Computational model of in vivo human energy metabolism during semistarvation and refeeding

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Hall, Kevin D. Computational model of in vivo human energy metabolism during semistarvation and refeeding. Am J Physiol Endocrinol Metab 291: E23–E37, 2006. First published January 31, 2006; doi:10.1152/ajpendo.00523.2005.—Changes in body weight and composition are the result of complex interactions among metabolic fluxes contributing to macronutrient balances. To better understand these interactions, a mathematical model was constructed that used the measured dietary macronutrient intake during semistarvation and refeeding as model inputs and computed whole body energy expenditure, de novo lipogenesis, and gluconeogenesis as well as turnover and oxidation of carbohydrate, fat, and protein. Published in vivo human data provided the basis for the model components that were integrated by fitting a few unknown parameters to the classic Minnesota human starvation experiment. The model simulated the measured body weight and fat mass changes during semistarvation and refeeding and predicted the unmeasured metabolic fluxes underlying the body composition changes. The resting metabolic rate matched the experimental measurements and required a model of adaptive thermogenesis. Refeeding caused an elevation of de novo lipogenesis that, along with increased fat intake, resulted in a rapid repletion and overshoot of body fat. By continuing the computer simulation with the prestarvation diet and physical activity, the original body weight and composition were eventually restored, but body fat mass was predicted to take more than one additional year to return to within 5% of its original value. The model was validated by simulating a recently published short-term caloric restriction study by Friedlander et al. (30), who measured changes of body weight, fat mass, resting metabolic rate, and nitrogen balance after energy intake was decreased by 40% and protein intake was maintained. No model parameters were altered for this simulation other than the initial baseline values.

Glossary of Model Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>BCM</td>
<td>Body cell mass in g</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight in g</td>
</tr>
<tr>
<td>CarbOx</td>
<td>Rate of carbohydrate oxidation in kcal/day</td>
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<tr>
<td>CI</td>
<td>Carbohydrate intake rate in kcal/day</td>
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<tr>
<td>DF</td>
<td>Rate of endogenous lipolysis in g/day</td>
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<tr>
<td>DG</td>
<td>Rate of glycogenolysis in g/day</td>
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<tr>
<td>DNL</td>
<td>Rate of de novo lipogenesis in kcal/day</td>
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<td>Rate of proteolysis in g/day</td>
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<tr>
<td>ECM</td>
<td>Extracellular water mass in g</td>
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<tr>
<td>F</td>
<td>Body fat mass in g</td>
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<tr>
<td>FatOx</td>
<td>Rate of fat oxidation in kcal/day</td>
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<tr>
<td>fC</td>
<td>Carbohydrate oxidation fraction</td>
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<tr>
<td>fF</td>
<td>Fat oxidation fraction</td>
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<tr>
<td>FI</td>
<td>Fat intake rate in kcal/day</td>
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<tr>
<td>fP</td>
<td>Protein oxidation fraction</td>
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<tr>
<td>G</td>
<td>Glycogen mass in g</td>
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<tr>
<td>G3P</td>
<td>Rate of glycerol 3-phosphate synthesis in kcal/day</td>
</tr>
<tr>
<td>GNF</td>
<td>Rate of gluconeogenesis from glycerol in kcal/day</td>
</tr>
<tr>
<td>GNGP</td>
<td>Rate of gluconeogenesis from protein in kcal/day</td>
</tr>
<tr>
<td>L</td>
<td>Lean body mass in g</td>
</tr>
<tr>
<td>MEI</td>
<td>Metabolizable energy intake in kcal/day</td>
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</table>

REGULATION OF BODY WEIGHT and composition is an issue of immense scientific, economic, and social importance. Obesity, anorexia nervosa, cachexia, and starvation are all life-threatening conditions of altered body composition fundamentally caused by a period of imbalance between energy intake and expenditure. But what determines the partitioning of energy between fat and lean tissue? How do dietary macronutrients contribute to energy partitioning? How does the interaction between in vivo metabolic fluxes finally integrate to provide regulation of body composition?

My goal was to develop a computational framework to study body composition regulation. I created mathematical models of the individual metabolic processes contributing to daily macronutrient balance that were based on published in vivo human data. Most model parameters were derived from the literature, and the model components were integrated by fitting a few unknown parameter values to match body composition data from a classic long-term feeding study known as the Minnesota human starvation experiment (36). The resulting computer simulation of the Minnesota experiment predicted the underlying adaptations of daily whole body energy expenditure and metabolic fluxes that were not measured.

A particularly important observation from the Minnesota human starvation experiment was that refeeding caused a rapid replenishment and overshoot of body fat mass, clearly an undesirable result with implications for obesity relapse as well as the treatment of malnutrition. Therefore, another goal of this study was to explain the physiological basis of the fat mass overshoot and predict whether or not it was a transient phenomenon.

Finally, to test the validity of the model, I simulated a recently published short-term caloric restriction study by Friedlander et al. (30), who measured changes of body weight, fat mass, resting metabolic rate, and nitrogen balance after energy intake was decreased by 40% and protein intake was maintained. No model parameters were altered for this simulation other than the initial baseline values.

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**RESULTS**

**Simulation of the Minnesota Human Starvation Experiment**

**Body weight and fat mass.** Figure 2 shows the model simulation along with the experimental measurements of body weight and fat mass during the Minnesota experiment. The

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**METHODS**

The **APPENDIX** provides a detailed description of the mathematical model along with the assumptions and supporting data from the literature. Because body composition changes occur over long time scales, the model does not attempt to simulate fluctuations of metabolism that occur within the course of a single day. Rather, the model was based on the concept of daily nutrient balance (27, 31), schematically depicted in Fig. 1. Daily fluxes were calculated by assuming that two-thirds of the day corresponded to the fed state and the remaining one-third corresponded to an overnight-fasted state.

The model inputs were the measured daily metabolizable energy intake of fat (FI), carbohydrate (CI), and protein (PI). The masses of body fat (F), glycogen (G), and protein (P) were depicted as circles in Fig. 1. The daily metabolic fluxes are represented by arrows in Fig. 1, where DNL is the rate of de novo lipogenesis, G3P is the glycerol 3-phosphate production rate, GNGF is the gluconeogenic rate from glycerol carbon, and GNGp is the gluconeogenic rate from amino acid carbon. CarbOx, FatOx, and ProtOx indicate the daily oxidation rates of carbohydrate, fat, and protein, respectively, which sum to the total daily energy expenditure (TEE; not shown).

Figure 1 is not intended to represent biochemical pathways and does not imply that macronutrients from the diet must first be converted to the storage pools F, G, or P before being oxidized or flow into gluconeogenic or lipogenic pathways. Rather, Fig. 1 indicates that changes of the body macronutrient pools result from net imbalances of the fluxes entering and exiting the pools. For example, an increase of P requires that PI be greater than the sum of GNGF and ProtOx.

The model of TEE included the resting metabolic rate (RMR), thermic effect of feeding (TEF), and energy expenditure of physical activity (PAE). RMR was determined by a weighted average of the organ basal metabolic rates (22) as well as the energy costs for gluconeogenesis, lipogenesis, and turnover of protein, triacylglycerol, and glycogen (9, 20). TEF was determined by macronutrient composition, and content of the diet (29), and PAE depended on the amount of daily physical activity and was proportional to the body weight (61).

A reduction of energy intake below what is required to maintain body weight causes a reduction of energy expenditure via a process called adaptive thermogenesis (16a, 19). Adaptive thermogenesis is believed to affect both RMR and PAE components of TEE (16a, 16b, 40), and I used the measured adaptation of RMR from the Minnesota experiment to investigate the relative changes of PAE vs. RMR and the contribution of adaptive thermogenesis.

The content of body protein, glycogen, and fat influenced the daily average rates of proteolysis, gluconeogenesis, and lipolysis, respectively. These rates, along with the macronutrient intakes, were used to determine the relative macronutrient oxidation rates according to equations presented in the **APPENDIX**. An important aspect of the macronutrient oxidation model was that, unlike carbohydrate and protein, fat intake did not directly stimulate its own oxidation (26, 55).

Glucose oxidation was driven by dietary changes as well as endogenous substrate delivery. GNGF varied directly with the dietary fat intake and the endogenous lipolysis rate (7), whereas GNGp depended upon the intake of carbohydrate and protein as well as the proteolysis rate. DNL was a function of the dietary carbohydrate intake and the glycogen content such that DNL became amplified in the case of saturated and high-carbohydrate intake (2).

The body weight was the sum of the body fat mass (F) and the lean mass (L). Lean mass was composed of bone, extracellular water, and the lean tissue (nonadipose) cell mass, including intracellular water, glycogen, and protein. On the basis of typical values reported for the intracellular composition (3), and assuming that water is associated with intracellular protein and glycogen in constant ratios, the lean tissue cell mass was computed from the body protein and glycogen masses.

Most model parameters were determined from published human data, and the few remaining parameters were chosen to minimize the mean squared difference between the simulation outputs and the data from the Minnesota experiment. In that study, macronutrient intakes were controlled, and body weight and composition changes were measured in 32 healthy young men over a period of ~1 yr (36). The subjects participated in a 24-wk semistarvation period and lost 24% of their body weight. Semistarvation was followed by a 12-wk controlled refeeding period. Twelve subjects went on to participate in an additional 8-wk ad libitum feeding phase. The model was applied to data taken from the 12 subjects that participated in the entire 20-wk refeeding protocol. To investigate how long it would take to return to the original body composition, I continued the simulation for an additional 19 mo with the dietary intake values given by those measured during the prestarvation period of the Minnesota experiment.

To simulate the caloric restriction study of Friedlander et al. (30), I chose the initial values for the body weight, fat mass, RMR, and the balanced diet to match the average subject during the baseline phase of the experiment. Next, the caloric restriction was imposed using the diet composition described by Friedlander et al., where the energy restriction came primarily from decreased fat and carbohydrate intake, whereas protein intake was held approximately constant.

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**E24**

**COMPUTATIONAL MODEL OF HUMAN ENERGY METABOLISM**

**NPRQ** Nonprotein respiratory quotient

**P** Protein mass in g

**PAE** Physical activity energy expenditure in kcal/day

**PI** Protein intake rate in kcal/day

**ProtOx** Rate of protein oxidation in kcal/day

**RMR** Resting metabolic rate in kcal/day

**RQ** Respiratory quotient

**SynthF** Rate of fat synthesis in g/day

**SynthG** Rate of glycogen synthesis in g/day

**SynthP** Rate of protein synthesis in g/day

**T** Adaptive thermogenesis

**TEE** Total energy expenditure in kcal/day

**TEF** Thermic effect of feeding in kcal/day

**TG** Triacylglycerol

**VCO2** Rate of carbon dioxide production in L/day

**VO2** Rate of oxygen consumption in L/day

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![Fig. 1. Schematic of the nutrient balance model. Changes in the body fat (F), glycogen (G), and protein (P) were determined by the balance of fat (FI), carbohydrate (CI), and protein intake (PI), gluconeogenesis from fat (GNGF) and protein (GNGp), de novo lipogenesis (DNL), glycerol 3-phosphate synthesis (G3P), and the oxidation of fat (FatOx), carbohydrate (CarbOx), and protein (ProtOx), respectively.](attachment:fig1.png)
model simulations matched the experimental data reasonably well during all feeding periods. The initial baseline feeding period resulted in a stable body weight and fat mass. The start of semistarvation induced a rapid decrease of body weight and fat mass that slowed and eventually reached 76 and 34% of their initial values, respectively. During refeeding, body weight and fat mass increased, with both recovering to about 80% of their initial masses by the end of the 12-wk controlled refeeding period. Ad libitum refeeding resulted in further increases of body weight and fat to finally achieve 104 and 145% of their initial values, respectively. Thus the simulation reproduced the overshoot of body fat mass. Continuing the simulation with the original balanced baseline diet and physical activity level eventually restored the original body weight and fat mass, but it took more than a year for the fat mass to decrease to within 5% of its original value (Fig. 3).

Energy expenditure. Figure 4A shows the simulated TEE in response to the metabolizable energy intake (MEI) during the Minnesota experiment. The initial baseline period was a state of energy balance where TEE was equal to MEI and body weight was maintained. At the onset of semistarvation, TEE dropped rapidly, primarily because PAE decreased by 17% and RMR decreased by 15% after the first week of semistarvation (Fig. 4B). Despite the drop of TEE at the beginning of semistarvation, TEE remained greater than MEI, and the resulting state of negative energy balance persisted until near the end of the semistarvation period where TEE eventually decreased to match MEI.

Figure 4B illustrates the components of TEE and shows that the simulated RMR closely matched the measured fall of RMR during semistarvation. However, despite the significant fall of RMR, the majority of the decrease of TEE was due to decreased PAE.

The refeeding periods induced a state of positive energy balance that allowed body weight and fat mass to be regained. The stepwise increases of MEI were met with parallel increases of TEE. The simulated RMR matched the measured values reasonably well, except at the onset of ad libitum feeding, when the measured RMR reached almost 2,200 kcal/day. However, the precision of this measurement was unclear because neither the uncertainty nor the individual subject data were reported.

Figure 5 depicts the measured RMR vs. L, along with the model simulation. The curve traced by RMR vs. L followed...
loop shown in Fig. 5, with the sequence of events indicated by the arrows on the curve traced during the simulation. The measured RMR when the lean mass was $51.3 \pm 1.5$ kg was significantly lower during semistarvation than during refeeding ($P < 0.0001$).

Initial, both RMR and lean body mass were constant. Semistarvation caused a rapid decrease of RMR due to adaptive thermogenesis, as indicated by the initial drop of the RMR vs. L curve. As the negative energy balance persisted, lean body mass decreased along with a concomitant decrease of RMR. The state of positive energy balance during refeeding resulted in repletion of lean mass and a parallel increase of RMR. In accordance with the data, the simulated RMR was greater during refeeding vs. semistarvation when compared at the same lean body mass. Specifically, the RMR after 12 wk of semistarvation was significantly lower than the RMR after 12 wk of refeeding despite the fact that both measurements occurred when lean mass was $51.3 \pm 1.5$ kg ($P < 0.0001$).

Macronutrient intake and oxidation. The macronutrient intake rates during the Minnesota experiment are depicted in Fig. 6A. The baseline diet, with an average basal carbohydrate intake ($CI_b$) = 1,826 kcal/day, fat intake ($FI_b$) = 1,343 kcal/day, and protein intake ($PI_b$) = 461 kcal/day, was provided for the initial weight maintenance period. Figure 6B shows the simulated oxidation rates of carbohydrate (CarbOx), fat (FatOx), and protein (ProtOx), which were approximately constant during the baseline period, with 52% of the TEE derived from carbohydrate oxidation, 38% from fat oxidation, and 10% from protein oxidation.

The semistarvation diet averaged $CI = 1,100$ kcal/day, $FI = 290$ kcal/day, and $PI = 195$ kcal/day for 24 wk (36). After the first week of semistarvation, the simulated CarbOx dropped by 35% and accounted for $\sim 42\%$ of the TEE. Small weekly variations of the experimental semistarvation diet were introduced, primarily via changes of carbohydrate intake, to obtain the desired rate of weight loss (36). The CarbOx followed carbohydrate intake during the remainder of the semistarvation period. The simulated ProtOx decreased by 12% after the first week of semistarvation and remained suppressed.

The simulated FatOx increased by 12% during the initial days of semistarvation. This increase was due to enhanced lipolysis associated with the reduced carbohydrate intake. After the first week of semistarvation, fat oxidation was 46% of the of the TEE. This led to a negative fat balance of more than 1,000 kcal/day that slowly became less negative as the semistarvation progressed and body fat was catabolized. At the end of the semistarvation period, all three macronutrient oxidation rates were roughly equal to their respective dietary intakes.

At the start of the controlled refeeding phase, carbohydrate oxidation increased by 30% and accounted for 64% of the TEE. Protein oxidation increased by 13% and accounted for 12% of the TEE, whereas fat oxidation sluggishly increased and accounted for 22% of the TEE. All macronutrient oxidation rates were less than their respective intake rates during the controlled refeeding period.

The final 8 wk of ad libitum feeding allowed the subjects to consume very large quantities of food, and they disproportionately increased their fat intake. Rapid adaptations of carbohydrate and protein oxidation accompanied their increased dietary intake. Fat oxidation continued its sluggish, almost linear increase during the entire refeeding phase, resulting in substantial differences between fat intake and oxidation, reaching almost 1,200 kcal/day during ad libitum feeding. Thus the difference between ad libitum fat intake and oxidation was the primary cause of the poststarvation body fat overshoot.

Gluconeogenesis. Figure 7 shows that GNGF and GNGP were initially constant at their baseline values, but the onset of semistarvation caused GNGF to decrease by 30%. This was caused by the reduced fat intake and the corresponding 78% decrease of exogenous glycerol, whereas endogenous glycerol gluconeogenesis increased by 23% due to increased adipose lipolysis (not shown). Despite the reduced protein intake at the onset of semistarvation, GNGP remained approximately con-
stant in the initial days of semistarvation due to the decrease of carbohydrate intake. Both GNGF and GNGP decreased slowly over the course of semistarvation as body fat and protein, respectively, were catabolized. Refeeding caused suppression of GNGP as carbohydrate intake increased. The inhibition of lipolysis upon refeeding and concomitant reduction of endogenous glycerol entering the gluconeogenic pathway was counterbalanced by the increase of exogenous glycerol from dietary fat. Therefore, GNGF was only slightly reduced upon refeeding and gradually increased as body fat, and thereby lipolysis, recovered.

Glycogen and DNL. Figure 8 shows a rapid initial drop of glycogen by \( \sim 100 \) g after the first week of semistarvation as expected. However, as the semistarvation progressed, the glycogen content surprisingly increased and eventually exceeded baseline levels by about 40%. This was caused by the progressive reduction of physical activity because glycogen remained low in simulations where the physical activity level was maintained throughout the semistarvation phase (not shown). The rate of glycogen increase during prolonged semistarvation was equivalent to an average positive carbohydrate balance of about 10 kcal/day. Given that the carbohydrate intake during the semistarvation period averaged 1,100 kcal/day, this implies that carbohydrate was remarkably well balanced to within 1% error. Refeeding initially induced further increases of glycogen content, but there was a trend toward normalization of glycogen, because carbohydrate oxidation was stimulated.

Figure 8 also shows that DNL mirrored the glycogen time course during the baseline and semistarvation periods. The basal DNL rate was about 100 kcal/day and decreased to 24 kcal/day after the first week of semistarvation. Controlled refeeding caused the DNL rate to increase to about 600 kcal/day and eventually exceeded 700 kcal/day during ad libitum feeding. Such high DNL rates were caused by the elevated glycogen content along with the high carbohydrate intake during refeeding. The elevated DNL was primarily responsible for the recovery of fat mass during controlled refeeding, because DNL was greater than the slight positive balance of fat intake over oxidation. However, during ad libitum feeding the situation was reversed, with DNL playing a secondary role to the drastically elevated fat intake.

Daily respiratory gas exchange. Figure 9 shows the simulated daily respiratory exchange of oxygen and carbon dioxide that was based upon the stoichiometry for carbohydrate, fat, and protein oxidation along with corrections introduced by gluconeogenic and lipogenic fluxes (21, 23). As expected from the above descriptions of energy expenditure and macronutrient oxidation, semistarvation caused a decrease of both oxygen consumption (\( \dot{V}O_2 \)) and carbon dioxide production (\( \dot{V}CO_2 \)), with a more rapid decrease of \( \dot{V}CO_2 \). Thus the resulting decrease of both the respiratory quotient (RQ) and the nonprotein respiratory quotient (NPRQ) reflected the increased reliance on fat oxidation. As semistarvation progressed, RQ and NPRQ gradually increased, whereas both \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) continued to decrease. This indicated that the contribution from fat oxidation was decreasing, because body fat was catabolized and energy expenditure continued to decrease. Refeeding caused
VO₂ and VCO₂ to increase, with VCO₂ increasing more rapidly as DNL was stimulated and the RQs transiently exceeded 1.

Triacylglycerol, protein, and glycogen turnover. Figure 10 shows the simulated daily turnover rates of triacylglycerol (TG), protein, and glycogen. Figure 10A shows that the daily average lipolysis and TG synthesis rates were initially balanced at 140 g/day. At the onset of semistarvation, lipolysis was stimulated by the reduction of carbohydrate intake and rose to 170 g/day, whereas TG synthesis dropped to 40 g/day primarily because of the decreased fat intake. The resulting state of negative fat balance caused body fat mass to decline and a concomitant progressive decrease of the lipolysis rate. Upon refeeding, TG synthesis increased, and the lipolysis rate was transiently depressed by the increased carbohydrate intake. However, the lipolysis rate gradually increased as fat mass was regained over the course of refeeding.

Protein degradation and synthesis were initially balanced at 300 g/day, as shown in Fig. 10B. Semistarvation caused daily protein synthesis to decrease immediately by 40 g/day followed by a gradual fall of the proteolysis rate caused by the decline of body protein. The difference between the proteolysis and protein synthesis rates during semistarvation was roughly constant, indicating that there was an approximately constant rate of protein catabolism. Refeeding caused protein synthesis to increase, and the resulting positive protein balance led to a gradual increase of the daily proteolysis rate.

Figure 10C shows the simulated daily glycogen turnover and illustrates that, except during transient changes of energy intake, glycogen synthesis and glycogenolysis rates were closely matched over the entire course of the study.

Nutrient balances. Figure 11 depicts the dynamic changes of the energy balance and individual macronutrient balances. Figure 11A shows that the fat balance closely tracked the energy balance, indicating that most of the energy deficit and surplus was accounted for by body fat changes. Figure 11B shows that long-term carbohydrate balance was more tightly controlled than protein balance because protein imbalances were sustained, whereas transient carbohydrate imbalances were quickly suppressed.

Simulation of Short-Term Caloric Restriction

Body weight and fat mass. Figure 12 shows the simulated changes of body weight and fat mass during 3 wk of caloric restriction by 40% of baseline energy requirements. Despite using model parameters derived from the Minnesota experiment, the model predictions matched the experimental measurements of Friedlander et al. (30), thereby providing support for the validity of the model. The body weight and fat mass had an almost linear decline during the 2 wk of caloric restriction, and the subjects lost 4 kg of body weight with slightly less than one-half coming from loss of body fat.

RMR and nitrogen balance. Figure 13 shows the predicted changes of RMR and nitrogen balance during the 3-wk caloric restriction, along with the experimental data. Again, the model
results match the experimental measurements and may help explain the observed negative nitrogen balance despite maintaining protein intake at baseline values. The model suggested that increased amino acid gluconeogenesis and oxidation resulted from the decreased carbohydrate intake and a concomitant fall of glycogen (not shown). Thus, whereas nitrogen intake was maintained, amino acid oxidation and gluconeogenesis increased, leading to negative nitrogen balance.

DISCUSSION

The Minnesota human starvation experiment is renowned for its comprehensive set of careful measurements over an extended duration of precisely controlled feeding. Such a study is unlikely to be repeated due to both its magnitude and the hardships endured by its subjects (36). At that time, it was not possible to measure all of the important metabolic fluxes participating in macronutrient balance. To address this issue, the present study introduced a computational model that integrates in vivo human data from a variety of published studies to predict the unmeasured daily rates of carbohydrate, fat, and protein turnover and oxidation, the TEE and its components, and the rates of gluconeogenesis and de novo lipogenesis.

Several investigators have used mathematical modeling to study the regulation of body weight (6, 37) and composition (4, 5, 11, 13, 14, 28, 38, 50, 64, 68). Most previous models of body weight and composition regulation assumed that the macronutrient composition of the diet had no effect on the partitioning of energy between lean and fat tissue, an assumption that runs counter to the nutrient balance concept (27, 31). Rather, most models defined a parameter or a simple function of initial body composition that determined that the fraction of energy imbalance partitioned toward deposition or mobilization of body protein vs. fat (11, 13, 14, 50). The physiological basis for this partitioning is unclear and begs the question of how body composition is regulated. A more recent model (28) incorporated carbohydrate and fat balances but ignored protein. A few previous models have also been applied to the data from the Minnesota experiment (4, 5, 37, 50), but the present study is the first to validate a human model by comparing model predictions with body composition and metabolic data from an independent human feeding study.

Previous mathematical models have represented RMR as a linear function of lean body mass (4, 5, 13, 14, 50, 64, 68), with coefficients occasionally significantly greater than those determined from cross-sectional analysis (16, 46). Such models fail to capture the loop traced by the RMR vs. L curve throughout semistarvation followed by refeeding. In a pair of elegant studies reanalyzing the Minnesota experiment, Dulloo et al. (17, 18) argued for the existence of an adaptive thermogenic mechanism to explain the measured RMR data. In agreement with these authors, the mathematical model presented here suggested that adaptive thermogenesis at the onset of semistarvation caused a rapid drop of RMR, which then decreased slowly as lean tissue was catabolized and protein turnover decreased. During refeeding, the level of adaptive thermogenesis and the energy costs of DNL and protein turnover were increased, resulting in a higher RMR at the same lean mass during refeeding vs. semistarvation.

The physiological mechanisms underlying adaptive thermogenesis are unknown. Several investigators (47, 50) have suggested that underfeeding causes metabolically active organs...
such as the liver, intestines, or kidneys to rapidly decrease their mass. Alternatively, the concentrations of circulating catecholamines and thyroid hormones have been observed to rapidly decrease with underfeeding (53, 65) and may reflect a reduction of sympathetic outflow and concomitant decrease of RMR. The present model was empirical and did not distinguish between these mechanisms.

Although changes of RMR contributed significantly to energy balance, the decrease of PAE during semistarvation was responsible for the majority of the slow decline of TEE. The physiological mechanisms underlying the changes of PAE are unclear. Because PAE for most common activities is proportional to body weight, the loss of body weight itself contributed to some decrease of PAE, but this was insufficient to account for the required decrease.

Adaptation of PAE has been hypothesized to involve altered energy efficiency of muscular work (16b, 40, 54). However, no change of physical activity energy efficiency was observed during treadmill tests at various stages of the Minnesota experiment (36, 58). Nevertheless, it is possible that changes of muscular work efficiency during typical daily activities may not have been reflected by the more physically demanding treadmill tests. Keys (36) noted that the subjects became apathetic and avoided voluntary physical activity toward the end of the semistarvation phase, but no systematic monitoring of physical activity was performed. Questionnaires completed by the subjects showed a progressive decrease of their physical “activity drive” over the course of semistarvation, which slowly improved with refeeding (36). Therefore, it is possible that PAE changes with semistarvation were the result of decreased voluntary as well as altered spontaneous physical activity expenditures such as fidgeting, posture control, and muscle tone (41).

The remarkable regulation of long-term carbohydrate balance was due to the limited whole body glycogen storage capacity. Therefore, relatively little energy could be accumulated or lost in the form of glycogen compared with protein or fat. However, large short-term changes of glycogen were permitted and led to potent modulation of both carbohydrate oxidation (24) and DNL (2). Thus glycogen feedback ensured that carbohydrate imbalances were only transient. In comparison, the relatively less significant short-term change of the body protein pool had little effect on protein oxidation so that protein imbalances were more sustained and led to long-term changes of the lean body mass. Unlike carbohydrate and protein, fat intake did not directly stimulate its own oxidation, and the relative change of the body fat pool only weakly affected fat oxidation (26, 55). Thus large fat imbalances were observed during semistarvation and refeeding, and these imbalances were sustained, resulting in significant changes of body fat mass.

The model predicted that the fat mass overshoot was not permanent provided that the original prestarvation diet and physical activity level were returned. However, recovery of the original body composition was predicted to take more than 1 yr. The predicted mechanism of the fat mass overshoot was an enhanced rate of de novo lipogenesis in the early refeeding period, followed by a dramatic increase of fat intake during ad libitum feeding. In contrast, Dulloo et al. (17, 18) postulated that the improved energy economy resulting from adaptive thermogenesis was somehow specifically channeled to accelerate fat mass gain during refeeding on the basis of a “fat stores memory.” The present study demonstrates that such a novel mechanism was not necessary to explain the data.

The present version of the computational model does not explicitly include the effects of hormones, but several hormonally-mediated effects are implicitly included. For example, insulin’s effect is implicit in the function of dietary carbohydrate oxidation, glycogen synthesis, and DNL. Future work will explicitly account for the effects of important hormones and will extend the model to study overfeeding and body composition regulation in altered metabolic states like obesity, anorexia nervosa, and cachexia.

APPENDIX

Detailed Description of the Mathematical Model

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. Because continued development of the model is part of an ongoing research program, additional relevant physiological data will be incorporated into the existing computational framework to improve the realism and predictive capabilities of the model.

The nutrient balance model depicted in Fig. 1 is an expression of the conservation of energy 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 the nutrient balance model was given by the following differential equations:

\[
\frac{dG}{dt} = C + GNG_F + GNG_P + DNPG(1 + h_p) + G(1 + h_C) - CarbOx
\]

\[
\frac{dF}{dt} = 3MFFAFI/MFG + DNLP - FatOx
\]

\[
\frac{dP}{dt} = PI - GNP - ProtOx
\]

where \(dG/dt\) is the rate of change of glycogen, \(dF/dt\) the rate of change of fat, and \(dP/dt\) the rate of change of protein. The energy densities of carbohydrate, fat, and protein, respectively (43). MFG = 860 g/mol and MFFA = 273 g/mol were the molecular masses of triacylglycerides and free fatty acids, respectively. The oxidation rates CarbOx, FatOx, and ProtOx summed to the total energy expenditure (TEE). Because 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 1 day. The nutrient balance equations were integrated using the 4th order Runge-Kutta algorithm with a timestep size of 0.1 days (52).

**Body Composition**

The body weight (BW) was the sum of the lean body mass (L) and the fat mass (F). L was computed using the following equation:

\[
L = BM + ECW + BCM
\]

\[
= BM + ECW + ICW + P + G + ICS
\]

where the lean mass is composed of bone mass (BM) extracellular water (ECW), and the body cell mass (BCM). BCM 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/BCM = 0.2, and the ICW fraction was ICW/BCM = 0.7 (3). ICW was directly related to P and G such that each gram of protein and glycogen was associated with hP = 2.7 and hG = 2 grams of water, respectively (45). ICW was a constant amount of intracellular water computed to attain the appropriate initial intracellular composition, assuming that G = 500 g.

I assumed that BM was 4% of the initial BW, as estimated by Keys (36). ECW varied slightly throughout the Minnesota experiment, increasing significantly at the end of the semistarvation phase (corresponding to clinical edema) and returning to baseline by the end of the refeeding period (36). I used the measured changes of ECW as a model input.

**Whole Body Total Energy Expenditure**

TEE was modeled by the following equation:

\[
\text{TEE} = \text{TEF} + \text{PAE} + \text{RMR} \tag{3}
\]

where TEF was the thermic effect of feeding, PAE was the energy expended for physical activity, 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.

**Thermal 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:

\[
\text{TEF} = \alpha_F \text{FI} + \alpha_p \text{PI} + \alpha_C \text{CI} \tag{4}
\]

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 (29).

**Adaptive Thermogenesis**

Energy imbalance causes an adaptation of metabolic rate that opposes weight change (16a, 16b, 19, 40). 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 (16a, 16b, 19, 40, 63, 66). The so-called adaptive thermogenesis is believed to affect both resting and nonresting energy expenditure (16a, 16b, 40) and has maximum amplitude during the dynamic phase of weight change (16a, 16b, 40, 65). Adaptive thermogenesis may also persist during weight maintenance at an altered BW, but the persistent effect has been debated (66). The non-RMR component of adaptive thermogenesis may reflect either altered efficiency or amount of muscular work (16a, 16b, 40, 41, 54).

The onset of adaptive thermogenesis is rapid and may correspond to altered levels of circulating thyroid hormones or catecholamines (53, 65). I defined a dimensionless adaptive thermogenesis parameter (T) that was generated by a first-order process in proportion to the departure from the baseline metabolizable energy intake (MBEi) = CBI + FIB + PIB:

\[
\frac{dT}{dt} = \frac{\lambda}{\tau} \left(\frac{\Delta \text{MEI}}{\text{MBEi}} - \frac{T}{\tau}\right) \tag{5}
\]

where \(\Delta \text{MEI}\) was the change from the baseline metabolizable energy intake, \(\tau = 7\) days was the estimated time constant for the onset of adaptive thermogenesis, and \(\lambda\) was a parameter to be 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 MEI and persisted as long as MEI was different from baseline. Importantly, the model allowed for the possibility that \(\lambda = 0\), meaning that no adaptive thermogenic mechanism was required to fit the data from the Minnesota experiment. The amount that the best fit value for \(\lambda\) differs from zero provides an indication of the extent of adaptive thermogenesis that occurred during the Minnesota experiment.

**Physical Activity Expenditure**

The energy expended for typical physical activities, such as walking or running, is proportional to the BW of the individual (61). Thus the following equation was used for the physical activity expenditure:

\[
\text{PAE} = \delta (1 + \sigma t) \text{BW} \tag{6}
\]

where \(\delta\) was the physical activity coefficient (in kcal·kg\(^{-1}\)·day\(^{-1}\)) that defined the daily physical activity level, and \(\text{BW} = L + F\) was the BW. The proportion of T that was allocated to the modification of PAE was determined by the parameter \(\sigma\) that was computed from the measured RMR data from the Minnesota experiment. The adaptation of PAE with T did not distinguish between altered efficiency vs. the amount of muscular work.

The activity coefficient, \(\delta\), in the Minnesota experiment was chosen to linearly decrease at the onset of semistarvation from its basal value, \(\delta_b\), to reach a minimum value, \(\delta_s\), by the end of the semistarvation period corresponding to the observed decrease of voluntary physical activity and the “activity drive” (36, 58). I used the measured RMR values during the baseline period and at the end of the semistarvation period to estimate the activity coefficients as follows:

\[
\delta = (\text{MEI} - \text{TEF} - \text{RMR})/\text{BW} \tag{7}
\]

where the index \(i\) indicates that the expression was used to compute the physical activity coefficient for measurements taken at either during the baseline period (\(i = b\)) or the end of the semistarvation period (\(i = s\)). While the MEI and expenditure were closely balanced during the baseline feeding period, this is only an approximation at the end of the semistarvation period, where BW was changing slowly. During the 20-wk refeeding phase, I assumed that \(\delta\) linearly returned to its basal value at the end of the refeeding period.

**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:

\[
\text{RMR} = \epsilon_r \text{M} + \epsilon_{BMC} (\text{BCM} - \text{M}) + \gamma_1 F + (1 - \epsilon_0) \text{DNL} \nonumber \]

\[
+ (1 - \epsilon_0)(\text{NGF}_p + \text{NGG}_p) + (\eta_p + \epsilon_0 \text{DF}_p + \eta_d \frac{dP}{dt} + \eta_d \text{DF}_d + \eta_0 \frac{dG}{dt}) \tag{8}
\]

where \(\epsilon_0 = 0.8\) was the efficiency of de novo lipogenesis (25), \(\epsilon_0 = 0.8\) was the efficiency of gluconeogenesis (9), and the constant \(\epsilon_r\) was a parameter chosen to ensure that the model achieved energy balance during the balanced baseline diet (see *Nutrient Balance Parameter Constraints* below).

The specific metabolic rate of adipose tissue was \(\gamma_F = 4.5\) kcal·kg\(^{-1}\)·day\(^{-1}\). The brain metabolic rate was \(\gamma_b = 240\) kcal·kg\(^{-1}\)·day\(^{-1}\), and its mass was \(M_b = 1.4\) kg, which does not change with weight gain or loss (22). The basal specific metabolic rate of the lean tissue cell mass, \(\gamma_{BMC} = 24\) kcal·kg\(^{-1}\)·day\(^{-1}\), was determined by the average organ masses and their specific metabolic rates according to the following equation:

\[
\gamma_{BMC} = \frac{\sum \gamma_i M_i}{\sum M_i} \tag{9}
\]

where \(\gamma_i\) and \(M_i\) are the average specific metabolic rate and mass,
respectively, of the organ indexed by \( i \). The organs included skeletal muscle \( (\gamma_{\text{SM}} = 13 \text{ kcal-kg}^{-1} \text{-day}^{-1}, M_{\text{SM}} = 28 \text{ kg}) \), liver \( (\gamma_{\text{L}} = 200 \text{ kcal-kg}^{-1} \text{-day}^{-1}, M_{\text{L}} = 1.8 \text{ kg}) \), kidney \( (\gamma_{\text{K}} = 440 \text{ kcal-kg}^{-1} \text{-day}^{-1}, M_{\text{K}} = 0.31 \text{ kg}) \), heart \( (\gamma_{\text{H}} = 440 \text{ kcal-kg}^{-1} \text{-day}^{-1}, M_{\text{H}} = 0.33 \text{ kg}) \), and residual lean tissue mass \( (\gamma_{\text{R}} = 12 \text{ kcal-kg}^{-1} \text{-day}^{-1}, M_{\text{R}} = 23.2 \text{ kg}) \), as provided by Elia (22). T affected the baseline specific metabolic rate for lean tissue cell mass according to the following equation:

\[
\gamma_{\text{BCM}} = \gamma_{\text{BCM}}^0 + (1 - \sigma)T
\]  

(10)

The last six terms of Eq. 8 accounted for the energy cost for turnover of protein, fat, and glycogen. Consider the energy cost for protein turnover with a synthesis rate \( \text{SynthP} \) and a degradation rate \( \text{DP} \), in g/day. I assumed that it cost \( \eta_{\text{P}} \text{SynthP} \) to synthesize \( P \) and that the energy required for degradation was \( \epsilon_{\text{P}} \text{DP} \). Because \( \text{DP}/dt = \text{SynthP} - \text{DP} \), the energy cost for protein turnover was given by \( \eta_{\text{P}} \text{SynthP} + \eta_{\text{D}} \text{DP}/dt \). Similar arguments led to the other terms of Eq. 8 representing the energy costs for fat and glycogen turnover, where the energy cost for degradation was negligible. The values for the parameters were: \( \eta_{\text{P}} = 0.18 \), \( \tau_{\text{P}} = 0.21 \), \( \epsilon_{\text{P}} = 0.17 \), and \( \eta_{\text{G}} = 0.86 \) kcal/g. These values were determined from the adenosine 5'-triphosphate (ATP) costs for the respective biochemical pathways (i.e., 8 ATP/TG synthesized, 2 ATP/glycosyl units of glycogen synthesized, 4 ATP/peptide bond synthesized + 1 ATP for amino acid transport, and 1 ATP/peptide bond hydrolyzed) (9, 20). I assumed that 19 kcal of macronutrient oxidation was required to synthesize 1 mol ATP (22).

**Daily Average Lipolysis**

The daily average lipolysis rate \( \text{DF} \) was modeled as

\[
\text{DF} = \hat{\text{DF}} \left[ \frac{\left( A_1 - B_1 \right) \times \exp\left( -k_1 \text{CI}/\text{CI}_b \right) + B_1 - 1}{\text{MAX}\left[ 1, \left( f/F_b \right)^{2/3} \right]} + 1 \right]
\]  

(11)

where \( \hat{\text{DF}} = 140 \text{ g/day} \) was the baseline daily average TG turnover rate given by 2/3 of the fed lipolysis rate plus 1/3 of the overnight-fasted lipolysis rate (34). The first \( \left( F/F_b \right)^{2/3} \) factor accounted for the dependence of the basal lipolysis rate on the total fat mass, and the 2/3 power reflected the hypothesis that basal lipolysis scales with adipocyte surface area (63).

The term in the square brackets accounted for the modulation of lipolysis by the carbohydrate intake. For example, complete starvation (CI = 0) stimulated average daily lipolysis by a factor of \( A_1 = 3.1 \), as computed by dividing the glyceral rate of appearance \( (R_g) \) after a 60-h fast (12) by the daily average glyceral \( R_g (34) \). Halving the carbohydrate intake increased the average lipolysis rate by factor of 1.4, as estimated by the increased area under the circulating free fatty acid curve after an isocaloric meal consisting of 33 vs. 66% carbohydrate (69). Given the above value for \( A_1 \), the effect of halving the carbohydrate content was modeled by choosing \( B_1 = 0.9 \). The following choice for \( k_1 \) ensured that the lipolysis rate was normalized for the baseline diet:

\[
k_1 = \ln \left( \frac{A_1 - B_1}{1 - B_1} \right)
\]  

(12)

Although obesity increases basal lipolysis, the stimulatory effect of decreased carbohydrate intake is impaired (70). This effect was modeled by dividing the exponential by the maximum of 1 and \( \left( F/F_b \right)^{2/3} \) such that the curve of lipolysis vs. CI becomes flattened as fat mass increases.

**Daily Average Proteolysis**

The daily average protein degradation rate \( \text{DP} \) was given by

\[
\text{DP} = \hat{\text{DP}} \left( \frac{P}{P_b} \right)
\]  

(13)

where \( \hat{\text{DP}} = 300 \text{ g/day} \) was the baseline daily protein turnover rate (62), and I assumed that the protein degradation rate was proportional to the normalized protein content of the body.

**Daily Average Glycogenolysis**

The daily average glycogen degradation rate \( \text{DG} \) was given by the following equation:

\[
\text{DG} = \hat{\text{DG}} \left( \frac{G}{G_b} \right)
\]  

(14)

where the baseline glycogen turnover rate, \( \hat{\text{DG}} = 180 \text{ g/day} \), 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 (44).

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

Mass conservation required that the daily average synthesis rates of fat, protein, and glycogen \( \text{SynthF}, \text{SynthP}, \), and \( \text{SynthG} \), respectively) were given by

\[
\text{SynthF} = \text{DF}_p + \frac{dF}{dt}
\]

\[
\text{SynthP} = \text{DP}_p + \frac{dP}{dt}
\]

\[
\text{SynthG} = \text{DG}_p + \frac{dG}{dt}
\]

(15)

**Glycerol 3-Phosphate Production**

Because adipose tissue lacks glycerol kinase, the glycerol 3-phosphate backbone of adipose TG is derived primarily from glucose. Thus the TG synthesis rate \( \text{SynthP} \) determined the rate of G3P according to

\[
\text{G3P} = \rho_{\text{SynthP}} \left( \frac{M_G}{M_{\text{TG}}} \right)
\]  

(16)

where \( M_G = 92 \text{ g/mol} \) and \( M_{\text{TG}} = 860 \text{ g/mol} \) are the molecular weights of glycerol and TG, respectively.

**Glycoler Gluconeogenesis**

Lipolysis of both endogenous and exogenous TG results in the release of glycerol that can be converted to glucose via gluconeogenesis (7). Recently, Trimmer et al. (60) demonstrated that glycerol disappearance could be fully accounted for by glucose production. Therefore, I assumed that all exogenous and endogenous glycerol entered the GNG pathway according to

\[
\text{GNG}_p = F_L \left( \frac{\rho_L M_{\text{G}}}{\rho_{\text{M}_{\text{TG}}} M_{\text{M}_{\text{TG}}}} \right) + \text{DP} \rho_{\text{C}} \left( \frac{M_{\text{C}}}{M_{\text{M}_{\text{TG}}}} \right)
\]  

(17)

Because glycerol cannot be used by adipose tissue for TG 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 nonadipose TG). 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 before oxidation. This error must be very small, because the total energy cost for glycerol GNG in the balanced state was only 25 kcal/day.

**Net Gluconeogenesis From Amino Acids**

The GNGp rate in the model referred to the net rate of gluconeogenesis from amino acid-derived carbon. Although all amino acids
except for 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 because the carbon skeleton of alanine is largely derived from carbohydrate precursors via skeletal muscle glycolysis (51). Nurjhan et al. (48) used a multiple isotopic tracer methodology to determine that the net alanine and gluconeogenic rate derived from amino acid carbon was 0.66 kcal/day in normal humans. Because the tracer techniques are known to underestimate gluconeogenesis by as much as 40% due to carbon exchange in the Krebs cycle (35), I estimated that the net basal gluconeogenic rate from amino acids \( GNG_P \) was 100 kcal/day.

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:

\[
GNG_P = GNGP \left( \frac{\Delta C}{C_{B}} + \frac{\Delta F}{F_{B}} + \frac{\Delta P}{P_{B}} \right)
\]

where the coefficients \( \Delta C = 0.5 \) and \( \Delta F = 0.3 \) were determined by solving Eq. 18 using two sets of data. The first measured a 56% increase in gluconeogenesis when protein intake was increased by a factor of 2.5-fold and carbohydrate intake was decreased by 20% (42). The second study measured a 42% decrease of alanine gluconeogenesis when both carbohydrate and protein were increased by 2.1-fold (15).

De Novo Lipogenesis

De novo lipogenesis (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 TG with a measured TG turnover rate of 50 g/day (57). Thus adipose DNL is about 94 kcal/day. Measurements of daily hepatic DNL in circulating very-low-density lipoproteins (VLDLs) have found that about 7% of VLDL TG occurs via DNL when consuming a basal diet of 30% fat, 50% carbohydrate, and 15% protein (33). Given that the daily VLDL TG secretion rate is about 33 g/day (56), the simulated O2 consumption (\( V\dot{O}_2 \)) and CO2 production (\( V\dot{CO}_2 \), in L/day) were computed according to

\[
\text{DNL} = \frac{\Delta C \times \text{G}/(\text{G}_0)^{d}}{(\text{G}/\text{G}_0)^{d} + K_{\text{DNL}}^{d}}
\]

I chose \( K_{\text{DNL}} = 2 \) 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 (1, 2, 33, 57).

Macronutrient Oxidation Rates

The whole body energy expenditure was equal to the sum of the carbohydrate, fat, and protein oxidation rates. I assumed that the minimum carbohydrate oxidation rate was equal to the sum of the gluconeogenic rates. Thus the remaining energy expenditure was apportioned between carbohydrate, fat, and protein oxidation according to the fractions \( f_C \), \( f_F \), and \( f_P \), respectively. Therefore, the substrate oxidation rates were given by

\[
\begin{align*}
\text{CarbOx} &= GNG_P + GNG_F + f_C(TEE - GNG_F - GNG_P) \\
\text{FatOx} &= f_F(TEE - GNG_F - GNG_P) \\
\text{ProtOx} &= f_P(TEE - GNG_P - GNG_F)
\end{align*}
\]

The substrate oxidation fraction for each macronutrient depends on a number of factors. First, increased lipolysis leads to concomitant increased fatty acid oxidation (12). Second, carbohydrate oxidation depends on the carbohydrate intake as well as the glycogen content (24, 39). Third, protein and carbohydrate intake directly stimulate protein and carbohydrate oxidation, respectively, but fat intake does not directly stimulate fat oxidation (26, 55). Fourth, I assumed that lean tissue supplies amino acids for oxidation in proportion to the proetolysis rate. Finally, although inactivity causes muscle wasting (8), increased physical activity may promote nitrogen retention (10, 59, 67), and the physical activity expenditure is primarily accounted for by increased oxidation of fat and carbohydrate (67). I modeled these effects by decreasing the fraction of energy expenditure derived from protein oxidation when physical activity increases.

On the basis of these physiological considerations, the substrate oxidation fractions were computed according to the following expressions:

\[
\begin{align*}
f_C &= w_C(D_F/D_{Net}) + w_PMAX[0,1 + S_C\Delta C/CI_b]/G/(G_{min} + G) \\
f_F &= w_F(D_P/D_{Net}) \\
f_P &= w_PMAX[0,1 + S_P\Delta P/PI_b]/S_Pexp(-k_A\delta/\theta_b)/Z
\end{align*}
\]

where the \( w \)’s and \( S \)’s were dimensionless model parameters, \( \Delta C \) and \( \Delta P \) were changes from \( C_0 \) and \( P_0 \), respectively. The small parameter, \( G_{\text{min}} = 1 \) g, was chosen such that carbohydrate oxidation was restrained when glycogen was depleted. 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.

Respiratory Gas Exchange

Oxidation of carbohydrate, fat, and protein was associated with consumption of oxygen (\( O_2 \)) and production of carbon dioxide (\( CO_2 \)) according to the stoichiometry of the net biochemical reactions (23):

\[
\begin{align*}
1 \text{ g carbohydrate} + 0.746 \text{ L } O_2 &\rightarrow 0.746 \text{ L } CO_2 + 0.6 \text{ g } H_2O \\
1 \text{ g fat} + 2.03 \text{ L } O_2 &\rightarrow 1.43 \text{ L } CO_2 + 1.09 \text{ g } H_2O \\
1 \text{ g protein} + 0.966 \text{ L } O_2 &\rightarrow 0.782 \text{ L } CO_2 + 0.45 \text{ g } H_2O + 0.16 \text{ g } N
\end{align*}
\]

Gluconeogenesis, lipogenesis, and G3P production also contribute to gas exchange, according to the following net reactions (21, 23):

\[
\begin{align*}
1 \text{ g protein} + 0.126 \text{ L } CO_2 &\rightarrow 0.16 \text{ g } N \\
1 \text{ g glycerol} + 0.133 \text{ L } O_2 &\rightarrow 0.17 \text{ g } H_2O \\
1 \text{ g carbohydrate} &\rightarrow 0.37 \text{ g fat} + 0.238 \text{ L } CO_2 + 0.2 \text{ g } H_2O \\
1 \text{ g carbohydrate} + 0.17 \text{ g } H_2O &\rightarrow 0.133 \text{ L } O_2
\end{align*}
\]

Oxidation of carbohydrate, fat, and protein can occur either directly or subsequent to intermediate exchange via lipogenesis or gluconeogenesis. In either case, the final ratio of \( CO_2 \) produced to \( O_2 \) consumed [i.e., the respiratory quotient (RQ)] is independent of any intermediate exchanges in accordance with the principles of indirect calorimetry (23).

The simulated \( O_2 \) consumption (\( V\dot{O}_2 \)) and \( CO_2 \) production (\( V\dot{CO}_2 \), in L/day) were computed according to
These equations were rearranged in matrix form as

\[
\begin{align*}
\mathbf{V}_\text{O}_2 &= 0.746 \frac{\text{CarbOx}}{\rho_c} + 2.03 \frac{\text{FatOx}}{\rho_f} + 0.966 \\
\mathbf{V}_\text{CO}_2 &= 0.746 \frac{\text{CarbOx}}{\rho_c} + 1.43 \frac{\text{FatOx}}{\rho_f} + 0.782 \frac{\text{ProtOx}}{\rho_p} + 0.133 \left( \frac{\text{GN}_G}{\rho_c} - \frac{G3P}{\rho_c} \right) \\
\text{ProtOx} &= 0.133 \left( \frac{\text{GN}_G}{\rho_c} - \frac{G3P}{\rho_c} \right) + 0.126 \left( \frac{\text{GN}_F}{\rho_p} + 0.238 \frac{\text{DNL}}{\rho_c} \right)
\end{align*}
\]

(24)


where the factor 6.25 was the number of grams of protein per gram of nitrogen.

**Nutrient Balance Parameter Constraints**

The initial feeding period of the Minnesota experiment provided a controlled diet for several weeks to maintain the baseline BW. I assumed that this basal diet achieved a state of nutrient and energy balance such that TEE = MEIb, where b refers to the balanced state. Therefore, at energy and nutrient balance

\[
\begin{align*}
\text{MEI}_b &= \text{TEF}_b + \text{PAE}_b + \text{RMR}_b \\
&= \text{TEF}_b + \delta_Bw_B \\
&+ E_0 + \gamma_Mn_M + \gamma_{\text{BCM}}(\text{BCM}_b - M_0) + \gamma_F_F \\
&+ (1 - \epsilon_g)DNL_a + (1 - \epsilon_g)( GN_{G}Gp + G_{N}Gp) \\
&+ (\epsilon_p + \epsilon_D)D_F + \epsilon_D F + \epsilon_D G
\end{align*}
\]

(26)

which can be solved for the constant Ec.

Nutrient balance implies that the left-hand sides of Eq. 1 are zero. Thus rearrangement of the nutrient balance equations gave

\[
\begin{align*}
\text{CarbOx}_b &= \text{Cl}_b + \GN_GF - \GN_G - \text{DNL}_a - \text{G3P}_a \\
\text{FatOx}_b &= 3\text{Mar}_P + \text{DNL}_a \\
\text{ProtOx}_b &= \text{Pb} - \GN_GP
\end{align*}
\]

(27)

By substituting Eqs. 20 and 21 at nutrient balance, I obtained

\[
\frac{w_F}{1 + w_F + w_G + w_C + w_F} = \left( \frac{\text{Mar}_P + \text{DNL}_a + \text{DNL}_a}{\text{Mar}_P + \text{DNL}_a + \text{Mar}_P + \text{DNL}_a} \right) = \zeta_F \\
\frac{w_G + w_C}{1 + w_F + w_G + w_C + w_F} = \left( \frac{\text{Mar}_P + \text{DNL}_a}{\text{Mar}_P + \text{DNL}_a + \text{Mar}_P + \text{DNL}_a} \right) = \zeta_C \\
\frac{w_C}{1 + w_F + w_G + w_C + w_F} = \left( \frac{\text{Mar}_P}{\text{Mar}_P + \text{DNL}_a + \text{Mar}_P + \text{DNL}_a} \right) = \zeta_F
\]

(28)

These equations were rearranged in matrix form as

\[
\begin{bmatrix}
(\zeta_F - 1) & \zeta_F & \zeta_F \\
\zeta_C & (\zeta_C - 1) & \zeta_C \\
\zeta_F & \zeta_F & (\zeta_F - 1)
\end{bmatrix}
\begin{bmatrix}
w_F \\
w_G + w_C \\
1 + w_F + w_C + w_F
\end{bmatrix} = \begin{bmatrix} 0 \\
0 \\
0
\end{bmatrix}
\]

(29)

Elementary algebra led to the following parameter constraints required to achieve nutrient balance:

\[
\begin{align*}
w_G &= \frac{\zeta_C}{\zeta_F} (1 + w_F) - w_C \\
w_F &= \left( \frac{\zeta_F}{1 - \zeta_F} \right) (1 + w_F)
\end{align*}
\]

(30)

**Carbohydrate Perturbation Constraint**

The parameters wc and Sc determined how the model adapted to changes of carbohydrate intake. I specified that an additional dietary carbohydrate intake (ΔCl) above baseline (Clb) resulted in an initial positive carbohydrate imbalance of κCΔCI, where 0 < κC < 1 specified the proportion of ΔCl directed towards glycogen storage. Thus the glycogen increment was ΔG = κCΔCl/ρc. The goal was to solve for the parameter Sc such that the correct amount of carbohydrate was oxidized and deposited as glycogen during short-term carbohydrate overfeeding. On the basis of the carbohydrate overfeeding study of Horton et al. (32), I chose κC = 0.5 when ΔCl = 1,500 kcal/day.

The change of total energy expenditure was given by

\[
\Delta\text{TEE}_{\text{ACI}} = \Delta\text{TEF} + \Delta\text{PAE} + \Delta\text{RMR}
\]

(31)

For a carbohydrate perturbation, the perturbed energy expenditure components were

\[
\Delta\text{TEF} = \alpha_C\Delta\text{Cl} \\
\Delta\text{PAE} = \delta_Bw_B\sigma\Delta\text{Cl} \\
\Delta\text{RMR} = \kappa_C\epsilon_F\Delta\text{Cl} + \gamma_{\text{BCM}}(1 - \sigma)\Delta\text{Cl} \\
+ (1 - \epsilon_g)\Delta\text{DNL} + (1 - \epsilon_g)\Delta\text{GNG}
\]

(32)\(\Delta\text{Cl} = \kappa_C\Delta\text{Cl} / \text{ME}_b \]

(33)\(\Delta\text{T} = \lambda\Delta\text{Cl} / \text{ME}_b \]

was the average value of the thermogenesis parameter (T) over 1 day and ΔDNL was computed at the midpoint of the glycogen increment according to

**Table 1. Model parameters determined from published data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ρF</td>
<td>9.4 kcal/g</td>
<td>Energy density of F</td>
</tr>
<tr>
<td>ρP</td>
<td>4.7 kcal/g</td>
<td>Energy density of P</td>
</tr>
<tr>
<td>ρc</td>
<td>4.2 kcal/g</td>
<td>Energy density of G</td>
</tr>
<tr>
<td>BM</td>
<td>2.7 kg</td>
<td>Bone mass</td>
</tr>
<tr>
<td>hF</td>
<td>2 g H2O/g</td>
<td>P hydration coefficient</td>
</tr>
<tr>
<td>hG</td>
<td>2.7 g H2O/g</td>
<td>G hydration coefficient</td>
</tr>
<tr>
<td>εF</td>
<td>0.18 kcal/g</td>
<td>F synthesis cost</td>
</tr>
<tr>
<td>εP</td>
<td>0.86 kcal/g</td>
<td>P synthesis cost</td>
</tr>
<tr>
<td>εC</td>
<td>0.17 kcal/g</td>
<td>P degradation cost</td>
</tr>
<tr>
<td>εG</td>
<td>0.21 kcal/g</td>
<td>G synthesis cost</td>
</tr>
<tr>
<td>δF</td>
<td>0.8</td>
<td>DNL efficiency</td>
</tr>
<tr>
<td>δP</td>
<td>0.8</td>
<td>DNL efficiency</td>
</tr>
<tr>
<td>δEC</td>
<td>0.025</td>
<td>TEF factor for F</td>
</tr>
<tr>
<td>δPC</td>
<td>0.075</td>
<td>TEF factor for Cl</td>
</tr>
<tr>
<td>δEC</td>
<td>0.25</td>
<td>TEF factor for P</td>
</tr>
<tr>
<td>γF</td>
<td>4.5 kcal•kg⁻¹•day⁻¹</td>
<td>Specific RMR for adipose</td>
</tr>
<tr>
<td>γG</td>
<td>24 kcal•kg⁻¹•day⁻¹</td>
<td>Basal specific RMR for BCM</td>
</tr>
<tr>
<td>ΔF</td>
<td>3.1</td>
<td>Minimum lipolysis change</td>
</tr>
<tr>
<td>ΔG</td>
<td>0.9</td>
<td>Basal GNGP</td>
</tr>
<tr>
<td>ΔCla</td>
<td>200 kcal/day</td>
<td>Effect of Cl on GNGP</td>
</tr>
<tr>
<td>ΔGp</td>
<td>0.3</td>
<td>Effect of P on GNGP</td>
</tr>
<tr>
<td>kDNL</td>
<td>2</td>
<td>Glycogen constant for DNL</td>
</tr>
<tr>
<td>d</td>
<td>4</td>
<td>Hill coefficient for DNL</td>
</tr>
</tbody>
</table>

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\[
\Delta \text{DNL} = \frac{(\text{CI}_b + \Delta \text{CI})(1 + G/2\text{G}_b)^d}{\text{K}_{\text{DNL}}^b + (1 + G/2\text{G}_b)^d} - \text{DNL}_b
\]  

(36)

The change of the gluconeogenic rate, \(\Delta \text{GNG}\), was given by

\[
\Delta \text{GNG} = \rho_C \left( \frac{M_C}{M_{\text{TG}}^b} \right) \Delta \text{P}_f \left[ [A_L - B_L] \times \exp \left[ -k_L (1 + \Delta \text{CI}/\text{CI}_b) \right] + B_L - 1 \right]
\]

(37)

The carbohydrate balance equation gave

\[
f_c(\text{MEI}_b + \Delta \text{TEE}_{\text{ACT}} - \text{GNG}_P - \text{GNG}_F - \Delta \text{GNG}) = \text{CI}_b + (1 - \kappa_c)\Delta \text{CI} - (\text{DNL}_b + \Delta \text{DNL}) - (\text{G3P}_b + \Delta \text{G3P})
\]

(38)

where

\[
\text{G3P}_b + \Delta \text{G3P} = \rho_C \left( \frac{M_C}{M_{\text{TG}}^b} \right) \left[ D_F + \frac{1}{\rho_F} [\text{FI}_b + \text{DNL}_b + \Delta \text{DNL}] - f_p(\text{MEI}_b + \Delta \text{TEE}_{\text{ACT}} - \text{GNG}_P - \text{GNG}_F - \Delta \text{GNG}) \right]
\]

(39)

and

\[
D_F = D_F \left[ [A_L - B_L] \times \exp \left[ -k_L (1 + \Delta \text{CI}/\text{CI}_b) \right] + B_L \right]
\]

(40)

Therefore,

\[
f_c - \left( \frac{\rho_C M_C}{\rho_F M_{\text{TG}}^b} \right)f_F = \Theta
\]

(41)

where \(\Theta\) was defined as:

\[
\Theta = \frac{\text{CI}_b + (1 - \kappa_c)\Delta \text{CI} - (1 + \rho_C M_C/\rho_F M_{\text{TG}}^b)(\text{DNL}_b + \Delta \text{DNL}) - \rho_C M_C/\rho_F M_{\text{TG}}^b(D_F + \text{FI}_b/\rho_F)}{\text{MEI}_b + \Delta \text{TEE}_{\text{ACT}} - \text{GNG}_P - \text{GNG}_F - \Delta \text{GNG}}
\]

(42)

Using Eq. 21, I solved Eq. 40 for \(S_c\), which gave the carbohydrate feeding constraint

\[
S_c = \frac{\text{Cl}_b \left( \Theta (1 + w_p) + (\Theta + \rho_C M_C/\rho_F M_{\text{TG}}^b) w_p \right) w_c}{(1 - \Theta) w_c - w_c - 1}
\]

(43)

where

\[
\Delta \text{RMR} = \frac{\kappa_p \text{TEF}}{\rho_F} \left[ \Delta \text{PI} + (1 - \epsilon_p)(\Delta \text{GNG}_P + \Delta \text{GNG}_F) + (1 - \epsilon_d)\Delta \text{DNL} \right]
\]

(49)

Table 3. Parameter values determined from energy balance, nutrient balance, protein, and carbohydrate perturbation constraints

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\delta_p)</td>
<td>26 kcal·kg(^{-1})·day(^{-1})</td>
<td>Basal physical activity</td>
</tr>
<tr>
<td>(\delta_s)</td>
<td>9 kcal·kg(^{-1})·day(^{-1})</td>
<td>Minimum physical activity</td>
</tr>
<tr>
<td>(E)</td>
<td>-270 kcal/day</td>
<td>Constant energy expenditure</td>
</tr>
<tr>
<td>(S_{\text{PT}})</td>
<td>7</td>
<td>Sensitivity of oxidation to PI changes</td>
</tr>
<tr>
<td>(S_{\text{CT}})</td>
<td>0.7</td>
<td>Sensitivity of oxidation to CI changes</td>
</tr>
<tr>
<td>(w_g)</td>
<td>1.7</td>
<td>Weighting of oxidation for glycogenolysis</td>
</tr>
<tr>
<td>(w_f)</td>
<td>4.1</td>
<td>Weighting of oxidation for lipolysis</td>
</tr>
</tbody>
</table>
Because the perturbed diet was isocaloric and there were no changes of physical activity,

$$\Delta P_{AE} = \Delta T = 0$$

(50)

Furthermore, I assumed that glycerol concentration would remain relatively unchanged with the isocaloric diet perturbation because $\Delta C_l$ was small and its effect counterbalanced by changes of GNG due to the large increase of protein intake. Therefore, I assumed that

$$\Delta DNL = \frac{\Delta C_l}{K_{\text{inst}} + 1}$$

(51)

Using Eq. 21, I solved Eq. 44 for $S_p$, which gave the following constraint:

$$S_p = \frac{P L}{\Delta P L} \left[ \frac{\Phi (w_F + w_G + w_C)}{1 - \Phi w_F} - 1 \right]$$

(52)

where $\Phi$ was defined as

$$\Phi = \frac{P L_F + (1 - k_F) \Delta P - GNGF - \Delta GNGF}{MEI_b + \Delta TEE_{\text{ep}} - GNGF - \Delta GNGF - GNGP - \Delta GNGP}$$

(53)

and

$$w_F = w_F [A_1 - B_1] \times \exp \left[-k_F (1 + \Delta C_l/C_l) + B_1 \right]$$

$$w_C = w_C (1 + S_1 \Delta C_l/C_l)$$

(54)

**Model Parameter Values**

The model parameter values listed above were obtained from the cited published literature and are listed in Table 1. The parameters $S_A$, $w_F$, $w_C$, $\alpha$, and $\sigma$ were determined using a downhill simplex algorithm (52) to minimize the sum of squares of weighted residuals between the simulation outputs and the data from the Minnesota human starvation experiment (36). I used the following measurement error estimates to define the weights for the parameter optimization algorithm: $\Delta B_W = 0.2 \text{ kg}$, $\Delta P_{FM} = 1 \text{ kg}$, and $\Delta R_{MR} = 50 \text{ kcal/day}$. The best fit parameter values are listed in Table 2, and the constrained parameters are listed in Table 3.

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**REFERENCES**


