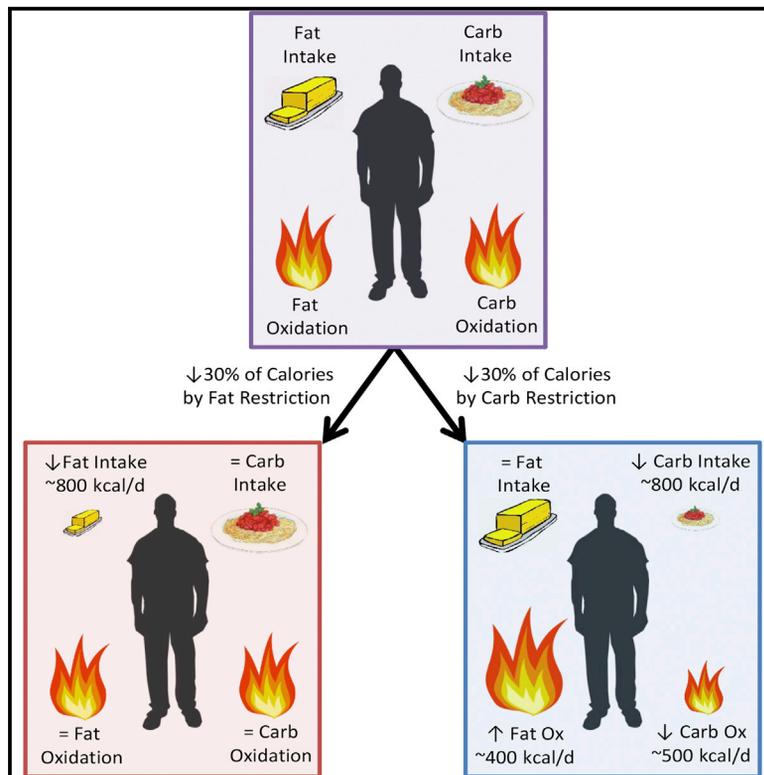


# Cell Metabolism

## Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity

### Graphical Abstract



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### In Brief

Hall et al. investigated 19 adults with obesity that selectively restricted dietary carbohydrate versus fat. Cutting carbohydrates increased net fat oxidation while equal calorie fat restriction had no effect. However, cutting fat resulted in more body fat loss than cutting carbohydrates. Mathematical model simulations predicted small long-term differences in body fat.

### Highlights

- 19 adults with obesity were confined to a metabolic ward for two 2-week periods
- Cutting carbohydrates increased net fat oxidation, but cutting fat by equal calories had no effect
- Cutting fat resulted in more body fat loss as measured by metabolic balance
- Mathematical model simulations predicted small long-term differences in body fat



# Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity

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## SUMMARY

Dietary carbohydrate restriction has been purported to cause endocrine adaptations that promote body fat loss more than dietary fat restriction. We selectively restricted dietary carbohydrate versus fat for 6 days following a 5-day baseline diet in 19 adults with obesity confined to a metabolic ward where they exercised daily. Subjects received both isocaloric diets in random order during each of two inpatient stays. Body fat loss was calculated as the difference between daily fat intake and net fat oxidation measured while residing in a metabolic chamber. Whereas carbohydrate restriction led to sustained increases in fat oxidation and loss of  $53 \pm 6$  g/day of body fat, fat oxidation was unchanged by fat restriction, leading to  $89 \pm 6$  g/day of fat loss, and was significantly greater than carbohydrate restriction ( $p = 0.002$ ). Mathematical model simulations agreed with these data, but predicted that the body acts to minimize body fat differences with prolonged isocaloric diets varying in carbohydrate and fat.

## INTRODUCTION

Weight loss diets often recommend targeted restriction of either carbohydrates or fat. While low-fat diets were popular in the latter part of the 20<sup>th</sup> century, carbohydrate restriction has regained popularity in recent years, with proponents claiming that the resulting decreased insulin secretion causes elevated release of free fatty acids from adipose tissue, increased fat oxidation and energy expenditure, and greater body fat loss than restriction of dietary fat (Ludwig and Friedman, 2014; Taubes, 2007, 2011; Westman et al., 2007). One influential author concluded that “any diet that succeeds does so because the dieter restricts fattening carbohydrates ... Those who lose fat on a diet do so because of what they are not

eating—the fattening carbohydrates” (Taubes, 2011). In other words, body fat loss *requires* reduction of insulinogenic carbohydrates. This extraordinary claim was based on the observation that even diets targeting fat reduction typically also reduce refined carbohydrates. Since the primary regulator of adipose tissue fat storage is insulin, and a reduction in refined carbohydrates reduces insulin, carbohydrate reduction alone may have been responsible for the loss of body fat—even with a low-fat diet.

While the first law of thermodynamics requires that all calories are accounted, could it be true that reducing dietary fat without also reducing carbohydrates would have no effect on body fat? Could the metabolic and endocrine adaptations to carbohydrate restriction result in augmented body fat loss compared to an equal calorie reduction of dietary fat?

Several randomized controlled trials have demonstrated greater short-term weight loss when advising obese patients to restrict dietary carbohydrates (Foster et al., 2010; Gardner et al., 2007; Shai et al., 2008), but such outpatient studies are difficult to interpret mechanistically because it is not currently possible to accurately measure adherence to the recommended diets since the instruments for assessing food intake rely on self-report and have been demonstrated to be biased (Winkler, 2005). Therefore, outpatient studies cannot determine to what extent any observed differences in weight loss are due to a metabolic advantage of reduced carbohydrate diets versus a greater reduction in overall energy intake.

We performed an in-patient metabolic balance study examining the effect of selective isocaloric reduction of dietary carbohydrate versus fat on body weight, energy expenditure, and fat balance in obese volunteers. A mechanistic mathematical model of human macronutrient metabolism (Hall, 2010) was used to design the study and predict the metabolic response to each diet before the study was conducted (Hall, 2012). Here, we report the results of this experiment and use the mathematical model to quantitatively integrate the data and make *in silico* predictions about the results of long-term diet studies that are not practical to perform in the real world. In agreement with our model simulations, we found that only the reduced carbohydrate diet led to significant changes in metabolic fuel selection, with sustained reductions of carbohydrate oxidation and increased fat

**Table 1. Body Composition, Energy Metabolism, and Macronutrient Intake during the Baseline Phase**

	All Subjects (n = 19)	Female (n = 9)	Male (n = 10)	p value (F versus M)
<b>Age (years)</b>	35.4 ± 1.74	32.7 ± 2.78	37.7 ± 2	0.15
<b>BW (kg)</b>	106 ± 3.8	103 ± 6.5	110 ± 4.3	0.38
<b>BMI (kg/m<sup>2</sup>)</b>	35.9 ± 1.1	37.9 ± 1.8	34.1 ± 1.1	0.082
<b>% body fat</b>	39.3 ± 2	46.5 ± 1.3	32.8 ± 1.8	<0.0001
<b>Fat mass (kg)</b>	42 ± 2.8	48.2 ± 3.9	36.4 ± 3.1	0.029
<b>SMR (kcal/day)</b>	1,770 ± 76	1,570 ± 100	1,950 ± 80	0.01
<b>24-hr EE (kcal/day)</b>	2,560 ± 110	2,240 ± 110	2,840 ± 120	0.0017
<b>24-hr RQ</b>	0.852 ± 0.0077	0.859 ± 0.0082	0.846 ± 0.013	0.41
<b>TEE (kcal/day)</b>	2,880 ± 160	2,500 ± 260	3,250 ± 120	0.026
<b>Energy intake (kcal/day)</b>	2,740 ± 100	2,460 ± 130	2,990 ± 110	0.0062
<b>CHO intake (g/day)</b>	343 ± 13	308 ± 17	374 ± 14	0.0065
<b>Fat intake (g/day)</b>	105 ± 4	95 ± 5.1	115 ± 4.6	0.0099
<b>Protein intake (g/day)</b>	104 ± 3.9	92.1 ± 4.5	114 ± 4.2	0.0024

Mean ± SEM.

oxidation. Remarkably, fat oxidation on the reduced-fat diet remained unchanged and resulted in a greater rate of body fat loss compared to the reduced carbohydrate diet, despite being equivalent in calories.

## RESULTS

### Baseline Data

We investigated ten male and nine female subjects who all had obesity with a BMI of (mean ± SEM) 35.9 ± 1.1 kg/m<sup>2</sup> (Table 1). While the men and women had similar body weight and BMI, the women had significantly higher body fat and lower rates of energy expenditure and food intake, as expected. All subjects were admitted to the metabolic unit at the NIH Clinical Center where they resided for a pair of 2-week inpatient periods separated by a 2- to 4-week washout period (Figure 1). The subjects exercised on a treadmill for 1 hr each day at a clamped pace and incline to maintain a relatively constant physical activity. For the first 5 days of each visit, they consumed a eucaloric baseline diet composed of 50% carbohydrate, 35% fat, and 15% protein with a total energy content of 2,740 ± 100 kcal/day, which was not significantly different from their average total energy expenditure (TEE) of 2,880 ± 160 kcal/day ( $p = 0.19$ ) as measured by the doubly labeled water method. During the days spent residing in a metabolic chamber, 24-hr energy expenditure (EE) was 2,560 ± 110 kcal/day, which was slightly less than the baseline energy intake ( $p = 0.001$ ) as well as TEE ( $p = 0.008$ ). This was likely due to decreased spontaneous physical activity when confined to the metabolic chamber, as confirmed by an overall 23.4% ± 4% reduction in accelerometer counts during chamber days (not shown,  $p < 0.0001$ ).

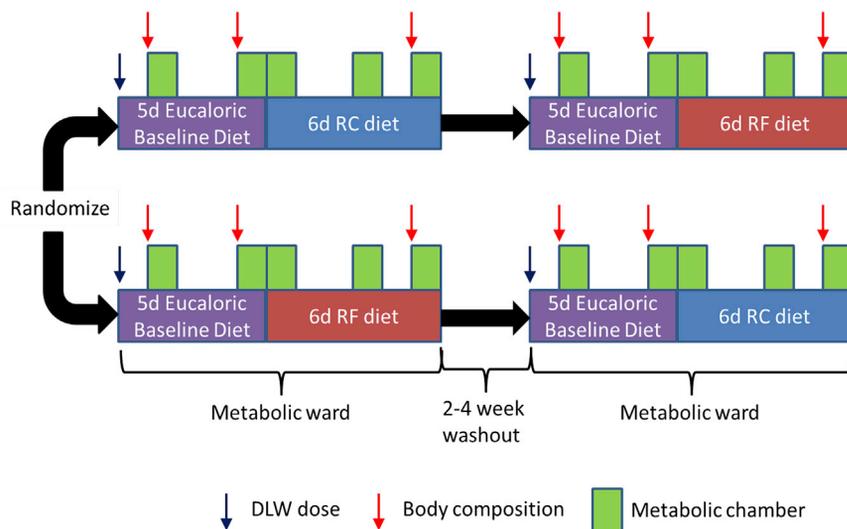
### Changes in Diet, Insulin Secretion, and Energy Metabolism

The experimental diets were designed such that they were 30% lower in calories than the baseline diet (Table 2), and the reduced carbohydrate (RC) and reduced fat (RF) diets led to selective reductions in carbohydrate intake and fat intake, respectively, whereas protein intake was practically unchanged from

baseline (Figure 2A). Note also that the RF diet did not have a decrease in sugar content compared to baseline (Table 2). This was important since a decrease in sugar content with the RF diet would be expected to decrease insulin secretion despite no change in total carbohydrate content compared to baseline. As a result, only the RC diet resulted in a 22.3% ± 7.0% decrease in daily insulin secretion ( $p = 0.001$ ) as measured by 24-hr urinary excretion of C-peptide and depicted in Figure 2B. Therefore, the experimental reduced-energy diets resulted in substantial differences in insulin secretion despite being isocaloric.

The 24-hr respiratory quotient (RQ) provides a measure of the overall metabolic fuel mixture being used by the body to produce energy, with RQ values approaching 1 indicating primarily carbohydrate oxidation and values near 0.7 indicating primarily fat oxidation. The 24-hr RQ was the primary endpoint of this study, and the mathematical model of human macronutrient metabolism predicted in advance that the RF diet would lead to no significant change in RQ whereas the RC diet would lead to a decrease in RQ (Hall, 2012). Figure 2C illustrates the 24-hr RQ data and mathematical model simulations in response to the RC and RF diets. In agreement with the model simulations, only the RC diet resulted in RQ changes, indicating a shift toward increased fat oxidation. In contrast, only the first day of the RF diet led to a significant increase in RQ from baseline ( $p < 0.0001$ ), but there was no significant change in RQ overall, implying that changes in dietary fat have little effect on carbohydrate or fat oxidation (Table 3).

Figure 2D illustrates that the RC and RF diets resulted in a reduction of energy intake by 810 ± 10 kcal/day from baseline. The diets resulted in minor changes in 24-hr EE (Figure 2E) and similar degrees of negative energy balance (Figure 2F). During the RC diet, the sleeping metabolic rate (SMR) and 24-hr EE were significantly decreased by 86.2 ± 25 kcal/day ( $p = 0.0034$ ) and 97.7 ± 23 kcal/day ( $p = 0.0007$ ), respectively, but were not significantly changed during the RF diet (Table 3). There was a trend for a greater degree of negative energy balance during the RF diet compared to the RC diet, but this was not statistically significant ( $p = 0.052$ ). Note that the model simulations



**Figure 1. Overview of the Study Design**

Adults with obesity were admitted to the metabolic unit at the NIH Clinical Center where they received a eucaloric baseline diet for 5 days followed by a 30% energy-restricted diet achieved either through selective reduction of fat (RF) or carbohydrate (RC) for a period of 6 days. Subjects spent 5 days residing in metabolic chambers and had a dose of doubly labeled water (DLW) administered on the first inpatient day. Body composition was assessed by dual-energy X-ray absorptiometry (DXA) during baseline and at the end of the reduced-energy diets. Subjects returned after a 2- to 4-week washout period to undergo the opposite RC or RF diet following the same 5-day baseline phase. The order of the RC and RF diet periods was randomized.

accounted for the observed differences in physical activity between chamber and non-chamber days (Figure 2E).

Only the RC diet led to significant sustained adaptations of carbohydrate and fat metabolism. At the end of the RC diet period, net fat oxidation increased by  $463 \pm 63$  kcal/day ( $p < 0.0001$ ) (Figure 2G) and net carbohydrate oxidation decreased by  $595 \pm 57$  kcal/day ( $p < 0.0001$ ) (Figure 2H). In contrast, only the first day of the RF diet led to a significant reduction in net fat oxidation by  $96 \pm 64$  kcal/day ( $p = 0.01$ ) (Figure 2G) and an increase in net carbohydrate oxidation of  $147 \pm 49$  kcal/day ( $p = 0.01$ ) (Figure 2H) compared to baseline. The mathematical model simulations agreed well with the observed changes in fat oxidation (Figure 2G), but slightly overestimated the decrease in carbohydrate oxidation during the RC diet (Figure 2H). The model also indicated that the RC diet would lead to increased net protein oxidation compared to the RF diet (Figure 2I), a trend that was apparent in the 24-hr urinary nitrogen data (Table 3).

The mean changes in overall energy expenditure, energy balance, 24-hr RQ, fat oxidation, and carbohydrate oxidation during the RC and RF diets are quantified in Table 3 and mirror the day-by-day results above that are presented in Figure 2.

### Macronutrient Balance and Body Composition Changes

Several days of the RF diet led to a steady fat imbalance of  $840 \pm 60$  kcal/day, or equivalently  $89 \pm 6$  g/day of body fat loss (Figure 3A), which was significantly greater than the steady rate of body fat loss of  $500 \pm 60$  kcal/day, or  $53 \pm 6$  g/day, achieved during the RC diet ( $p = 0.0002$ ) (Figure 3A). In contrast, the RC diet led to significantly greater transient carbohydrate imbalance (Figure 3B) with little difference in protein balance (Figure 3C) compared with the RF diet.

Figure 3D shows that the greater net fat imbalance during the RF versus RC diet led to  $\sim 80\%$  greater cumulative body fat loss, such that by the end of the 6-day period, the RF diet resulted in  $463 \pm 37$  g of fat loss compared to  $245 \pm 21$  g of fat loss with the RC diet ( $p < 0.0001$ ) (Table 3). Dual-energy X-ray absorptiometry (DXA) is a widely used clinical method for estimating body fat percentage, which was measured before and after the RC and

RF diets (Figure 1). While both diets led to significant decreases in DXA-determined fat mass compared to baseline ( $p < 0.002$ ) (Table 3 and Figure 3B), DXA was not sufficiently sensitive to detect a significant difference in fat mass change between the RC and RF diets. Figure 3C illustrates that both diets led to weight loss ( $p < 0.0001$ ), with the RC diet resulting in greater weight loss than the RF diet ( $p = 0.02$ ). The mathematical model simulations closely matched the cumulative fat loss measurements for both diets (Figure 3D). While the simulated weight loss with the RC diet was close to the observed value, the RF diet led to substantially more weight loss than was predicted by the model. This was likely due to body water losses that took place via mechanisms outside the scope of the current model (see the Supplemental Information for a full description of the model).

Table 4 presents the baseline overnight-fasted plasma measurements along with the changes in response to the RC and RF diets. Both RC and RF diets appeared to significantly decrease plasma C-peptide, insulin, insulin resistance, leptin, adiponectin, total cholesterol, and HDL. Plasma HDL and total cholesterol decreased to a greater extent with the RF diet, and LDL decreased only with the RF diet. Plasma TG decreased only with the RC diet. Plasma  $\beta$ -hydroxybutyrate and ghrelin increased only with the RC diet. Plasma GIP increased with the RC diet and decreased with the RF diet. Sex-specific data are presented in Table S2.

Figure 3G illustrates the mathematical model simulations of 6 months of selective isocaloric restriction of dietary fat versus carbohydrate at the level implemented during the inpatient study. The model predicted that the RF diet would lead to approximately 3 kg more body fat loss after 6 months of perfect adherence to the isocaloric diets.

Since it might be possible that different ratios of carbohydrate and fat would lead to different results, we simulated body weight and fat mass changes after 6 months of eating a variety of 30% reduced-energy isocaloric diets varying in carbohydrate and fat, with protein fixed at baseline levels as illustrated in Figure 3H. The model predicted that weight loss increased with decreasing carbohydrate. However, body fat loss was relatively insensitive

**Table 2. Nutrient Content of the Baseline and Reduced-Carbohydrate and Reduced-Fat Diets**

	Baseline Diet	RC Diet	RF Diet
<b>Energy (kcal)</b>	2,740	1,918	1,918
<b>Energy density (kcal/g)</b>	1.27	1.36	0.79
<b>Protein (g)</b>	101	101	105
<b>Fat (g)</b>	109	108	17
<b>Carbohydrate (g)</b>	350	140	352
<b>Total fiber (g)</b>	24	16	21
<b>Sugars (g)</b>	152	37	170
<b>Saturated fat (g)</b>	39	36	4
<b>Monounsaturated fat (g)</b>	43	40	4
<b>Polyunsaturated fat (g)</b>	21	24	4
<b>Cholesterol (mg)</b>	472	522	189
<b>Sodium (mg)</b>	4,514	4,514	4,533
<b>Protein (% energy)</b>	14.5	20.9	21.1
<b>Fat (% energy)</b>	35.3	50.1	7.7
<b>Carbohydrate (% energy)</b>	50.2	29	71.2
<b>Saturated fat (% energy)</b>	13.2	17.3	1.9
<b>Monounsaturated fat (% energy)</b>	14.6	19.7	2.1
<b>Polyunsaturated fat (% energy)</b>	7	11.9	1.9
<b>Omega-3 fatty acids (g)</b>	2.2	2.9	0.6
<b>Omega-6 fatty acids (g)</b>	18.1	21.3	3.5
<b>Omega-6:Omega-3 ratio</b>	8.3	7.4	6

RC, reduced carbohydrate; RF, reduced fat.

to isocaloric substitutions of dietary fat and carbohydrate, suggesting that the body acts to minimize differences in fat loss when the diet calories and protein are held constant. In fact, the experimental RC and RF diets resulted in close to the maximum predicted differences in body fat loss. In other words, the modest differences in body fat loss achieved by the diets used in our experiment are probably greater than would be observed with other ratios of carbohydrate and fat.

Figure 3I shows the simulated average changes in fat balance and total energy expenditure during the 6-month simulations. There was a reciprocal relationship between fat balance and energy expenditure such that greater suppression of expenditure results in a lower rate of fat loss. Changes in whole-body metabolic fluxes, thermic effect of food, and body composition generated by isocaloric variations in carbohydrate and fat were responsible for the simulated differences in energy expenditure (not shown).

## DISCUSSION

This study demonstrated that, calorie for calorie, restriction of dietary fat led to greater body fat loss than restriction of dietary carbohydrate in adults with obesity. This occurred despite the fact that only the carbohydrate-restricted diet led to decreased insulin secretion and a substantial sustained increase in net fat oxidation compared to the baseline energy-balanced diet.

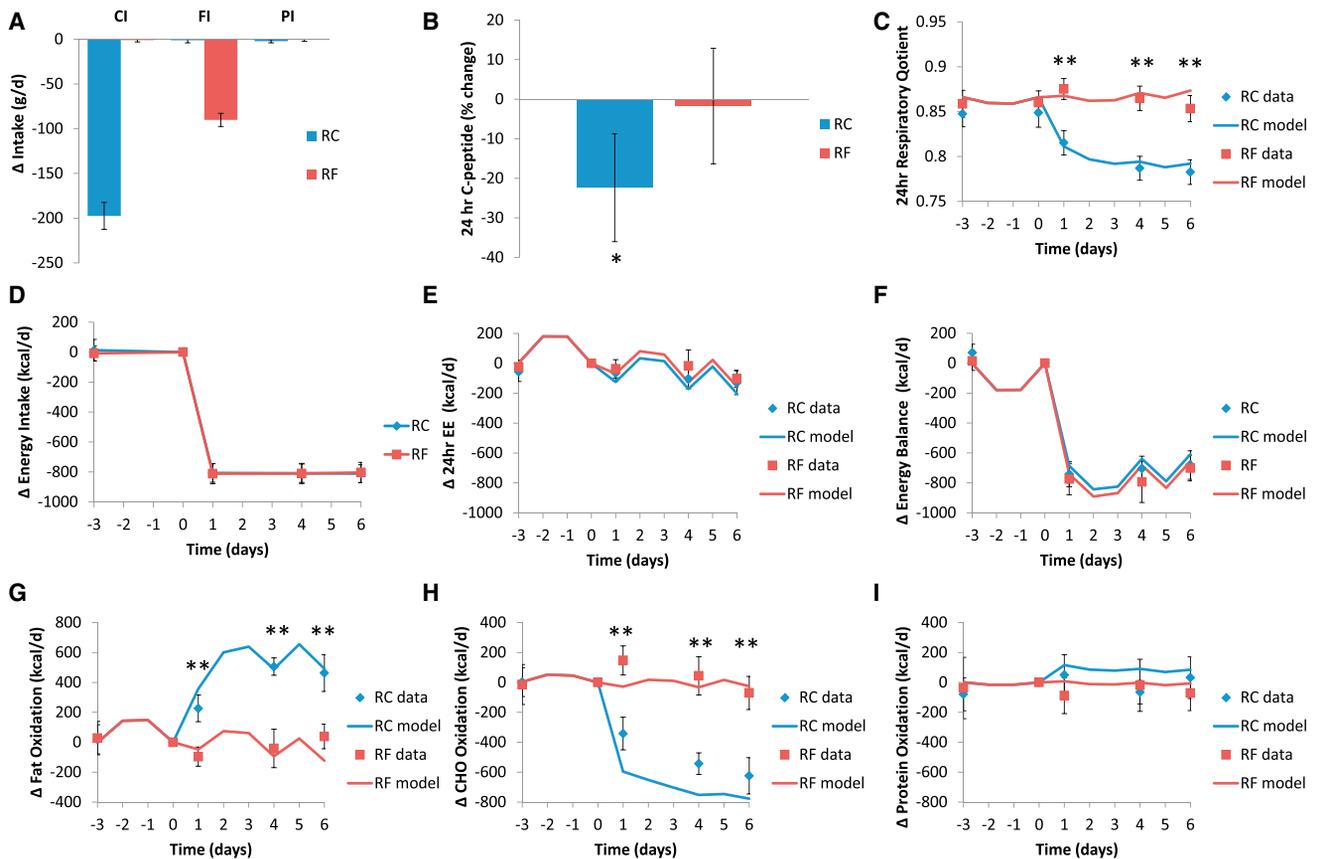
In contrast to previous claims about a metabolic advantage of carbohydrate restriction for enhancing body fat loss (Ludwig and Friedman, 2014; Taubes, 2007, 2011; Westman et al., 2007), our data and model simulations support the opposite conclusion

when comparing the RF and RC diets. Furthermore, we can definitively reject the claim that carbohydrate restriction is required for body fat loss (Taubes, 2011).

Dietary fat contributed only about 8% to the total energy content of the RF diet, making it a very low-fat diet. The RF diet did not reduce refined carbohydrates from baseline and resulted in no significant changes in 24-hr insulin secretion. In contrast, carbohydrates were about 29% of the energy content of the RC diet with a mean absolute carbohydrate intake of about 140 g/day, which induced a substantial drop in 24-hr insulin secretion. Thus, while the RC diet qualifies as a low-carbohydrate diet, it was clearly not a very low-carbohydrate diet, which typically requires carbohydrates to be less than 50 g/day (Westman et al., 2007). Given the composition of the baseline diet, it was not possible to design an isocaloric very low-carbohydrate diet without also adding fat or protein. We decided against such an approach due to the difficulty in attributing any observed effects of the diet to the reduction in carbohydrate as opposed to the addition of fat or protein.

Randomized controlled trials often involve hundreds or thousands of subjects prescribed to follow different diet regimens, with investigators providing instructions and support to participants on how to eat the prescribed diets. However, there is little evidence that people actually adhere to the diet prescriptions. Such studies actually test the effects of different diet prescriptions rather than the effects of different diets and cannot shed much light on the underlying physiology. As an alternative, controlled feeding studies can provide more useful physiological information, but diet adherence is often poor in outpatient studies even when participants are provided with all of their food (Das et al., 2007). Therefore, inpatient feeding studies are required to properly control the diets and measure physiological effects, but such studies are very expensive and labor intensive, making them typically small in size.

Previous inpatient controlled-feeding studies have employed isocaloric reduced-energy diets with fixed protein and varying in carbohydrate and fat to investigate differences in weight loss (Anderson, 1944; Bell et al., 1969; Bogardus et al., 1981; Bortz et al., 1967, 1968; Fletcher et al., 1961; Golay et al., 1996; Kekwick and Pawan, 1956; Kinsell et al., 1964; Lewis et al., 1977; Miyashita et al., 2004; Olesen and Quaade, 1960; Pilkington et al., 1960; Rabast et al., 1979, 1981; Rumpler et al., 1991; Vazquez and Adibi, 1992; Vazquez et al., 1995; Werner, 1955; Yang and Van Itallie, 1976). Only two of these previous studies investigated more subjects per diet group than the present study (Golay et al., 1996; Rabast et al., 1979). Unlike the current study, all previous studies altered multiple macronutrients from their baseline values rather than selectively restricting individual macronutrients. Nevertheless, many studies agreed with our data showing greater weight loss with reduced carbohydrate diets (Anderson, 1944; Bell et al., 1969; Bogardus et al., 1981; Bortz et al., 1967, 1968; Kekwick and Pawan, 1956; Lewis et al., 1977; Olesen and Quaade, 1960; Pilkington et al., 1960; Rabast et al., 1979, 1981; Werner, 1955; Yang and Van Itallie, 1976). However, several studies did not detect significant differences in weight loss (Fletcher et al., 1961; Golay et al., 1996; Kinsell et al., 1964; Miyashita et al., 2004; Rumpler et al., 1991; Vazquez and Adibi, 1992; Vazquez et al., 1995). Often, the greater weight losses with the low-carbohydrate diets were attributed to sodium and



**Figure 2. Changes in Daily Diet, Insulin Secretion, and Energy Metabolism**

(A) The reduced carbohydrate (RC) diet achieved 30% energy restriction via selective reduction in carbohydrate intake (CI) whereas the isocaloric reduced-fat (RF) diet resulted from selective reduction of fat intake (FI). Protein intake (PI) was unchanged from baseline on both diets.

(B) Insulin secretion throughout the day was assessed by 24-hr urinary C-peptide excretion and was significantly reduced only following the RC diet.

(C) The 24-hr respiratory quotient was practically unchanged during the RF diet but fell during the RC diet, indicating an increased reliance on fat oxidation.

(D) Energy intake was reduced equivalently during the RC and RF diets.

(E) Energy expenditure as measured in the metabolic chamber (24-hr EE) decreased with the RC diet but not the RF diet.

(F) Energy balance was similar between RF and RC diets.

(G) Net fat oxidation increased substantially during the RC diet and reached a plateau after several days, whereas the RF diet appeared to have little effect.

(H) Net carbohydrate oxidation decreased during the RC diet and was relatively unchanged during the RF diet apart from a slight initial increase on the first day.

(I) Net protein oxidation was not significantly altered by the RF or RC diets.

Mean  $\pm$  95% CI. \* indicates a significant difference from baseline at  $p = 0.001$ ; \*\* indicates a significant difference between RC and RF at  $p < 0.0001$ .

water imbalances (Anderson, 1944; Bell et al., 1969; Bortz et al., 1967, 1968; Lewis et al., 1977; Olesen and Quaade, 1960; Pilkington et al., 1960; Werner, 1955; Yang and Van Itallie, 1976). Furthermore, nitrogen balance measurements in several previous studies have suggested greater lean tissue loss with low-carbohydrate diets (Bell et al., 1969; Bortz et al., 1967, 1968; Vazquez and Adibi, 1992; Vazquez et al., 1995).

Fat loss is a more important goal than weight loss in the treatment of obesity. Five of the previous inpatient feeding studies attempted to measure differences in body fat resulting from varying carbohydrate and fat, but no significant differences were found (Bogardus et al., 1981; Golay et al., 1996; Miyashita et al., 2004; Rumpel et al., 1991; Yang and Van Itallie, 1976). Most of these studies used body composition assessment methodologies to measure body fat changes (Bogardus et al., 1981; Golay et al., 1996; Miyashita et al., 2004; Rumpel et al., 1991). But even high-precision methods, such as DXA, may lack the

sensitivity to detect small differences in body fat change (Hind et al., 2011; Lohman et al., 2009; Müller et al., 2012). Indeed, retrospective analysis of our data suggests that the minimum detectable difference between the diets for body fat mass using DXA was  $\sim 0.4$  kg. Thus, we suspect that the DXA measurements of fat mass change in the present study were insufficiently sensitive to detect differences between the diets. Furthermore, DXA may provide inaccurate results in situations of dynamic weight change and shifting body fluids (Lohman et al., 2000; Müller et al., 2012; Pourhassan et al., 2013; Valentine et al., 2008). This could be especially important with diets differing in their level of carbohydrate restriction since greater losses of body water are likely with lower levels of dietary carbohydrate.

The most sensitive method for detecting the rate of body fat change requires calculating daily fat balance as the difference between fat intake and net fat oxidation (i.e., fat oxidation minus de novo lipogenesis) measured by indirect calorimetry while

**Table 3. Body Composition and Energy Metabolism Changes following the Isocaloric Reduced-Carbohydrate and Reduced-Fat Diets**

All Subjects	Δ RC diet (n = 19)	p value	Δ RF diet (n = 17)	p value	p value (RC versus RF)
<b>BW (kg)</b>	-1.85 ± 0.15	<0.0001	-1.3 ± 0.16	<0.0001	0.022
<b>BMI (kg/m<sup>2</sup>)</b>	-0.615 ± 0.067	<0.0001	-0.387 ± 0.071	<0.0001	0.028
<b>% Body fat<sup>a</sup></b>	0.161 ± 0.15	0.3073	-0.072 ± 0.16	0.66	0.24
<b>Fat mass (kg)<sup>a</sup></b>	-0.529 ± 0.13	0.0015	-0.588 ± 0.14	0.001	0.78
<b>SMR (kcal/day)</b>	-86.2 ± 25	0.0034	4.33 ± 26	0.87	0.0024
<b>24-hr EE (kcal/day)</b>	-97.7 ± 23	0.0007	-49.6 ± 24	0.058	0.099
<b>Energy balance (kcal/day)</b>	-707 ± 35.9	<0.0001	-765 ± 36.6	<0.0001	0.052
<b>24-hr RQ</b>	-0.0552 ± 0.003	<0.0001	0.00453 ± 0.0031	0.16	<0.0001
<b>24-hr fat ox (kcal/day)</b>	403 ± 30	<0.0001	-31.2 ± 31	0.33	<0.0001
<b>24-hr CHO Ox (kcal/day)</b>	-520 ± 33	<0.0001	43.9 ± 35	0.22	<0.0001
<b>24-hr urinary N (g/day)</b>	0.754 ± 1.1	0.48	-2.43 ± 1.1	0.037	0.095
<b>Cumulative fat imbalance (g)</b>	-245 ± 21	<0.0001	-463 ± 37	<0.0001	<0.0001

RC, reduced carbohydrate; RF, reduced fat. The data were analyzed using a repeated-measures mixed model controlling for sex and order effects and are presented as least-squares mean ± SEM. The p values refer to the diet effects and were not corrected for multiple comparisons.

<sup>a</sup>One female subject had changes in DXA % body fat data that were not physiological and were clear outliers, so these data were excluded from the analyses.

residing in a metabolic chamber. At the end of the diet periods, our study had a minimum detectable difference in daily fat balance of 220 kcal/day (or 23 g/day) and the cumulative fat loss had a minimum detectable difference of 110 g. The observed differences in fat balance and cumulative body fat loss between RC and RF diets were substantially larger than these values and were statistically significant. While the fat balance method does not determine the anatomical location of lost fat, decreased adipose tissue triglyceride likely makes up the majority. Any additional loss of ectopic fat from liver or skeletal muscle would likely be even more beneficial.

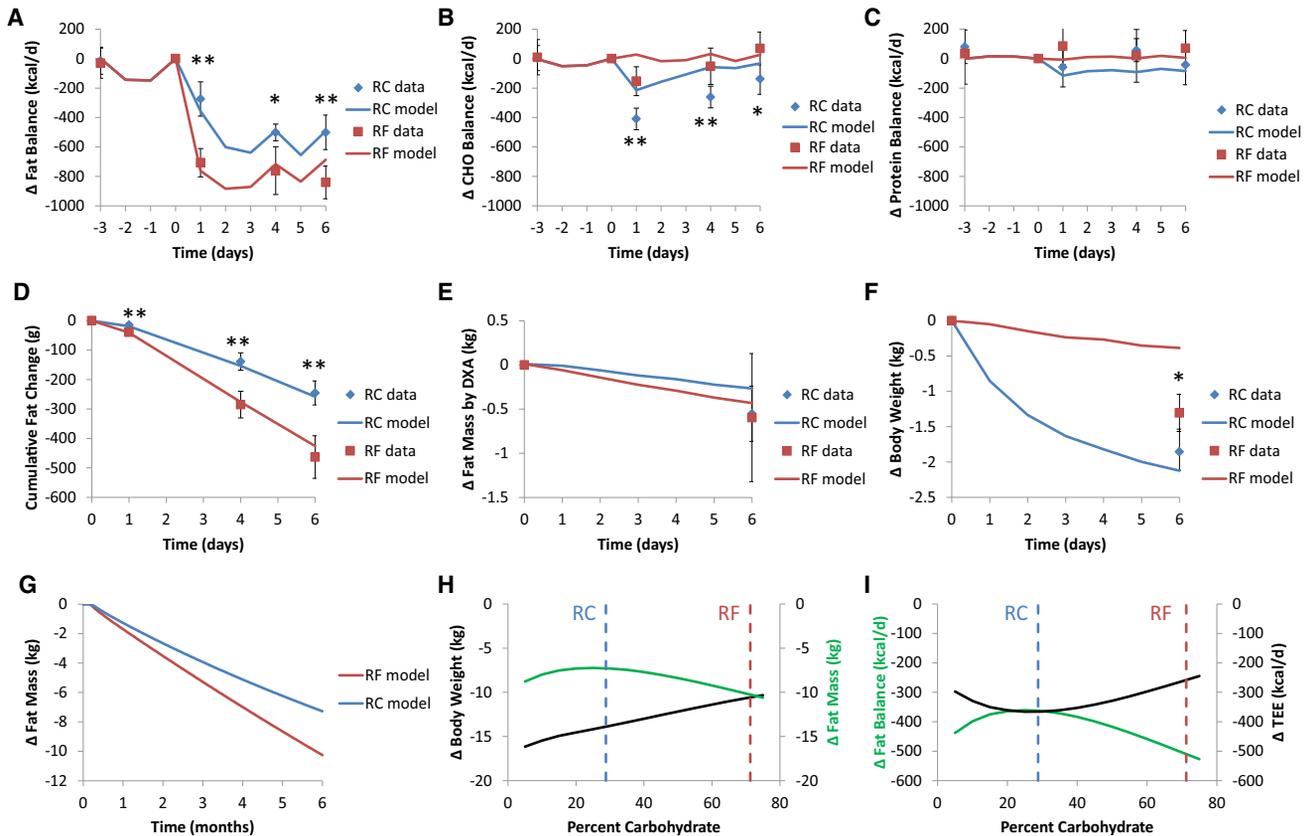
Model simulations suggest that the differences in fat loss were due to transient differences in carbohydrate balance along with persistent differences in energy and fat balance. The model also implicated small persistent changes in protein balance resulting from the fact that dietary carbohydrates preserve nitrogen balance to a greater degree than fat (Bell et al., 1969; Vazquez and Adibi, 1992; Vazquez et al., 1995). The timing and magnitude of the observed change in net fat oxidation and fat balance with the RC diet were accurately simulated by the model and indicated that the adaptation to the experimental carbohydrate restriction achieved a plateau after several days. In contrast, the RF diet led to little adaptation with a relatively constant net fat oxidation rate, thereby leading to a greater fat imbalance compared to the RC diet.

Our relatively short-term experimental study has obvious limitations in its ability to translate to fat mass changes over prolonged durations. It could be argued that perhaps the fat balance and body fat changes would converge with continuation of the diets over the subsequent weeks. However, this would require that the net fat oxidation rate somehow increase above the observed plateau with the RC diet, and/or the RF diet would have to result in a swifter decrease in fat oxidation. Neither of these possibilities was apparent in the data and did not occur in the mathematical model simulations of prolonged diet periods. If such a convergence in body fat loss were to occur with prolonged RC and RF diets, the physiological mechanism is unclear.

The mathematical model simulations suggest that the diet with selective reduction in fat would continue to outpace the reduced carbohydrate diet over 6 months. However, further reducing dietary carbohydrate from the RC diet (with a corresponding addition of fat to maintain calories) was predicted to decrease body fat to a greater extent than the experimental RC diet. Very low carbohydrate diets were predicted to result in fat losses comparable to low fat diets. Indeed, the model simulations suggest that isocaloric reduced-energy diets over a wide range of carbohydrate and fat content would lead to only small differences in body fat and energy expenditure over extended durations. In other words, while the present study demonstrated the theoretical possibility that isocaloric diets differing in carbohydrate and fat can result in differing body fat losses, the body acts to minimize such differences. The endocrine and metabolic adaptations that allow for the relative insensitivity of body fat to dietary macronutrient composition may themselves have effects on health over the long term, but this was not investigated in the present study.

Translation of our results to real-world weight-loss diets for treatment of obesity is limited since the experimental design and model simulations relied on strict control of food intake, which is unrealistic in free-living individuals. While our results suggest that the experimental reduced-fat diet was more effective at inducing body fat loss than the reduced-carbohydrate diet, diet adherence was strictly enforced. We did not address whether it would be easier to adhere to a reduced-fat or a reduced-carbohydrate diet under free-living conditions. Since diet adherence is likely the most important determinant of body fat loss, we suspect that previously observed differences in weight loss and body fat change during outpatient diet interventions (Foster et al., 2010; Gardner et al., 2007; Shai et al., 2008) were primarily due to differences in overall calorie intake rather than any metabolic advantage of a low-carbohydrate diet.

In summary, we found that selective reduction of dietary carbohydrate resulted in decreased insulin secretion, increased fat oxidation, and increased body fat loss compared to a eucaloric baseline diet. In contrast, selective isocaloric reduction of dietary



**Figure 3. Macronutrient Balance and Body Composition Changes**

(A) Daily fat balance was negative for both the RF and RC diets, indicating loss of body fat. The RF diet led to consistently greater fat imbalance compared with the RC diet.

(B) Net carbohydrate balance was more negative for the RC diet compared to the RF diet and returned toward balance at the end of the study with both diets.

(C) Protein balance tended to be lower for the RC diet compared to the RF diet.

(D) Cumulative fat balance indicated that both the RF and RC diets led to body fat loss, but the RF diet led to significantly more fat loss than the RC diet.

(E) Fat mass change as measured by DXA revealed significant changes from baseline, but did not detect a significant difference between RF and RC diets.

(F) The RC and RF diets both led to weight loss, but significantly more weight was lost following the RC diet.

(G) Mathematical model simulations of 6 months of perfect adherence to the RC and RF diets predicted slightly greater fat mass loss with the RF diet compared with the RC diet.

(H) Simulating 6 months of adherence to a 30% reduced-energy diet varying in carbohydrate and fat percentage, but with protein fixed at baseline, indicated that weight loss was linearly related to carbohydrate content, but fat mass was non-monotonic and relatively unaffected by carbohydrate content.

(I) Model simulated changes in average fat balance and total energy expenditure (TEE) were reciprocally related and non-monotonic with respect to carbohydrate content. The experimental RC and RF diets are indicated by the vertical dashed lines.

Mean  $\pm$  95% CI. \*\* indicates  $p < 0.001$  between RC and RF. \* indicates  $p = 0.004$  between RC and RF.

fat led to no significant changes in insulin secretion or fat oxidation compared to the eucaloric baseline diet, but significantly more body fat was lost than during the carbohydrate-restricted diet.

## EXPERIMENTAL PROCEDURES

### Study Protocol

The study protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NCT00846040). Nine women and ten men with body mass indices (BMI)  $> 30$  kg/m<sup>2</sup> provided informed consent and were admitted to the NIH Metabolic Clinical Research Unit (MCRU). Participants were excluded if they were not weight stable ( $> \pm 5$  kg in the past 6 months), had diabetes, were menopausal or pregnant or breastfeeding (women), had impaired physical mobility, showed evidence of diseases or were taking medications interfering with study outcomes, had allergies to food or local anesthetics, engaged in regular excessive

use of caffeinated drinks and alcohol, had eating disorders and/or psychiatric disorders, or had strict dietary concerns (vegetarian or kosher diet).

Each inpatient visit included a 5-day baseline and a 6-day calorie-restricted dietary intervention. Subjects were fed an energy-balanced diet (50% carbohydrate, 35% fat, 15% protein) for 5 days followed by random assignment to isocaloric removal of 30% of total energy, either by a 60% reduction of dietary carbohydrate (RC) or 85% reduction of dietary fat (RF) for 6 days. Diets were designed using ProNutra software (version 3.4, Viocare, Inc.).

All subjects were confined to the metabolic ward throughout the study with no access to outside food. Subjects knew that it was imperative that they eat all of the food provided and nothing else. If they were not able to eat a study food, they were instructed to notify the study dietitian immediately so that other arrangements could be considered. Dietitians and health technicians met with the subjects regularly to discuss the diet and assess compliance. Visitors were allowed to meet with study subjects in a common area under observation of the nursing and/or research staff to avoid the exchange of food or beverages. Meals were consumed in a common area or in patient rooms with open doors.

**Table 4. Overnight-Fasted Plasma Hormone and Metabolite Levels**

All Subjects	Baseline	N	Δ RC diet	p value	N	Δ RF diet	p value	N	p value (RC versus RF)
<b>Glucose (mg/dl)</b>	87.5 ± 1.2	19	-2.69 ± 1.7	0.13	19	-7.1 ± 1.7	0.0008	17	0.025
<b>Glycerol (mg/l)</b>	9.77 ± 1.3	19	1.35 ± 1.5	0.39	19	-0.328 ± 1.6	0.84	17	0.32
<b>BHB (mM)</b>	0.0682 ± 0.009	19	0.0883 ± 0.014	<0.0001	19	0.00569 ± 0.015	0.71	17	<0.0001
<b>Cholesterol (mg/dl)</b>	179 ± 5.8	18	-8.47 ± 2.8	0.01	15	-19.1 ± 2.6	<0.0001	16	0.024
<b>TG (mg/dl)</b>	101 ± 11	18	-17.5 ± 5	0.0044	15	-4.3 ± 4.8	0.39	16	0.012
<b>LDL (mg/dl)</b>	114 ± 4.2	18	-1.77 ± 2.6	0.52	15	-11.4 ± 2.5	0.0006	16	0.032
<b>HDL (mg/dl)</b>	44.8 ± 2.4	18	-2.67 ± 0.66	0.0013	16	-7.27 ± 0.62	<0.0001	16	<0.0001
<b>Leptin (ng/ml)</b>	21.5 ± 2.7	19	-3.89 ± 0.81	0.0002	19	-2.89 ± 0.86	0.0039	17	0.39
<b>Ghrelin (pg/ml)</b>	23.7 ± 1.6	19	7.18 ± 2.9	0.026	18	-3.58 ± 3.2	0.28	15	0.022
<b>MCP-1 (pg/ml)</b>	150 ± 11	19	1.96 ± 4.9	0.69	19	2.41 ± 5.1	0.64	17	0.95
<b>GIP (pg/ml)</b>	30.9 ± 3.7	19	4.42 ± 3.2	0.18	19	-4.94 ± 3.3	0.16	17	0.021
<b>GLP-1 (pg/ml)</b>	37.8 ± 4.4	19	0.543 ± 0.75	0.48	19	0.628 ± 0.79	0.44	17	0.95
<b>C-peptide (ng/ml)</b>	1.42 ± 0.13	19	-0.133 ± 0.045	0.009	19	-0.179 ± 0.047	0.0017	17	0.52
<b>PYY (pg/ml)</b>	125 ± 15	17	4.44 ± 2.8	0.14	17	-1.38 ± 3.1	0.66	15	0.24
<b>Insulin (μU/ml)</b>	12.6 ± 2	19	-2.76 ± 0.77	0.0024	18	-2.04 ± 0.8	0.021	17	0.48
<b>PP (ng/ml)</b>	54.6 ± 24	19	-1.02 ± 5.6	0.86	18	0.511 ± 6	0.93	16	0.88
<b>Adiponectin (mg/dl)</b>	0.978 ± 0.13	17	-0.118 ± 0.035	0.0037	17	-0.126 ± 0.035	0.0024	17	0.73
<b>Resistin (ng/ml)</b>	56.2 ± 13	17	8.99 ± 3.7	0.028	17	4.2 ± 3.8	0.28	17	0.26
<b>PAI-1 (ng/ml)</b>	36 ± 6.6	17	-3.47 ± 3.3	0.31	17	-7.12 ± 3.3	0.048	17	0.25
<b>Cortisol (pg/ml)</b>	4,490 ± 690	19	703 ± 540	0.21	19	-494 ± 570	0.4	17	0.074
<b>CRP (mg/l)</b>	1.18 ± 0.2	17	-0.018 ± 0.11	0.87	16	-0.0887 ± 0.12	0.46	14	0.55
<b>HOMA-IR</b>	2.72 ± 0.43	19	-0.489 ± 0.15	0.0054	18	-0.541 ± 0.15	0.0028	17	0.8

Overnight-fasted plasma hormone and metabolite levels during the last 3 days of the baseline diet and their changes over the last 3 days of the isocaloric reduced-carbohydrate (RC) and reduced-fat (RF) diets. The data were analyzed using a repeated-measures mixed model controlling for sex and order effects and are presented as least-squares mean ± SEM. The p values refer to the diet effects and were not corrected for multiple comparisons.

All meal trays were checked after consumption and any food that was not consumed at a given meal was weighed back by the dietitians and subsequent meals were modified to adjust for previously uneaten food, if necessary.

Every day, subjects completed 60 min of treadmill walking at a fixed self-selected pace and incline determined during the screening visit. Physical activity was quantified with activity monitors using high sampling frequencies (32 samples per second in the chamber, minute-to-minute sampling other times) during all waking periods using small, portable pager-type accelerometers (Mini-Mitter, A Respironics Company) worn on the hip.

Volunteers were readmitted after a 2- to 4-week washout period to repeat the 5-day balanced diet followed by the alternate 6-day RF or RC diet. Both in-patient visits were carried out during the follicular phase of the menstruation cycle in the female subjects. One male subject erroneously received the RF diet on the first day of the RC study period and one female subject erroneously received the RF diet on the final day of the RC study period. These data were retained in the analyses and their removal did not affect the statistical significance of any comparisons. Two male subjects dropped out of the study after completing the first inpatient stay on the RC diet and therefore did not contribute data to the RF diet phase.

#### Respiratory Quotient, Energy Expenditure, and Net Macronutrient Oxidation Rates

The primary aim of the study was to measure differences in the 24-hr respiratory quotient during the RC and RF diets as measured in a metabolic chamber for at least 23 continuous hours on days 2 and 5 of the baseline diet and days 1, 4, and 6 of the reduced-energy diets. We extrapolated the chamber measurements to represent 24-hr periods by assuming that the mean of the measured periods was representative of the 24-hr period. The mechanistic mathematical model described below was used to design and power the study as well as predict this primary outcome.

Energy expenditure (EE) and net macronutrient oxidation rates were calculated using the coefficients derived by Livesey and Elia (Livesey and Elia, 1988)

such that the indirect calorimetry equations for net fat, carbohydrate, and protein oxidation were:

$$\begin{aligned}\text{FatOx}_{\text{net}}(\text{g}) &= 1.63 \times \text{VO}_2(\text{L}) - 1.64 \times \text{VCO}_2(\text{L}) - 1.84 \times \text{N}(\text{g}) \\ \text{CarbOx}_{\text{net}}(\text{g}) &= -3.10 \times \text{VO}_2(\text{L}) + 4.46 \times \text{VCO}_2(\text{L}) - 3.68 \times \text{N}(\text{g}) \\ \text{ProtOx}_{\text{net}}(\text{g}) &= 6.25 \times \text{N}(\text{g})\end{aligned}$$

where  $\text{VO}_2$  and  $\text{VCO}_2$  were the volumes of oxygen consumed and carbon dioxide produced, respectively, and N was the 24-hr urinary nitrogen excretion measured by chemiluminescence (Antek MultiTek Analyzer, PAC).

The net macronutrient oxidation rates determined by the indirect calorimetry equations above include the influence of gluconeogenesis (GNG) from amino acids and de novo lipogenesis (DNL) from carbohydrates (Frayn, 1983). In other words, the net fat oxidation rate determined by the equation above is actually the difference between fat oxidation and DNL. Similarly, the net carbohydrate oxidation rate is the sum of carbohydrate oxidation and DNL minus GNG, and the net protein oxidation rate is the sum of protein oxidation and GNG. Therefore, the macronutrient balances calculated by subtracting the net oxidation rates from macronutrient intake rates are given by:

$$\begin{aligned}\text{FatBal} &= \text{FI} - \text{FatOx}_{\text{net}} = \text{FI} - \text{FatOx} + \text{DNL} \\ \text{CarbBal} &= \text{CI} - \text{CarbOx}_{\text{net}} = \text{CI} - \text{CarbOx} + \text{GNG} - \text{DNL} \\ \text{ProtBal} &= \text{PI} - \text{ProtOx}_{\text{net}} = \text{PI} - \text{ProtOx} - \text{GNG}\end{aligned}$$

where FI, CI, and PI are the metabolizable fat, carbohydrate, and protein intake rates, respectively. Summing the above equations gives the energy balance equation:

$$\begin{aligned}\text{EnergyBal} &= \text{FI} + \text{CI} + \text{PI} - (\text{FatOx}_{\text{net}} + \text{CarbOx}_{\text{net}} + \text{ProtOx}_{\text{net}}) \\ \text{EnergyBal} &= \text{EI} - \text{EE}\end{aligned}$$

Therefore, energy expenditure (EE) was determined by summing the net macronutrient oxidation rates and, using the indirect calorimetry equations

above along with the energy densities of fat, carbohydrate and protein, results in the following equation for EE:

$$EE(\text{kcal}) = 3.88 \times VO_2(L) + 1.08 \times VCO_2(L) - 1.52 \times N(g)$$

One male subject was not compliant during the 24-hr urine collection procedure, so we assumed nitrogen balance for this subject when calculating macronutrient oxidation rates and energy expenditure. Sleeping metabolic rate (SMR) was determined during chamber periods of zero physical activity between 2 a.m. and 5 a.m.

On the morning of the first day of both in-patient periods, subjects drank from a stock solution of 1.5 g per kg body weight of 10%  $^{18}\text{O}$ -enriched  $\text{H}_2\text{O}$  and 0.08 g of 99%-enriched  $^2\text{H}_2\text{O}$  per kg of body weight followed by 100–200 ml tap water to rinse the dose container. Spot urine samples were collected daily. Isotopic enrichments of urine samples were measured by dual inlet chromium reduction and continuous-flow  $\text{CO}_2$  equilibration isotope ratio mass spectrometry. The  $\text{CO}_2$  production rate was estimated from the differential disappearance of the two isotopes ( $k_O$  and  $k_D$ ) over the baseline period according to the equation by Speakman (Speakman, 1997):

$$r\text{CO}_2 = N(0.48123 \times k_O - 0.48743 \times k_D)$$

where  $N = (N_O + N_D / R_{\text{dilspace}}) / 2$  and  $R_{\text{dilspace}}$  was calculated as the mean of the  $N_D / N_O$  values.

The average total energy expenditure (TEE) during each baseline period was calculated as:

$$TEE(\text{kcal/d}) = [3.85 / \text{RQ} + 1.08] \times r\text{CO}_2(\text{L/d})$$

where the respiratory quotient, RQ, was calculated as the average RQ measured during the metabolic chamber days.

### Anthropometry and Body Composition

Body weight (Scale-Tronix 5702) and height (Seca 242) were measured to the nearest 0.1 kg and 0.1 cm, respectively, with subjects wearing light clothes and following an overnight fast. Since body composition assessment methods are insufficiently sensitive to measure short-term body fat change during active energy imbalance (Lohman et al., 2000; Müller et al., 2012; Pourhassan et al., 2013; Valentine et al., 2008), body fat change was determined using cumulative net fat balance as determined by indirect calorimetry. Nevertheless, body fat percentage was also measured using dual-energy X-ray absorptiometry scanner (Lunar iDXA, GE Healthcare). One female subject had fat mass changes measured via DXA that were not physiological and were clear outliers. These data were excluded from the analyses.

### Analytical Measurements

Blood was drawn into EDTA-coated tubes containing DPPIV (EMD-Millipore) and protease inhibitors (S-cocktail, Sigma-Aldrich). Samples were processed immediately after blood collection and stored at  $-80^\circ\text{C}$  for the subsequent measurement of biomarkers.

Plasma ghrelin (active), GLP-1 (active), pancreatic polypeptide (PP), PYY, leptin, MCP-1, C-peptide, insulin, and GIP were measured using the Milliplex magnetic bead human metabolic hormone multiplex panel (HMHMAG-34K; EMD-Millipore) and plasma adiponectin, resistin, and PAI-1 were measured using the Milliplex magnetic bead human serum adipokine multiplex panel A (HADK1-61K-A; EMD-Millipore). Both assays are based on the Luminex xMAP technology. The intra- and inter-assay CV were 5.8% and 4.9% for ghrelin (active), 6.8% and 3.4% for GLP-1 (active), 4.9% and 4.6% for PP, 4.4% and 3.8% for PYY, 7.3% and 5.9% for leptin, 4.0% and 3.5% for MCP-1, 3.0% and 4.6% for c-peptide, 4.4% and 5.2% for insulin, and 3.2% and 5.0% for GIP, respectively.

Beta hydroxybutyrate (BHB), glucose, and glycerol were measured using colorimetric kits from Cayman Chemical Co. The intra- and inter-assay CV were 5.0% and 3.6% for BHB, 3.6% and 3.9% for glucose and 4.0%, and 2.1% for glycerol respectively. Cortisol was measured using an ELISA from Cayman Chemical Company. The intra- and inter-assay CV were 3.6% and 3.9%, respectively.

Twenty-four-hour urinary C-peptide excretion was measured during chamber days using an ELISA from Mercodia. The intra- and inter-assay CV were 5.1% and 6.7%, respectively.

### Mathematical Modeling

A detailed description of the mathematical model is presented in the [Supplemental Information](#). The model quantitatively tracks the metabolism of all three dietary macronutrients and simulates how diet changes result in adaptations of whole-body energy expenditure, metabolic fuel selection, and alterations in the major whole-body fluxes contributing to macronutrient balance (Hall, 2010). Other than the initial conditions for body composition and energy expenditure and the physical activity differences between chamber and non-chamber days, no model parameters were adjusted to fit the data from this study. Model simulations were used to design the study and the successful predictions of the observed 24-hr RQ changes were included in the clinical protocol (NCT00846040).

### Statistical Analyses

Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc.). The baseline data are presented as mean  $\pm$  SEM and were analyzed by analysis of variance (PROC GLM, SAS). The data tables present least-squares mean  $\pm$  SEM and were analyzed using a repeated-measures mixed model with a covariance structure of compound symmetry (PROC MIXED, SAS). We controlled for sex and order effects by including these parameters in the statistical model. The figures depict mean  $\pm$  95% CI at each time point and two-sided t tests were used to compare the diet groups. Outliers were identified by Cook's distance with a cutoff of  $4/n$ , where  $n$  is the number of observations. Significance was declared at  $p < 0.05$ . Retrospective calculations of minimal detectable effect sizes were performed using the measured variances with a type I error probability of 0.05 and 80% power for pairwise comparison of 17 subjects (PROC POWER, SAS).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Model Description and five tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cmet.2015.07.021>.

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**Cell Metabolism, Volume 22**

**Supplemental Information**

**Calorie for Calorie, Dietary Fat Restriction  
Results in More Body Fat Loss than Carbohydrate  
Restriction in People with Obesity**

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## Supplementary Model Description Related to Experimental Procedures.

### GLOSSARY OF MODEL VARIABLES

<i>BM</i>	Bone mineral mass in g
<i>BW</i>	Body weight in g
<i>CarbOx</i>	Rate of carbohydrate oxidation in kcal/d
<i>CI</i>	Digestible carbohydrate intake rate in kcal/d
<i>D<sub>F</sub></i>	Rate of endogenous lipolysis in g/d
<i>D<sub>G</sub></i>	Rate of glycogenolysis in g/d
<i>DNL</i>	Rate of de novo lipogenesis in kcal/d
<i>D<sub>P</sub></i>	Rate of proteolysis in g/d
<i>ECF</i>	Extracellular fluid mass in g
<i>ECP</i>	Extracellular protein mass in g
<i>EI</i>	Digestible energy intake in kcal/d
<i>F</i>	Body fat mass in g
<i>FA</i>	Fatty acid contribution to body fat mass in g
<i>FatOx</i>	Rate of fat oxidation in kcal/d
<i>f<sub>C</sub></i>	Carbohydrate oxidation fraction
<i>f<sub>F</sub></i>	Fat oxidation fraction
<i>FFM</i>	Fat-free body mass in g
<i>FI</i>	Digestible fat intake rate in kcal/d
<i>f<sub>P</sub></i>	Protein oxidation fraction
<i>g</i>	Body glycerol in g
<i>G</i>	Body glycogen mass in g
<i>G3P</i>	Rate of glycerol 3-phosphate synthesis in kcal/d
<i>GNG<sub>F</sub></i>	Rate of gluconeogenesis from glycerol in kcal/d
<i>GNG<sub>P</sub></i>	Rate of gluconeogenesis from protein in kcal/d
<i>ICS</i>	Intracellular solid mass in g
<i>ICW</i>	Intracellular water mass in g
<i>KetOx</i>	Rate of ketone oxidation in kcal/d
<i>KTG</i>	Rate of ketogenesis in kcal/d
<i>KU<sub>excr</sub></i>	Rate of ketone excretion in kcal/d
<i>LCM</i>	Lean tissue cell mass in g
<i>M<sub>g</sub></i>	Molar mass of glycerol in g
<i>M<sub>G</sub></i>	Molar mass of glucose in g
<i>M<sub>F</sub></i>	Molar mass of fat in g
<i>M<sub>FA</sub></i>	Molar mass of fatty acids in g
<i>N<sub>excr</sub></i>	Nitrogen excretion rate in g/d
<i>NPRQ</i>	Non-protein respiratory quotient
<i>P</i>	Intracellular protein mass in g
<i>PAE</i>	Physical activity energy expenditure in kcal/d
<i>PI</i>	Digestible protein intake rate in kcal/d
<i>ProtOx</i>	Rate of protein oxidation in kcal/d
<i>RMR</i>	Resting metabolic rate in kcal/d
<i>RQ</i>	Respiratory quotient

$Synth_F$	Rate of fat synthesis in g/d
$Synth_G$	Rate of glycogen synthesis in g/d
$Synth_P$	Rate of protein synthesis in g/d
$T$	Adaptive thermogenesis
$TEE$	Total energy expenditure in kcal/d
$TEF$	Thermic effect of feeding in kcal/d
$VCO_2$	Rate of carbon dioxide production in L/d
$VO_2$	Rate of oxygen consumption in L/d

## DETAILED MATHEMATICAL MODEL DESCRIPTION

The individual components of the mathematical model were based on a variety of published, *in vivo* human data as described below. Each model component was relatively simple and only the most important physiological effectors have been incorporated. Since continued development of the model is part of an ongoing research program, additional relevant physiological data will be incorporated within the existing computational framework to improve the realism and predictive capabilities of the model.

### Macronutrient Balance

The concept of macronutrient balance is an expression of energy conservation such that changes of the body's energy stores were given by the sum of fluxes entering the pools minus the fluxes exiting the pools. Thus, the mathematical representation of macronutrient balance was given by the following differential equations:

$$\begin{aligned}
 \rho_C \frac{dG}{dt} &= CI - DNL + GNG_P + GNG_F - G3P - CarbOx \\
 \rho_P \frac{dP}{dt} &= PI - GNG_P - ProtOx \\
 \left[ \rho_F - \rho_C \left( \frac{M_g}{M_F} \right) \right] \frac{dF}{dt} &= \rho_{FA} \frac{dFA}{dt} \\
 &= \left( 1 - \frac{\rho_C M_g}{\rho_F M_F} \right) FI + \varepsilon_d DNL \\
 &\quad - KU_{excr} - (1 - \varepsilon_k) KTG - KetOx - FatOx
 \end{aligned} \tag{1}$$

The macronutrient intake rates,  $CI$  and  $FI$ , refer to the digestible energy intake of carbohydrate and fat, respectively, whereas  $PI$  refers to the digestible energy intake of protein corrected for the obligatory formation of ammonia with protein metabolism. The energy cost of ureagenesis is accounted for separately as described below.

The energy densities of carbohydrate, protein, and fat were  $\rho_C = 3.7$  kcal/g,  $\rho_P = 4.7$  kcal/g, and  $\rho_F = 9.4$  kcal/g, respectively (Livesey and Elia, 1988). The fat balance equation accounts for both the glycerol,  $g$ , and fatty acid,  $FA$ , components of body fat,  $F$ . Because  $\rho_F dF/dt = \rho_{FA} dFA/dt + \rho_C dg/dt$  and  $dg/dt = (M_g/M_F) dF/dt$ , we can

express the rate of change in body fat as a proportion of the rate of change in fatty acids. Accordingly, the first term on the right side of the fat balance equation represents the fatty acid portion of  $FI$ . The energy density of glycerol was assumed to be equal to  $\rho_C$  and the fatty acid energy density was  $\rho_{FA} = (\rho_F \times M_F - \rho_C \times M_g) / (3 \times M_{FA}) = 9.42$  kcal/g, where  $M_g = 92$  g/mol,  $M_F = 860$  g/mol and  $M_{FA} = 274$  g/mol were the molecular masses of glycerol, triacylglyceride and fatty acids, respectively.

The efficiency of *de novo* lipogenesis,  $DNL$ , was represented as the dimensionless parameter  $\varepsilon_d = 0.95$  which is the enthalpy of combustion of 0.37 g of fat divided by the enthalpy of combustion of the 1 g of glucose used to produce the fat (Elia and Livesey, 1988). The efficiency of ketogenesis,  $KTG$ , was represented by the parameter  $\varepsilon_k = 0.77$  calculated as the enthalpy of combustion of 4.5 moles of acetoacetate divided by the enthalpy of combustion of 1 mole of stearic acid used to produce the ketones (Blaxter, 1989) which are subsequently oxidized,  $KetOx$ , to provide energy. The value  $(1 - \varepsilon_k)KTG$  is equivalent to the beta oxidation heat production during the calculated rate of ketogenesis. When the ketogenic rate increases, ketones are excreted in the urine at the rate  $KU_{excr}$ . The oxidation rates  $CarbOx$ ,  $FatOx$ ,  $KetOx$ , and  $ProtOx$ , summed to the total energy expenditure,  $TEE$ , less the small amount heat produced via flux through ketogenic and lipogenic pathways.

#### *Body Composition*

The body weight,  $BW$ , was the sum of the fat-free mass,  $FFM$ , and the body fat mass,  $FM$ .  $FFM$  was computed using the following equation:

$$\begin{aligned} FFM &= BM + ECF + ECP + LCM \\ &= BM + ECF + ECP + ICW + P + G + ICS \\ &= BM + ECF + ECP + \hat{ICW} + P(1 + h_p) + G(1 + h_G) + ICS \end{aligned} \quad [2]$$

where the fat-free mass is composed of bone mineral,  $BM$ , extracellular fluid,  $ECF$ , extracellular protein,  $ECP$ , and the lean tissue cell mass,  $LCM$ .  $LCM$  is composed of intracellular water,  $ICW$ , glycogen,  $G$ , and protein,  $P$ , as well as a small contribution from nucleic acids and other intracellular solids,  $ICS$ . The protein fraction of the lean tissue cell mass was  $P/LCM = 0.25$  and that the initial intracellular water fraction was  $ICW/LCM = 0.7$  (Brozek et al., 1963; Wang et al., 2004).  $ICW$  was then calculated dynamically from  $P$  and  $G$  such that each gram of protein and glycogen was associated with  $h_p$  and  $h_G$  grams of water, respectively.  $\hat{ICW}$  was a constant amount of intracellular water computed to attain the appropriate initial intracellular composition assuming that  $G = 500$ g,  $h_G = 2.7$ , and  $h_p = 1.6$  (Brozek et al., 1963; Hall, 2008; McBride et al., 1941).

The initial  $ECF$  was calculated via the regression equations of Silva et al. (Silva et al., 2007). It is well-known that both dietary sodium and carbohydrate rapidly affect body fluid balance and the corresponding changes of  $ECF$  were calculated as follows:

$$\frac{dECF_{fast}}{dt} = \frac{1}{[Na]} \left( \Delta Na_{diet} - \xi_{Na} (ECF - ECF_{init}) - \xi_{CI} (1 - CI/CI_b) \right)$$

$$\tau_{BW} \frac{dECF_{slow}}{dt} = \xi_{BW} (BW - BW_{init}) - ECF_{slow} \quad [3]$$

$$ECF = ECF_{init} + ECF_{fast} + ECF_{slow}$$

where  $[Na] = 3.22$  g/L is the extracellular sodium concentration,  $\Delta Na_{diet}$  is the change of dietary sodium in mg/d, and  $ECF_{slow}$  is the slow increment in  $ECF$  that occurs with body weight change on a very long time scale of  $\tau_{BW} \sim 200$  days. The value of  $\xi_{BW} = 0.17$  L/kg was chosen so that the steady state increment of  $ECF$  with weight change matched the regression equations of Silva et al. (Silva et al., 2007). The value of  $\xi_{Na}$  was chosen according to the data of Andersen et al. (Andersen et al., 2002) who measured a change of sodium excretion of 5 g/d following an infusion of 1.7 L of isotonic saline. Therefore, I assumed that a 5 g/d change in sodium intake would be balanced by a 1.7 L change of  $ECF$  giving  $\xi_{Na} = 3$  g/L/d. This value is also in line with the  $\sim 1$  kg of body weight increase observed by Heer et al. when increasing dietary sodium from a very low 1.1 g/d baseline diet by  $\Delta Na_{diet} = 3.5$  g/d to approach a typical sodium intake (Heer et al., 2009). Interestingly, Heer et al. observed that further increases in dietary sodium to very high levels ( $\sim 12$  g/d) do not necessarily lead to further increases  $ECF$ . This saturation effect is not included in the model equations 3 and therefore does not apply to very high sodium intake.

Dietary carbohydrate influences sodium homeostasis and  $ECF$ , likely through insulin's effect on renal sodium metabolism (DeFronzo, 1981). Stinebaugh and Schloeder observed that removing dietary carbohydrate results in a doubling of the cumulative sodium excretion when compared to a very low sodium diet alone (Stinebaugh and Schloeder, 1966). Therefore, assuming that the low sodium diet resulted in  $\Delta Na_{diet} = -4$  g/d, then  $\xi_{CI} = 4$  g/d corresponding to complete fasting and removal of all dietary carbohydrate.

I assumed that  $BM$  was 4% of the initial body weight (Wang et al., 2003) and the  $ECP$  was assumed to be a constant determined by the initial  $BM$  and  $ECF$  values as determined by the regression equations of Wang et al. (Wang et al., 2003).

#### *Whole-body Total Energy Expenditure*

Total energy expenditure,  $TEE$ , was modeled by the following equation:

$$TEE = TEF + PAE + RMR \quad [4]$$

where  $TEF$  was the thermic effect of feeding,  $PAE$  was the energy expended for physical activity and exercise, and  $RMR$  was the remainder of the whole-body energy expenditure defined as the resting metabolic rate. Explicit equations for each component of energy expenditure follow.

### *Thermic Effect of Feeding*

Feeding induces a rise of metabolic rate associated with the digestion, absorption, and short-term storage of macronutrients and was modeled by the following equation:

$$TEF = \alpha_F FI + \alpha_P PI + \alpha_C CI \quad [5]$$

where  $\alpha_F = 0.025$ ,  $\alpha_P = 0.25$ , and  $\alpha_C = 0.075$  defined the short-term thermic effect of fat, protein, and carbohydrate feeding (Blaxter, 1989).

### *Adaptive Thermogenesis*

Energy imbalance causes an adaptation of metabolic rate that opposes weight change (Doucet et al., 2003; Doucet et al., 2001; Leibel et al., 1995). Whether or not the adaptation of energy expenditure is greater than expected based on body composition changes alone has been a matter of some debate (Flatt, 2007; Major et al., 2007; Weinsier et al., 2000). The so-called adaptive thermogenesis is believed to affect both resting and non-resting energy expenditure and has maximum amplitude during the dynamic phase of weight change. Adaptive thermogenesis also persists during weight maintenance at an altered body weight (Rosenbaum et al., 2008). The non-RMR component of adaptive thermogenesis may reflect either altered efficiency or amount of muscular work (Levine, 2004; Levine et al., 1999; Levine et al., 2005; Rosenbaum et al., 2003).

The onset of adaptive thermogenesis is rapid and may correspond to altered levels of circulating thyroid hormones or catecholamines (Rosenbaum et al., 2000; Weinsier et al., 2000). I defined a dimensionless adaptive thermogenesis parameter,  $T$ , which was generated by a first order process in proportion to the departure from the baseline energy intake  $EI_b = CI_b + FI_b + PI_b$ :

$$\tau_T \frac{dT}{dt} = \begin{cases} \lambda_1 (\Delta EI / EI_b) - T, & \text{if } EI < EI_b \\ \lambda_2 (\Delta EI / EI_b) - T, & \text{else} \end{cases} \quad [6]$$

where  $\Delta EI$  was the change from the baseline energy intake,  $\tau_T = 7$  days was the estimated time constant for the onset of adaptive thermogenesis, and the parameters  $\lambda_1$  and  $\lambda_2$  quantified the effect of underfeeding and overfeeding, respectively, and were determined from the best fit to the Minnesota experiment data. The adaptive thermogenesis parameter,  $T$ , acted on both the RMR and PAE components of energy expenditure as defined below. This simple model assumed that adaptive thermogenesis reacted to perturbations of  $EI$  and persisted as long as  $EI$  was different from baseline. Importantly, the model allowed for the possibility that no adaptive thermogenic mechanism was required to fit the data from the Minnesota experiment. The amount that the best fit values for the  $\lambda_1$  and  $\lambda_2$  parameters differ from zero provides an indication of the extent of adaptive thermogenesis that occurred during the underfeeding and overfeeding phases the Minnesota experiment, respectively.

### Physical Activity Expenditure

The energy expended for typical physical activities is proportional to the body weight of the individual (Blaxter, 1989; van der Walt and Wyndham, 1973). Low intensity physical activities may be subject to the effects of adaptive thermogenesis, whereas higher intensity exercise appears to not be affected (Rosenbaum et al., 2003). Therefore, the following equation was used for the physical activity expenditure:

$$PAE = \delta(1 + \sigma T)BW + \upsilon BW \quad [7]$$

where  $\delta$  was the non-exercise physical activity coefficient (in kcal/kg/d),  $\upsilon$  was the exercise coefficient (in kcal/kg/d), and  $BW = L + F$  was the body weight. The proportion of adaptive thermogenesis,  $T$ , that was allocated to the modification of non-exercise physical activity energy expenditure was determined by the parameter  $\sigma$ . The adaptation of  $PAE$  with  $T$  did not distinguish between altered efficiency versus amount of muscular work.

### Resting Metabolic Rate

$RMR$  includes the energy required to maintain irreversible metabolic fluxes such as *de novo* lipogenesis and gluconeogenesis, as well as the turnover costs for protein, fat, and glycogen. The following equation included these components:

$$\begin{aligned} RMR = & E_c + \gamma_B M_B + \gamma_{FFM} \left[ FFM - M_B - \Delta G(1 + h_g) - (ECF - ECF_{init}) \right] + \gamma_F F \\ & + (1 - \varepsilon_d) DNL + (1 - \varepsilon_g)(GNG_F + GNG_P) + \pi_K (1 - \varepsilon_K) KTG \\ & + \eta_N N_{excr} + (\eta_P + \varepsilon_P) D_P + \eta_P \frac{dP}{dt} + \eta_F D_F + \eta_F \frac{dF}{dt} + \eta_G D_G + \eta_G \frac{dG}{dt} \end{aligned} \quad [8]$$

where  $\varepsilon_g = 0.8$  was the efficiency of gluconeogenesis (Blaxter, 1989). The parameter  $\pi_K = 0.9$  represents the proportion of the observed increase in hepatic oxygen consumption as a result of the change in beta-oxidation associated with varying rates of ketogenesis (Scholz et al., 1984). In other words, hepatic energy expenditure increases during ketogenesis by an amount that is proportional to the ketogenic rate and this helps ameliorate previously proposed limitations on ketogenic rate resulting from the necessity of the liver to dispose of reducing equivalents generated during beta oxidation of fatty acids (Flatt, 1972). The constant  $E_c$  was a parameter chosen to ensure that the model achieved energy balance during the balanced baseline diet (see the section on Nutrient Balance Parameter Constraints below). The rate of nitrogen excretion was calculated as  $N_{excr} = (\text{ProtOx} + GNG_P) / 6.25 \rho_P$ , where the factor 6.25 was the number of grams of protein per gram of nitrogen.

The specific metabolic rate of adipose tissue was  $\gamma_F = 4.5$  kcal/kg/d. The brain metabolic rate was  $\gamma_B = 240$  kcal/kg/d and its mass was  $M_B = 1.4$  kg which does not change with weight gain or loss (Elia, 1992). The baseline specific metabolic rate of the fat-free mass,  $\hat{\gamma}_{FFM} = 19$  kcal/kg/d, was determined by the specific metabolic rates of the organs

multiplied by the rate of change of the organ mass with fat-free mass change according to the following equation:

$$\hat{\gamma}_{FFM} = \sum_i \gamma_i \frac{dM_i}{dFFM} \quad [9]$$

where  $\gamma_i$  and  $M_i$  are the average specific metabolic rate and mass of the organ indexed by  $i$ , respectively. The organs included skeletal muscle ( $\gamma_{SM} = 13$  kcal/kg/d,  $M_{SM} = 28$  kg,  $dM_{SM}/dFFM = 0.59$ ), liver ( $\gamma_L = 200$  kcal/kg/d,  $M_L = 1.8$  kg,  $dM_L/dFFM = 0.017$ ), kidney ( $\gamma_K = 440$  kcal/kg/d,  $M_K = 0.31$  kg,  $dM_K/dFFM = 0.0038$ ), heart ( $\gamma_H = 440$  kcal/kg/d,  $M_H = 0.33$  kg,  $dM_H/dFFM = 0.0029$ ), and residual lean tissue mass ( $\gamma_R = 12$  kcal/kg/d,  $M_R = 23.2$  kg,  $dM_R/dFFM = 0.37$ ) as provided by Elia (Elia, 1992) and the relationship between the organ masses and FFM was determined from cross-sectional body composition data (Gallagher, 2008). It remains to be determined whether longitudinal organ mass changes follow the cross-sectional relationship with FFM changes.

I assumed that there was no effect on resting metabolic rate arising solely from changes of glycogen content,  $\Delta G$ , and its associated water or changes of ECF. Adaptive thermogenesis affected the baseline specific metabolic rate for lean tissue cell mass according to the following equation:

$$\gamma_{FFM} = \hat{\gamma}_{FFM} [1 + (1 - \sigma)T] \quad [10]$$

The last seven terms of equation 8 accounted for the energy cost for urea synthesis and nitrogen excretion as well as the turnover of protein, fat, and glycogen. Urea synthesis requires 4 moles of ATP per mole of nitrogen excreted (Pattabiraman, 1995) and was represented by the parameter  $\eta_N = 5.4$  kcal per gram of excreted nitrogen,  $N_{excr}$ . To calculate the energy cost for protein turnover, consider that the whole-body protein pool turns over with a synthesis rate  $Synth_P$  and a degradation rate  $D_P$  (in g/day). I assumed that it cost  $\eta_P Synth_P$  to synthesize  $P$  and that the energy required for degradation was  $\varepsilon_P D_P$ . Since  $dP/dt = Synth_P - D_P$ , the energy cost for protein turnover was given by  $(\eta_P + \varepsilon_P)D_P + \eta_P dP/dt$ . Similar arguments led to the other terms of equation 8 representing the energy costs for fat and glycogen turnover where the energy cost for degradation was negligible. The values for the parameters were:  $\eta_F = 0.18$  kcal/g,  $\eta_G = 0.21$  kcal/g,  $\varepsilon_P = 0.54$  kcal/g, and  $\eta_P = 1.34$  kcal/g. These values were determined from the adenosine triphosphate (ATP) costs for the respective biochemical pathways (Blaxter, 1989; Elia et al., 1987) (i.e., 8 ATP per triglyceride synthesized, 2 ATP per glycosyl unit of glycogen synthesized, 4 ATP per peptide bond synthesized plus 1 ATP for amino acid transport, and 1 ATP per peptide bond hydrolyzed). I assumed that 19 kcal of macronutrient oxidation was required to synthesize 1 mol ATP (Elia, 1992).

### Daily Average Lipolysis Rate

The daily average lipolysis rate,  $D_F$ , was modeled as:

$$D_F = \hat{D}_F \left( \frac{F}{F_{Keys}} \right)^{\frac{1}{3}} [L_{diet} + L_{PA}] \quad [11]$$

where  $\hat{D}_F = 150$  g/d is the mean of the fed and overnight fasted lipolysis rates (Jensen, 1999). The  $(F/F_{Keys})^{1/3}$  factor accounted for the dependence of the basal lipolysis rate on the total fat mass normalized by the initial fat mass of the average Minnesota experiment subject,  $F_{Keys}$ . The 1/3 power reflects the scaling of basal lipolysis with the adipocyte diameter (Jacobsson and Smith, 1972) and matches the FFA rate of appearance (Ra) data as a function of body fat mass observed by Lillioja et al. (Lillioja et al., 1986). The effect of diet on lipolysis,  $L_{diet}$ , is primarily determined by the carbohydrate content of the diet via insulin and I modeled this effect as follows:

$$\tau_L \frac{dL_{diet}}{dt} = 1 + \frac{K_L^{S_L} [(L_{max} - L_{min}) \times \exp(-k_L CI/CI_b) + L_{min} - 1]}{K_L^{S_L} + \text{MAX} \left\{ 0, (F_{init}/F_{Keys} - 1)^{S_L} \right\}} - L_{diet} \quad [12]$$

The following choice for  $k_L$  ensured that the lipolysis rate was normalized for the baseline diet:

$$k_L = \ln \left( \frac{L_{max} - L_{min}}{1 - L_{min}} \right) \quad [13]$$

Complete starvation ( $CI = 0$ ) stimulated average daily lipolysis by a factor of  $L_{max} = 3$  as computed by the 3 fold increase of glycerol Ra over the course of prolonged fasting (Bortz et al., 1972; Elia et al., 1987; Klein et al., 1990; Klein et al., 1989; Klein et al., 1993; Klein and Wolfe, 1992; Klein et al., 1986). Furthermore, the lipolysis rate reaches half of its maximum value after about 1 day of fasting (Klein et al., 1993), so  $\tau_L = 1/\ln(2) = 1.44$  days. Halving the carbohydrate content of the diet increased the average lipolysis rate by factor of 1.4 as estimated by the increased area under the circulating FFA curve following an isocaloric meal consisting of 33% versus 66% carbohydrate (Wolever et al., 1995). Given the above value for  $L_{max}$ , the effect of halving the carbohydrate content was modeled by choosing  $L_{min} = 0.87$ .

While obesity increases basal lipolysis, the stimulatory effect of decreased carbohydrate intake is impaired (Wolfe et al., 1987). This effect was modeled in equation 12 by setting  $K_L = 4$  and  $S_L = 2$  such that the curve of lipolysis versus  $CI$  becomes flattened as fat mass increases and matches the data of Klein et al. where long-term versus short-term fasting stimulated lipolysis to a lesser degree in obese versus lean subjects (Klein et al., 1988).

Physical activity and exercise are known to stimulate lipolysis and this was modeled in equation 11 by the factor  $L_{PA}$  as follows:

$$L_{PA} = \psi \left( \frac{\delta + \nu}{\delta_{init} + \nu_{init}} - 1 \right) \quad [14]$$

where  $\psi = 0.4$  was the best fit value for the measured effect of graded exercise to increase lipolysis rates determined by FFA Ra measurements (Friedlander et al., 1998; Klein et al., 1994; Romijn et al., 1993; Wolfe et al., 1990).

#### *Daily Average Ketogenesis Rate*

The daily rate of ketogenesis was modeled as a function of the daily lipolysis rate, the protein content in the diet, and the glycogen level as follows:

$$KTG = \rho_K D_F \left( \frac{3M_{FA}}{M_F} \right) \left[ A_K \left( \frac{L_{diet} + L_{PA}}{K_K + L_{diet} + L_{PA}} \right) \exp \left( -k_p \frac{PI}{PI_b} \right) \exp \left( -k_G \frac{G}{G_{init}} \right) \right] \quad [15]$$

where  $\rho_K = 4.45$  kcal/g is the average energy density of ketones calculated as average enthalpy of combustion of beta-hydroxybuterate and acetoacetate in a 2:1 ratio (Blaxter, 1989).  $A_K = 0.8$  is the maximum fraction of FFA from lipolysis converted to ketones when  $PI = G = 0$  (Balasse and Fery, 1989). When protein intake was at normal levels, I assumed that the maximum fraction of FFA converted to ketones was 0.4 since a protein modified fast decreases circulating ketone levels by half compared with fasting alone (Vazquez et al., 1985). Therefore,  $k_p = \ln(0.8/0.4) = 0.69$ . When both glycogen and protein intake are at normal levels, I assumed that the maximum fraction of FFA converted to ketones was 0.2 and therefore  $k_G = \ln(0.4/0.2) = 0.69$  and when the lipolysis rate was normal I assumed that 10% of FFA from lipolysis were converted to ketones such that  $K_K = 1$  (Balasse and Fery, 1989).

#### *Daily Average Ketone Excretion Rate*

Ketones are excreted in the urine when circulating levels cross the renal threshold for reuptake. I assumed that:

$$KU_{excr} = \left\{ \begin{array}{ll} 0, & \text{if } KTG/\rho_K < KTG_{thresh} \\ \frac{\rho_K KU_{max} (KTG/\rho_K - KTG_{thresh})}{(KTG_{max} - KTG_{thresh})}, & \text{else} \end{array} \right\} \quad [16]$$

Where  $KTG_{thresh} = 70$  g/d,  $KU_{max} = 20$  g/d and  $KTG_{max} = 400$  g/d such that the urinary excretion of ketones,  $KU_{excr}$ , matches the excretion data during various ketone infusion rates as measured by Wildenhoff et al. and Sapir & Owen (Sapir and Owen, 1975; Wildenhoff, 1977).

### *Ketone Oxidation Rate*

Since ketones cannot be stored in the body in any significant quantity, I assumed that once a ketone is produced it is either oxidized or excreted. Therefore,

$$KetOx = KTG - KU_{excr} \quad [17]$$

### *Daily Average Proteolysis Rate*

The daily average protein degradation rate,  $D_P$ , was given by:

$$D_P = \hat{D}_P \left[ \left( \frac{P}{P_{Keys}} \right) + \chi \left( \frac{\Delta PI}{PI_b} \right) \right] \quad [18]$$

where  $\hat{D}_P = 300$  g/d was the baseline daily protein turnover rate (Wagenmakers, 1999) and I assumed that the protein degradation rate was proportional to the normalized protein content of the body (Welle, 1999). While it is possible that the protein content of the diet may directly influence protein turnover as represented by the parameter  $\chi$  (Reeds and Fuller, 1983), the balance of the current data suggests that  $\chi = 0$  (Garlick et al., 1999; Pacy et al., 1994). Nevertheless, I included this parameter in the model to allow for this possibility should new data provide further evidence for such an effect.

### *Daily Average Glycogenolysis Rate*

The daily average glycogen degradation rate,  $D_G$ , was given by the following equation:

$$D_G = \hat{D}_G \left( \frac{G}{G_{Keys}} \right) \quad [19]$$

where the baseline glycogen turnover rate,  $\hat{D}_G = 180$  g/d, was determined by assuming that 70% was from hepatic glycogenolysis and 30% from skeletal muscle with the hepatic contribution computed as 2/3 of the fed plus 1/3 of the overnight fasted hepatic glycogenolysis rate (Magnusson et al., 1994).

### *Daily Average Fat, Protein, and Glycogen Synthesis Rates*

Mass conservation required that the daily average synthesis rates of fat, protein, and glycogen ( $Synth_F$ ,  $Synth_P$ , and  $Synth_G$ , respectively) were given by:

$$\begin{aligned} Synth_F &= D_F + \frac{dF}{dt} \\ Synth_P &= D_P + \frac{dP}{dt} \\ Synth_G &= D_G + \frac{dG}{dt} \end{aligned} \quad [20]$$

### *Glycerol 3-P Production Rate*

Because adipose tissue lacks glycerol kinase, the glycerol 3-P backbone of adipose triglyceride is derived primarily from glucose. Thus, the fat synthesis rate,  $Synth_F$ , determined the rate of glycerol 3-P production,  $G3P$ , according to:

$$G3P = \rho_C Synth_F \left( \frac{M_g}{M_F} \right) \quad [21]$$

### *Glycerol Gluconeogenesis Rate*

Lipolysis of both endogenous and exogenous triglyceride results in the release of glycerol that can be converted to glucose via gluconeogenesis (Baba et al., 1995). Trimmer *et al.* demonstrated that glycerol disappearance could be fully accounted for by glucose production (Trimmer et al., 2001). Therefore, I assumed that all exogenous and endogenous glycerol entered the GNG pathway according to:

$$GNG_F = FI \left( \frac{\rho_C M_g}{\rho_F M_F} \right) + D_F \rho_C \left( \frac{M_g}{M_F} \right) \quad [22]$$

Since glycerol cannot be used by adipose tissue for fat synthesis due to lack of glycerol kinase, all glycerol released by lipolysis is eventually oxidized (apart from a negligibly small amount incorporated in altered pool sizes of non-adipose triglyceride). By assuming that all glycerol enters the GNG pathway, any model error was limited to an overestimate of the energy expenditure associated with glycerol's initial conversion to glucose prior to oxidation. This error must be very small since the total energy cost for glycerol GNG in the basal state was only 25 kcal/d.

### *Gluconeogenesis from amino acids*

The  $GNG_P$  rate in the model referred to the net rate of gluconeogenesis from amino acid-derived carbon. While all amino acids except leucine and lysine can be used as gluconeogenic substrates, the primary gluconeogenic amino acids are alanine and glutamine. Much of alanine gluconeogenesis does not contribute to the net amino acid gluconeogenic rate since the carbon skeleton of alanine is largely derived from carbohydrate precursors via skeletal muscle glycolysis (Perriello et al., 1995). In an extensive review of hepatic amino acid metabolism, Jungas estimated that the net basal gluconeogenic rate from amino acids,  $\hat{G}NG_P$ , was ~300 kcal/d (Jungas et al., 1992).

Several factors may regulate  $GNG_P$ , but for simplicity I have assumed that  $GNG_P$  was proportional to the normalized proteolysis rate and was influenced by the diet as follows:

$$GNG_P = MAX \left\{ 0, \hat{G}NG_P \left[ \left( \frac{D_P}{\hat{D}_P} \right) - \Gamma_C \left( \frac{\Delta CI}{CI_b} \right) + (\Gamma_P + \chi) \left( \frac{\Delta PI}{PI_b} \right) \right] - S_{GNG} \Delta GNG_F \right\} \quad [23]$$

The coefficients  $\Gamma_c = 0.46$  and  $\Gamma_p = 0.37$  were determined by solving equation 23 using three sets of data. The first measured an initial nitrogen balance of -4 g/d upon removal of baseline dietary carbohydrate while keeping dietary protein at baseline values with about 20 g/d of dietary fat (Hoffer et al., 1984). I assumed that the negative nitrogen balance was largely driven by increased amino acid gluconeogenesis and thereby determined the value of  $\Gamma_c$ . The parameter  $S_{GNG}$  determined how amino acid gluconeogenesis was influenced by glycerol gluconeogenesis and a value of  $S_{GNG} = 0.5$  allowed for the appropriate sparing of nitrogen over time (Hoffer et al., 1984). The third study found a 56% increase of gluconeogenesis when protein intake was increased by a factor of 2.5 fold while carbohydrate intake was decreased by 20% and fat intake was unchanged (Linn et al., 2000). Given the value of  $\Gamma_c$ , these data determined the value of the sum  $\Gamma_p + \chi$  and therefore  $\Gamma_p$ .

#### *De Novo Lipogenesis Rate*

*DNL* occurs in both the liver and adipose tissue. Under free-living conditions, adipose *DNL* has recently been measured to contribute about 20% of new triglyceride with a measured triglyceride turnover rate of about 50 g/d (Strawford et al., 2004). Thus, adipose *DNL* is about 94 kcal/d. Measurements of daily hepatic *DNL* in circulating very low-density lipoproteins (VLDL) have found that about 7% of VLDL triglyceride occurs via *DNL* when consuming a basal diet of 30% fat, 50% carbohydrate, and 15% protein (Hudgins et al., 2000). Given that the daily VLDL triglyceride secretion rate is about 33 g/d (Sidossis et al., 2004), this corresponds to a hepatic *DNL* rate of about 22 kcal/d. For an isocaloric diet of 10% fat, 75% carbohydrate, and 15% protein, hepatic *DNL* increases to 113 kcal/d (Hudgins et al., 2000).

When carbohydrate intake is excessively large and glycogen is saturated, *DNL* can be greatly amplified (Acheson et al., 1988). Therefore, I modeled *DNL* as a Hill function of the normalized glycogen content with a maximum *DNL* rate given by the carbohydrate intake rate:

$$DNL = \frac{CI \times (G/G_{init})^d}{(G/G_{init})^d + K_{DNL}^d} \quad [24]$$

I chose  $K_{DNL} = 2$  and  $d = 4$  such that the computed *DNL* rate corresponded with measured *in vivo* *DNL* rates for experimentally determined carbohydrate intakes and estimated glycogen levels (Aarsland et al., 1997; Acheson et al., 1988; Hudgins et al., 2000; Strawford et al., 2004).

#### *Macronutrient Oxidation Rates*

The whole-body energy expenditure rate, *TEE*, was accounted for by the sum of the heat produced from carbohydrate, fat, ketone, and protein oxidation plus the heat produced via flux through the *DNL* (Flatt, 1970) and ketogenesis pathways. Furthermore, I assumed that the minimum carbohydrate oxidation rate was equal to the sum of the gluconeogenic rates less the flux required to produce glycerol 3-phosphate. Thus, the remaining energy

expenditure,  $T\tilde{E}E$ , was apportioned between carbohydrate, fat, and protein oxidation according to the fractions  $f_C$ ,  $f_F$ , and  $f_P$ , respectively.

$$\begin{aligned}
 CarbOx &= GNG_f + GNG_p - G3P + f_C \times T\tilde{E}E \\
 FatOx &= f_F \times T\tilde{E}E \\
 ProtOx &= f_P \times T\tilde{E}E
 \end{aligned} \tag{25}$$

where

$$T\tilde{E}E = TEE - (1 - \varepsilon_d) DNL - (1 - \varepsilon_k) KTG - KetOx - GNG_f - GNG_p + G3P \tag{26}$$

The substrate oxidation fraction for each macronutrient depends on a number of factors. First, increased lipolysis leads to concomitant increased fatty acid oxidation (Carlson et al., 1994). Second, carbohydrate oxidation depends on the carbohydrate intake as well as the glycogen content (Fery et al., 2003; Laurent et al., 2000). Third, protein and carbohydrate intake directly stimulate protein and carbohydrate oxidation, respectively, but fat intake has a minimal direct effect to stimulate fat oxidation (Flatt et al., 1985; Schutz et al., 1989). Fourth, I assumed that lean tissue supplies amino acids for oxidation in proportion to the proteolysis rate. Finally, while inactivity causes muscle wasting (Blanc et al., 1998; Stein et al., 1999), increased physical activity may promote nitrogen retention (Butterfield and Calloway, 1984; Todd et al., 1984; Welle, 1999) and the physical activity expenditure is primarily accounted for by increased oxidation of fat and carbohydrate (Welle, 1999). I modeled these effects by decreasing the fraction of energy expenditure derived from protein oxidation as physical activity increases.

Based on these physiological considerations, the substrate oxidation fractions were computed according to the following expressions:

$$\begin{aligned}
 f_C &= \frac{w_G(D_G/\hat{D}_G) + w_C MAX\{0, (1 + S_C \Delta CI/CI_b)\} G / (G_{\min} + G)}{Z} \\
 f_F &= \frac{w_F(D_F/\hat{D}_F) + S_F \Delta FI/FI_b}{Z} \\
 f_P &= \frac{w_P MAX\{0, (1 + P_{sig})\} + (D_P/\hat{D}_P) S_A \exp(-k_A(\delta + \nu)/(\delta_b + \nu_b))}{Z} \\
 \tau_{PI} \frac{dP_{sig}}{dt} &= S_P \Delta PI/PI_b - P_{sig} \\
 S_P &= \begin{cases} S_P^+, & \text{if } \Delta PI > 0 \\ S_P^-, & \text{else} \end{cases}
 \end{aligned} \tag{27}$$

where the  $w$ 's and  $S$ 's were dimensionless model parameters,  $\Delta CI$ ,  $\Delta FI$  and  $\Delta PI$  were changes from the basal carbohydrate intake,  $CI_b$ , fat intake,  $FI_b$ , and protein intake,  $PI_b$ ,

respectively. The small parameter,  $G_{min} = 10$  g, was chosen such that carbohydrate oxidation was restrained as glycogen decreases and prevents glycogen from becoming negative. The signal for dietary protein intake perturbations,  $P_{sig}$ , changes with a time constant of  $\tau_{PI} = 1.1$  days in accordance with the data of Rand et al. (Rand et al., 1976) and the model allows for an asymmetry between positive and negative perturbations of dietary protein. To normalize for the baseline physical activity, the constant  $k_A$  was chosen such that  $k_A = \ln(S_A)$ .  $Z$  was a normalization factor equal to the sum of the numerators so that the sum of the fractions  $f_C$ ,  $f_F$ , and  $f_P$  was equal to 1.

#### *Nutrient Balance Parameter Constraints*

The baseline diet may not necessarily result in macronutrient or energy balance. Therefore, I defined the following parameters to specify the initial degree of imbalance for fat, carbohydrate, and protein,  $F_{imbal}$ ,  $G_{imbal}$ , and  $P_{imbal}$ , respectively. Therefore, the baseline diet satisfies the following relationship  $TEE + F_{imbal} + G_{imbal} + P_{imbal} = EI_b$ , where the subscript  $b$  refers to the baseline state. Therefore, by explicitly expressing the total energy expenditure in the initial baseline state I derived the following expression:

$$\begin{aligned}
EI_b &= TEF_b + PAE_b + RMR_b + F_{imbal} + G_{imbal} + P_{imbal} \\
&= TEF_b + (\delta_b + \nu_b) BW_b \\
&\quad + E_c + \gamma_B M_B + \gamma_{FFM} (FFM_b - M_B) + \gamma_F F_b \\
&\quad + (1 - \varepsilon_d) DNL_b + (1 - \varepsilon_g) (GNG_{Finit} + GNG_{Pinit}) + \pi_K (1 - \varepsilon_k) KTG_b \\
&\quad + (\eta_P + \varepsilon_P) D_{Pinit} + \eta_F D_{Finit} + \eta_G D_{Ginit} + (1 + \eta_F / \rho_F) F_{imbal} + (1 + \eta_G / \rho_C) G_{imbal} + (1 + \eta_P / \rho_P) P_{imbal} \\
&\quad + \eta_N \frac{(PI_b - P_{imbal})}{6.25 \rho_P}
\end{aligned} \tag{28}$$

which was solved for the constant  $E_c$ .

Assuming negligible baseline ketone excretion, rearrangement of the nutrient balance equations gave:

$$\begin{aligned}
CarbOx_b &= CI_b - DNL_b + GNG_{Pinit} + GNG_{Finit} - G3P_b - G_{imbal} \\
FatOx_b &= (1 - \rho_C M_g / \rho_F M_F) FI_b + \varepsilon_d DNL_b - (1 - \varepsilon_k) KTG_b - KetOx_b - F_{imbal} \\
ProtOx_b &= PI_b - GNG_{Pinit} - P_{imbal}
\end{aligned} \tag{29}$$

Next, I defined the following parameters:

$$\begin{aligned}\zeta_F &\equiv \frac{(1 - \rho_C M_g / \rho_F M_F) FI_b + \varepsilon_d DNL_b - (1 - \varepsilon_k) KTG_b - KetOx_b - F_{imbal}}{\Omega} \\ \zeta_C &\equiv \frac{(CI_b - DNL_b - G_{imbal})}{\Omega} \\ \zeta_P &\equiv \frac{(PI_b - GNG_{P_{init}} - P_{imbal})}{\Omega}\end{aligned}\quad [30]$$

where  $\Omega$  was given by:

$$\begin{aligned}\Omega &\equiv EI_b - (1 - \varepsilon_d) DNL_b - (1 - \varepsilon_k) KTG_b - KetOx_b - GNG_{F_{init}} - GNG_{P_{init}} + G3P_b \\ &\quad - G_{imbal} - F_{imbal} - P_{imbal}\end{aligned}\quad [31]$$

By substituting equations 25 and 27 at the initial state, I obtained:

$$\begin{aligned}\frac{w_F (F_{init} / F_{Keys})^{1/3}}{(P_{init} / P_{Keys}) + w_P + w_G (G_{init} / G_{Keys}) + w_C (G_{init} / (G_{init} + G_{min})) + w_F (F_{init} / F_{Keys})^{1/3}} &= \zeta_F \\ \frac{w_G (G_{init} / G_{Keys}) + w_C (G_{init} / (G_{init} + G_{min}))}{(P_{init} / P_{Keys}) + w_P + w_G (G_{init} / G_{Keys}) + w_C (G_{init} / (G_{init} + G_{min})) + w_F (F_{init} / F_{Keys})^{1/3}} &= \zeta_C \\ \frac{(P_{init} / P_{Keys}) + w_P}{(P_{init} / P_{Keys}) + w_P + w_G (G_{init} / G_{Keys}) + w_C (G_{init} / (G_{init} + G_{min})) + w_F (F_{init} / F_{Keys})^{1/3}} &= \zeta_P\end{aligned}\quad [32]$$

where  $F_{init}$ ,  $G_{init}$ , and  $P_{init}$  were the initial values for body fat, glycogen, and protein, respectively. Elementary algebra led to the following parameter constraints required to achieve the specified macronutrient imbalance:

$$\begin{aligned}w_G &= \frac{\zeta_C / \zeta_P (P_{init} / P_{Keys} + w_P) (G_{Keys} / G_{init})}{1 + w_{C:G} (G_{Keys} / (G_{init} + G_{min}))} \\ w_F &= \left( \frac{\zeta_F}{1 - \zeta_F} \right) \left( 1 + \frac{\zeta_C}{\zeta_P} \right) \left( \frac{P_{init}}{P_{Keys}} + w_P \right) \left( \frac{F_{Keys}}{F_{init}} \right)^{1/3}\end{aligned}\quad [33]$$

where  $w_{C:G} = w_C / w_G$ .

### Carbohydrate Perturbation Constraint

The parameters  $w_C$  and  $S_C$  determined how the model adapted to changes of carbohydrate intake. I specified that an additional dietary carbohydrate intake,  $\Delta CI$ , above baseline,  $CI_b$ , resulted in an initial positive carbohydrate imbalance of  $\kappa_C \Delta CI$ , where  $0 < \kappa_C < 1$  specified the proportion of  $\Delta CI$  directed towards glycogen storage. Thus, the glycogen increment was  $\Delta G = \kappa_C \Delta CI / \rho_C$  after one day. The goal was to solve for the parameter  $S_C$  such that the correct amount of carbohydrate was oxidized and deposited as glycogen during short-term carbohydrate overfeeding. Based on the carbohydrate overfeeding study of McDevitt *et al.*, I chose  $\kappa_C = 0.6$  when  $\Delta CI = 510$  kcal/d (McDevitt *et al.*, 2000).

The change of total energy expenditure was given by:

$$\Delta TEE = \Delta TEF + \Delta PAE + \Delta RMR \quad [34]$$

For a carbohydrate perturbation, the perturbed energy expenditure components were:

$$\Delta TEF = \alpha_C \Delta CI \quad [35]$$

$$\Delta PAE = \delta_b BW_b \sigma \Delta T \quad [36]$$

$$\Delta RMR = \frac{\kappa_C \eta_G}{\rho_C} \Delta CI + \eta_G \hat{D}_G \left( \frac{\Delta G}{G_{Keys}} \right) + \hat{\gamma}_{FFM} (FFM_b - M_B) (1 - \sigma) \Delta T + (1 - \varepsilon_d) \Delta DNL + (1 - \varepsilon_g) \Delta GNG \quad [37]$$

where

$$\Delta T = \lambda_2 \frac{\Delta CI}{EI_b} (1 - \tau_T + \tau_T \exp(-1/\tau_T)) \quad [38]$$

was the average value of the thermogenesis parameter,  $T$ , over one day and

$\Delta DNL$  was computed according to:

$$\Delta DNL = \frac{(CI_b + \Delta CI) (1 + \Delta G / G_{init})^d}{K_{DNL}^d + (1 + \Delta G / G_{init})^d} - DNL_b \quad [39]$$

The change of the gluconeogenic rate,  $\Delta GNG$ , was given by:

$$\begin{aligned} \Delta GNG &= -\Gamma_c \left( \frac{\Delta CI}{CI_b} \right) G\hat{N}G_p \\ &+ (1 - S_{GNG}) \rho_C \left( \frac{M_G}{M_F} \right) \hat{D}_F \left( \frac{F_{init}}{F_{Keys}} \right)^{\frac{1}{3}} \frac{K_L^{S_L} \left[ (L_{max} - L_{min}) \times \exp(-k_L (1 + \Delta CI / CI_b)) + L_{min} - 1 \right]}{K_L^{S_L} + \text{MAX} \left\{ 0, (F_{init} / F_{Keys} - 1)^{S_L} \right\}} \end{aligned} \quad [40]$$

The carbohydrate balance equation 1 and the carbohydrate oxidation equation 25 gave:

$$f_c = \frac{CI_b + (1 - \kappa_c) \Delta CI - (DNL_b + \Delta DNL)}{T\tilde{E}E} \quad [41]$$

Since I assumed that the baseline diet results in a state of energy balance, I obtained the following equation for  $T\tilde{E}E$ :

$$\begin{aligned} T\tilde{E}E &= EI_b + \Delta TEE - (1 - \varepsilon_d) DNL - (1 - \varepsilon_k) KTG \\ &- KetOx - GNG_F - GNG_p + G3P \end{aligned} \quad [42]$$

For simplicity, I assumed that:

$$\begin{aligned} G3P &= G3P_b \\ KTG &= KetOx = KTG_b \end{aligned} \quad [43]$$

The carbohydrate oxidation fraction on the first day was equivalent to the parameter  $\Theta$  defined as:

$$\Theta \equiv \frac{CI_b + (1 - \kappa_c) \Delta CI - (DNL_b + \Delta DNL)}{T\tilde{E}E} \quad [44]$$

Therefore, using equation 27, I solved equation 41 for  $S_C$  which gave the carbohydrate feeding constraint:

$$S_C = \frac{CI_b}{\Delta CI} \left[ \frac{\Theta \left( P_{init} / P_{Keys} + w_P + \tilde{w}_F + \tilde{w}_G \right) - \tilde{w}_G}{(1 - \Theta) \tilde{w}_C} - 1 \right] \quad [45]$$

where

$$\begin{aligned}
\tilde{w}_F &= w_F \left( \frac{F_{init}}{F_{Keys}} \right)^{\frac{1}{3}} \left[ 1 + \frac{K_L^{S_L} \left[ (L_{max} - L_{min}) \times \exp(-k_L (1 + \Delta CI / CI_b)) + L_{min} - 1 \right]}{K_L^{S_L} + \text{MAX} \left\{ 0, (F_{init} / F_{Keys} - 1)^{S_L} \right\}} \right] \\
\tilde{w}_C &= w_C \left( \frac{G_{init} + \Delta G}{G_{min} + G_{init} + \Delta G} \right) \\
\tilde{w}_G &= w_G \left( \frac{G_{init} + \Delta G}{G_{Keys}} \right)
\end{aligned} \tag{46}$$

### Protein Perturbation Constraint

The parameters  $w_P$  and  $S_p^+$  determined how the model adapted short-term substrate oxidation rates to changes of protein intake. In a meticulous study of whole-body protein balance, Oddoye and Margen measured nitrogen balance in subjects consuming isocaloric diets with moderate or high protein content (Oddoye and Margen, 1979). These studies found that ~90% of the additional dietary nitrogen on the high protein diet was rapidly excreted such that  $\kappa_P = 0.07$  when  $\Delta PI = 640$  kcal/d,  $\Delta CI = -310$  kcal/d, and  $\Delta FI = -330$  kcal/d.

To compute the value for  $S_p^+$  to match the data of Oddoye and Margen (Oddoye and Margen, 1979), I began with the protein balance equations 1 and 27 to derive:

$$f_p = \frac{PI_b + (1 - \kappa_P) \Delta PI - G \hat{N} G_P - \Delta G N G_P}{T \tilde{E} E} \tag{47}$$

where the changes of gluconeogenic rates were given by:

$$\begin{aligned}
\Delta G N G_P &= G \hat{N} G_P \left[ (\Gamma_P + \chi) \frac{\Delta PI}{PI_b} - \Gamma_C \frac{\Delta CI}{CI_b} \right] - S_{GNG} \Delta G N G_F \\
\Delta G N G_F &= \Delta FI \left( \frac{\rho_C M_G}{\rho_F M_F} \right) \\
&+ \rho_C \left( \frac{M_G}{M_F} \right) \left( \frac{F_{init}}{F_{Keys}} \right)^{\frac{1}{3}} \frac{K_L^{S_L} \left[ (L_{max} - L_{min}) \times \exp(-k_L (1 + \Delta CI / CI_b)) + L_{min} - 1 \right]}{K_L^{S_L} + \text{MAX} \left\{ 0, (F_{init} / F_{Keys} - 1)^{S_L} \right\}}
\end{aligned} \tag{48}$$

The change of total energy expenditure was given by:

$$\Delta TEE = \Delta TEF + \Delta PAE + \Delta RMR \tag{49}$$

where

$$\Delta TEF = \alpha_C \Delta CI + \alpha_F \Delta FI + \alpha_P \Delta PI \quad [50]$$

and

$$\begin{aligned} \Delta RMR = & (1 - \varepsilon_d) \Delta DNL + (1 - \varepsilon_g) (\Delta GNG_F + \Delta GNG_P) + \frac{\eta_N (1 - \kappa_P) \Delta PI}{6.25 \rho_P} \\ & + (\eta_P + \varepsilon_P) \hat{D}_P \left( \frac{\kappa_P \Delta PI}{\rho_P P_{Keys}} + \chi \frac{\Delta PI}{PI_b} \right) + \frac{\eta_P \kappa_P}{\rho_P} \Delta PI + \frac{\eta_G \kappa_C}{\rho_C} \Delta CI \left( 1 + \frac{\hat{D}_G}{G_{Keys}} \right) \end{aligned} \quad [51]$$

Since the perturbed diet was isocaloric and there were no changes of physical activity,

$$\Delta PAE = \Delta T = 0 \quad [52]$$

I also assumed that:

$$G3P = G3P_b$$

$$KTG = KetOx = KTG_b$$

$$\Delta G \approx G_{init} (\Delta CI + \Delta GNG_P + \Delta GNG_F) / (CI_b + G\hat{N}G_P + G\hat{N}G_F) \quad [53]$$

Therefore the change in DNL is given by:

$$\Delta DNL = \frac{(CI_b + \Delta CI) (1 + \Delta G / G_{init})^d}{K_{DNL}^d + (1 + \Delta G / G_{init})^d} - \frac{CI_b}{K_{DNL}^d + 1} \quad [54]$$

Using equation 27, I solved equation 47 for  $S_P$  which gave the following constraint:

$$S_P^+ = \frac{PI_b}{\Delta PI} \left[ \frac{\Phi (\tilde{w}_F + \tilde{w}_G + \tilde{w}_C)}{(1 - \Phi) w_P} - \frac{(P_{init} / P_{Keys} + \chi \Delta PI / PI_b)}{w_P} - 1 \right] \quad [55]$$

where  $\Phi$  was defined as:

$$\Phi \equiv \frac{PI_b + (1 - \kappa_P) \Delta PI - G\hat{N}G_P - \Delta GNG_P}{T\tilde{E}E} \quad [56]$$

and

$$\begin{aligned}\tilde{w}_F &= w_F \left( \frac{F_{init}}{F_{Keys}} \right)^{\frac{1}{3}} \frac{K_L^{S_L} \left[ (L_{max} - L_{min}) \times \exp(-k_L (1 + \Delta CI / CI_b)) + L_{min} - 1 \right]}{K_L^{S_L} + \text{MAX} \left\{ 0, (F_{init} / F_{Keys} - 1)^{S_L} \right\}} + S_F \Delta FI / FI_b \\ \tilde{w}_C &= w_C (1 + S_C \Delta CI / CI_b) \left( \frac{G_{init} + \Delta G}{G_{min} + G_{init} + \Delta G} \right) \\ \tilde{w}_G &= w_G (G_{init} + \Delta G) / G_{Keys}\end{aligned}\tag{57}$$

### Physical Inactivity Constraint

The parameter  $S_A$  defines how the protein oxidation fraction depends of physical activity and exercise. Stein et al. observed that 17 days of bed rest without a change of diet resulted in an average negative N balance  $N_{bal} = -2$  g/d (Stein et al., 1999). From the protein balance equation,

$$f_P = \frac{PI_b - G \hat{N} G_P (P_{init} / P_{Keys}) - 6.25 \rho_P N_{bal}}{T \tilde{E} E}\tag{58}$$

where

$$\Delta TEE = -(\delta_b + \upsilon_b) BW_{init} - \eta_N N_{bal}\tag{59}$$

We assume that the about half of the energy savings due to inactivity will initially be deposited as glycogen such that  $\Delta G = \Delta TEE / 2 \rho_C$ .

From the macronutrient oxidation equations 27,

$$f_P = \frac{w_P + S_A (P_{init} / P_{Keys})}{w_P + S_A (P_{init} / P_{Keys}) + \tilde{w}_C + \tilde{w}_G + \tilde{w}_F}\tag{60}$$

where

$$\begin{aligned}\tilde{w}_G &= w_G \left( \frac{G_{init} + \Delta G}{G_{init}} \right) \\ \tilde{w}_C &= w_C \left( \frac{G_{init} + \Delta G}{G_{min} + G_{init} + \Delta G} \right) \\ \tilde{w}_F &= w_F (1 - \psi) \left( \frac{F_{init}}{F_{Keys}} \right)^{\frac{1}{3}}\end{aligned}\tag{61}$$

This results in the following expression for  $S_A$ :

$$S_A = MAX \left\{ 1, \left( \frac{P_{Keys}}{P_{init}} \right) \left[ \frac{\Xi}{1-\Xi} (\tilde{w}_C + \tilde{w}_G + \tilde{w}_F) - w_P \right] \right\} \quad [62]$$

where

$$\Xi \equiv \frac{PI_b - G\hat{N}G_P \left( P_{init} / P_{Keys} \right) - 6.25 \rho_P N_{bal}}{TEE} \quad [63]$$

### Energy Conservation

To explicitly demonstrate that the model obeys the first law of thermodynamics, add equations 1 to give:

$$\begin{aligned} & \rho_C \frac{dG}{dt} + \rho_P \frac{dP}{dt} + \rho_F \frac{dF}{dt} \\ &= \left( \rho_C \frac{M_g}{M_F} \right) \frac{dF}{dt} + CI + PI + \left( 1 - \frac{\rho_C M_g}{\rho_F M_F} \right) FI - KU_{excr} \\ & - (CarbOx + ProtOx + FatOx + KetOx) - (1 - \varepsilon_d) DNL - (1 - \varepsilon_k) KTG + GNG_F - G3P \end{aligned} \quad [64]$$

Using equations 25 and 26, we can replace the sum of the macronutrient oxidation rates with  $TEE - KetOx - (1 - \varepsilon_d) DNL - (1 - \varepsilon_k) KTG$ . Furthermore, replacing  $GNG_F$  and  $G3P$  using equations 21 and 22 along with the fact that  $Synth_F = D_F + dF/dt$  from equation 20 gives the following energy balance equation:

$$\rho_C \frac{dG}{dt} + \rho_P \frac{dP}{dt} + \rho_F \frac{dF}{dt} = CI + PI + FI - TEE - KU_{excr} \quad [65]$$

Therefore, the macronutrient balance model always obeys the law of energy conservation.

### Respiratory Gas Exchange

Assuming that respiratory gas exchange and nitrogen excretion arise solely due to macronutrient oxidation, the standard indirect calorimetry equations are:

$$\begin{aligned} VO_2 &= a \times CarbOx + b \times FatOx + d \times ProtOx \\ VCO_2 &= e \times CarbOx + g \times FatOx + h \times ProtOx \\ N &= ProtOx/i \end{aligned} \quad [66]$$

In matrix form, these equations can be written as:

$$\begin{bmatrix} a & b \\ e & g \end{bmatrix} \begin{bmatrix} \text{CarbOx} \\ \text{FatOx} \end{bmatrix} = \begin{bmatrix} VO_2 - di \times N \\ VCO_2 - hi \times N \end{bmatrix} \quad [67]$$

Therefore, the carbohydrate and fat oxidation rates can be solved by matrix inversion:

$$\begin{bmatrix} \text{CarbOx} \\ \text{FatOx} \end{bmatrix} = \frac{1}{(ag - be)} \begin{bmatrix} g & -b \\ -e & a \end{bmatrix} \begin{bmatrix} VO_2 - di \times N \\ VCO_2 - hi \times N \end{bmatrix} \quad [68]$$

The macronutrient oxidation rates as a function of oxygen consumption, carbon dioxide production, and nitrogen excretion are as follows:

$$\begin{aligned} \text{CarbOx} &= \frac{g}{(ag - be)} VO_2 - \frac{b}{(ag - be)} VCO_2 + \frac{(bh - dg)}{(ag - be)} (i \times N) \\ \text{FatOx} &= \frac{-e}{(ag - be)} VO_2 + \frac{a}{(ag - be)} VCO_2 + \frac{(de - ah)}{(ag - be)} (i \times N) \\ \text{ProtOx} &= i \times N \end{aligned} \quad [69]$$

The energy expenditure is calculated by multiplying the oxidation rates by their corresponding energy densities:

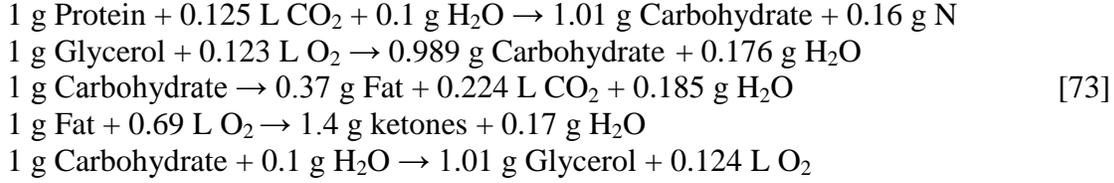
$$EE = \rho_C \times \text{CarbOx} + \rho_F \times \text{FatOx} + \rho_P \times \text{ProtOx} \quad [70]$$

$$\begin{aligned} EE &= \rho_C \left[ \frac{g}{(ag - be)} VO_2 - \frac{b}{(ag - be)} VCO_2 + \frac{(bh - dg)}{(ag - be)} (i \times N) \right] \\ &+ \rho_F \left[ \frac{-e}{(ag - be)} VO_2 + \frac{a}{(ag - be)} VCO_2 + \frac{(de - ah)}{(ag - be)} (i \times N) \right] \\ &+ \rho_P (i \times N) \end{aligned} \quad [71]$$

The coefficients for gas exchange and nitrogen excretion are determined by collecting terms:

$$EE = \left[ \frac{\rho_C g - \rho_F e}{(ag - be)} \right] VO_2 + \left[ \frac{\rho_F a - \rho_C b}{(ag - be)} \right] VCO_2 + \left[ \rho_C \frac{(bh - dg)}{(ag - be)} + \rho_F \frac{(de - ah)}{(ag - be)} + \rho_P \right] (i \times N) \quad [72]$$

Gluconeogenesis, lipogenesis, ketogenesis (with subsequent excretion), and glycerol 3-P production also contribute to respiratory gas exchange according to the following net reactions (Elia and Livesey, 1988; Ferrannini, 1988; Frayn, 1983):



Oxidation of carbohydrate, fat, and protein can either occur directly, or subsequent to intermediate exchange via lipogenesis or gluconeogenesis. In either case, the final ratio of CO<sub>2</sub> produced to O<sub>2</sub> consumed (i.e., the respiratory quotient) is independent of any intermediate exchanges in accordance with the principles of indirect calorimetry (Elia and Livesey, 1988; Ferrannini, 1988; Frayn, 1983).

The simulated O<sub>2</sub> consumption (VO<sub>2</sub>) and CO<sub>2</sub> production (VCO<sub>2</sub>) (in L/day) were computed according to:

$$\begin{aligned}
VO_2 &= 0.746 \frac{CarbOx}{\rho_C} + 2.03 \frac{FatOx}{\rho_{FA}} + 1.036 \frac{ProtOx}{\rho_P} + 0.123 \left( \frac{GNG_F}{\rho_C} - \frac{G3P}{\rho_C} \right) + 0.3 \frac{KU_{excr}}{\rho_K} \\
VCO_2 &= 0.742 \frac{CarbOx}{\rho_C} + 1.41 \frac{FatOx}{\rho_{FA}} + 0.851 \frac{ProtOx}{\rho_P} - 0.125 \frac{GNG_P}{\rho_P} + 0.224 \frac{DNL}{\rho_C}
\end{aligned} \tag{74}$$

where we have used the fact that the STP molar volume of O<sub>2</sub> and CO<sub>2</sub> are 22.392 L and 22.261 L, respectively.

The respiratory quotient, RQ, was computed by dividing VCO<sub>2</sub> by VO<sub>2</sub>. When comparing model predictions for fuel selection with experiments employing 24-hr room indirect calorimetry, I used the simulated VCO<sub>2</sub> and VO<sub>2</sub> values from equation 74 and computed macronutrient oxidation and energy expenditure rates using equations 69 and 72 as if they were the actual indirect calorimetry measurements.

#### *Model Parameter Values and Numerical Integration*

Since body composition changes take place on the time scale of weeks, months, and years, the model was targeted to represent daily changes of energy metabolism and not fluctuations of metabolism that occur within a day. The nutrient balance equations were integrated using the 4<sup>th</sup> order Runge-Kutta algorithm with a timestep size of 0.1 days (Press et al., 1986). The model parameter values listed above were obtained from the cited published literature and are listed in **Table S3**. The parameters,  $S_p^-$ ,  $w_P$ ,  $w_{C:G}$ ,  $\lambda_1$ ,  $\lambda_2$ , and  $\sigma$  were determined using a downhill simplex algorithm (Press et al., 1986) implemented using Berkeley Madonna software (version 8.3; <http://www.berkeleymadonna.com>) to minimize the sum of squares of weighted residuals between the simulation outputs and the data from the Minnesota human starvation experiment (Keys, 1950). I used the following measurement error estimates to define the weights for the parameter optimization algorithm:  $\delta_{BW} = 0.2$  kg,  $\delta_{FM} = 0.5$  kg, and  $\delta_{RMR} = 50$  kcal/d. The best fit parameter values are listed in **Table S4**, and the constrained parameters are listed in **Table S5**.

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**Table S3.** Model parameters determined from published data.

<u>Parameter</u>	<u>Value</u>	<u>Description</u>
$\rho_C$	3.7 kcal/g	Energy density of <i>G</i>
$\rho_F$	9.4 kcal/g	Energy density of <i>F</i>
$\rho_{FA}$	9.42 kcal/g	Energy density of <i>FA</i>
$\rho_P$	4.7 kcal/g	Energy density of <i>P</i>
$\rho_K$	4.45 kcal/g	Energy density of ketones
$h_P$	1.6 g H <sub>2</sub> O/g	<i>P</i> hydration coefficient
$h_G$	2.7 g H <sub>2</sub> O/g	<i>G</i> hydration coefficient
$\eta_F$	0.18 kcal/g	<i>F</i> synthesis cost
$\eta_P$	1.34 kcal/g	<i>P</i> synthesis cost
$\varepsilon_P$	0.54 kcal/g	<i>P</i> degradation cost
$\eta_G$	0.21 kcal/g	<i>G</i> synthesis cost
$\eta_N$	5.4 kcal/g	Urea synthesis cost per g Nitrogen
$\varepsilon_d$	0.95	<i>DNL</i> efficiency
$\varepsilon_g$	0.8	<i>GNG</i> efficiency
$\varepsilon_k$	0.77	<i>KTG</i> efficiency
$\pi_K$	0.9	Hepatic <i>KTG</i> expenditure increment
$\alpha_F$	0.025	<i>TEF</i> factor for <i>FI</i>
$\alpha_C$	0.075	<i>TEF</i> factor for <i>CI</i>
$\alpha_P$	0.25	<i>TEF</i> factor for <i>PI</i>
$\gamma_F$	4.5 kcal/kg/d	Specific <i>RMR</i> for Adipose
$\hat{\gamma}_{FFM}$	19 kcal/kg/d	Basal specific <i>RMR</i> for <i>FFM</i>
$\zeta_{BW}$	0.17 L/kg	<i>ECF</i> response to <i>BW</i> changes
$\zeta_{CI}$	4 g/d	<i>ECF</i> response to <i>CI</i> changes
$\zeta_{Na}$	3 g/L/d	<i>ECF</i> response to sodium changes
$\tau_{BW}$	200 days	<i>ECF</i> response time for <i>BW</i> changes
$\tau_T$	7 days	Response time for <i>T</i> changes
$\hat{D}_G$	180 g/d	Baseline Glycogenolysis rate
$K_{DNL}$	2	Glycogen constant for <i>DNL</i>
$d$	4	Hill coefficient for <i>DNL</i>
$\hat{D}_F$	150 g/d	Baseline Lipolysis rate
$L_{max}$	3	Maximum Lipolysis change
$L_{min}$	0.87	Minimum Lipolysis change
$\tau_L$	1.44 days	Response time for Lipolysis
$K_L$	4	Body fat constant for Lipolysis
$S_L$	2	Hill coefficient for Lipolysis
$\psi$	0.4	<i>PA</i> effect on Lipolysis
$A_K$	0.8	Maximum <i>KTG</i> fraction
$k_P$	0.69	Effect of <i>PI</i> on <i>KTG</i>
$k_G$	0.69	Effect of <i>G</i> on <i>KTG</i>
$K_K$	1	Sets basal <i>KTG</i> rate
$KTG_{thresh}$	70 g/d	Renal threshold <i>KTG</i> rate
$KU_{max}$	20 g/d	Maximum ketone excretion
$KTG_{max}$	400 g/d	Maximum <i>KTG</i>
$\hat{D}_P$	300 g/d	Baseline Proteolysis rate
$\chi$	0	Effect of <i>PI</i> on protein turnover
$\hat{GNG}_P$	300 kcal/d	Baseline <i>GNG<sub>P</sub></i> rate
$S_{GNG}$	0.5	Effect of <i>GNG<sub>F</sub></i> changes on <i>GNG<sub>P</sub></i>
$\Gamma_C$	0.46	Effect of <i>CI</i> on <i>GNG<sub>P</sub></i>
$\Gamma_P$	0.31	Effect of <i>PI</i> on <i>GNG<sub>P</sub></i>
$\tau_{PI}$	1.1 days	Response time of <i>ProtOx</i> to <i>PI</i> changes
$S_F$	0	Sensitivity of <i>FatOx</i> to <i>FI</i> changes

**Table S4.** Parameter values fit to the Minnesota experiment data.

<u>Parameter</u>	<u>Value</u>	<u>Description</u>
$\lambda_1$	0.8	Underfeeding adaptive thermogenesis
$\lambda_2$	0.1	Overfeeding adaptive thermogenesis
$\sigma$	0.6	Thermogenesis effect on <i>PAE</i> vs. <i>RMR</i>
$w_P$	1.2	Weighting of <i>ProtOx</i> for basal <i>PI</i>
$w_{C:G}$	1.0	Ratio of <i>CarbOx</i> weighting parameters
$S_P^-$	0.85	Sensitivity of <i>ProtOx</i> to reduced <i>PI</i>

**Table S5.** Parameter values determined from constraints on energy balance, nutrient balance, physical inactivity as well as perturbations of dietary protein and carbohydrate. These parameters are calculated according to the initial conditions corresponding to different subject groups. The values listed are for the average Minnesota experiment subject.

<u>Parameter</u>	<u>Value</u>	<u>Description</u>
$S_p^+$	2.5	Sensitivity of <i>ProtOx</i> to increased <i>PI</i>
$S_C$	1.4	Sensitivity of <i>CarbOx</i> to <i>CI</i> changes
$w_G$	6.6	Weighting of <i>CarbOx</i> for Glycogenolysis
$w_F$	11	Weighting of <i>FatOx</i> for Lipolysis
$E_c$	-520 kcal/d	Constant energy expenditure offset