean ± SE	KD Mean ± SE	KD-BD Mean ± SE	P-value
EI (kcal/d)	2740±114	2732±117	-8±5	0.17
$EE_{chamber}$ (kcal/d)	2632±110	2651±95	19±31	0.55
EE_{bal} (kcal/d)	3182±176	3027±167	-155±125	0.23
EE_{DLW} (kcal/d)	2905±118	3093±130	189±86	0.044
$EE_{DLW\Delta RQ}$ (kcal/d)	2992±132	3106±132	114±91	0.23

Supplementary Table 3

N=16 (excluding subject B)	BD Mean ± SE	KD Mean ± SE	KD-BD Mean ± SE	P-value
EI (kcal/d)	2736±114	2726±117	-10±5	0.06
$EE_{chamber}$ (kcal/d)	2628±110	2649±95	21±32	0.52
EE_{bal} (kcal/d)	3160±181	3006±170	-155±125	0.24
EE_{DLW} (kcal/d)	2995±130	3146±152	151±63	0.031
$EE_{DLW\Delta RQ}$ (kcal/d)	3080±138	3157±153	78±69	0.28

Supplementary Table 4

N=15 (excluding subjects A and B)	BD Mean ± SE	KD Mean ± SE	KD-BD Mean ± SE	P-value
EI (kcal/d)	2738±122	2728±125	-10±5	0.064
$EE_{chamber}$ (kcal/d)	2635±118	2650±102	15±33	0.65
EE_{bal} (kcal/d)	3212±185	3041±177	-171±133	0.22
EE_{DLW} (kcal/d)	2933±122	3059±134	126±62	0.063
$EE_{DLW\Delta RQ}$ (kcal/d)	3025±136	3072±136	46±65	0.49