Changes in macronutrient balance during over- and underfeeding assessed by 12-d continuous whole-body calorimetry

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ABSTRACT Alterations in energy balance must be accommodated by adjustments in the net storage of the major energy-yielding macronutrients: carbohydrate, protein, and fat. This study used continuous whole-body calorimetry to measure changes in energy expenditure and substrate oxidation during a 12-d imposed energy imbalance in six lean men on mixed diets (overfeeding: 16.5 MJ/d, +33%, n = 3; underfeeding: 3.5 MJ/d, −67%, n = 3). Changes in total energy expenditure (TEE) and its components were modest; TEE changed by +6.2% (overfeeding) and −10.5% (underfeeding). In consequence, body weight changed by +2.90 and −3.18 kg. Marked changes in metabolic fuel selection occurred over the course of the study. Carbohydrate intake (540 and 83 g/d for overfeeding and underfeeding, respectively) exerted direct autoregulatory feedback on carbohydrate oxidation (551 and 106 g/d at day 12 for overfeeding and underfeeding, respectively). Subjects were close to balance by day 5. Changes in protein oxidation were small and not sufficient to prevent the oxidation of body protein mass, or its accretion, in response to energy deficit or surplus. Fat oxidation (59 and 177 g/d for overfeeding and underfeeding, respectively) was not sensitive to dietary fat intake (150 and 20 g/d, for overfeeding and underfeeding, respectively), rather, its oxidation was inversely related to the oxidation of other substrates. Changes in fat balance accounted for 74.1% and 84.0% of the energy imbalance during overfeeding and underfeeding, respectively. This study shows a clear oxidative hierarchy for the macronutrients. Metabolic fuel selection is dominated by the need to maintain carbohydrate balance. This induces inappropriate counterregulatory alterations in fat oxidation during energy surplus. Am J Clin Nutr 1996;64:259–66.

KEY WORDS Overfeeding, underfeeding, macronutrient balance, whole-body calorimetry

INTRODUCTION Numerous studies have examined the metabolic response to energy deficit or surplus, and evidence has been sought for autoregulatory mechanisms, which may defend the body against periods of energy imbalance. These have shown that adaptive changes in energy expenditure are small and that changes in body weight and composition are the primary mechanisms by which the body accommodates energy excess or inadequacy.

More recently, there has been an increase in interest in the mechanisms by which the individual macronutrients are balanced (1–3). In particular, there is increasing awareness that the development of obesity might more usefully be viewed as a failure to maintain fat balance and, hence, as a failure to maintain energy balance.

The energy balance equation can be reformulated in terms of separate balance equations for the individual macronutrients as shown in Figure 1. This greatly aids the understanding of the underlying control processes because these differ radically between the different macronutrients (3). The figure describes an oxidative hierarchy in which the macronutrients that are least easily stored assume priority in the metabolic fuel mixture being combusted (3, 4). Alcohol in the bloodstream dominates oxidative pathways because it cannot be stored and must be detoxified (5, 6). Ingestion of alcohol elicits a rapid rise in alcohol combustion that is maintained until all alcohol is cleared. Carbohydrate oxidation also shows tight autoregulatory linkage with carbohydrate intake because the capacity for glycogen storage is limited (7–10) and de novo lipogenesis is likely to be quantitatively unimportant (11, 12). Protein oxidation (and hence nitrogen excretion) is likewise linked to protein intake (13). The relative positions of protein and carbohydrate in this hierarchy remain a matter of debate (3, 4). In complete contrast with the other macronutrients, there appears to be virtually no autoregulatory linkage between fat intake and fat oxidation (14–16). Indeed, because fat oxidation is suppressed by high intakes of the other macronutrients, it tends to decline under conditions of energy excess in a manner that does not assist in the restoration of balance (9, 10). This lack of autoregulatory control is the main physiologic reason why fat balance is so often poorly regulated.

In conditions of long-term or severe energy imbalance the contribution of glycogen stores becomes trivial and most of the imbalance is reflected by fat stores. However, in the shorter-term, changes in energy status can be accommodated through a...
Stimulating subsequent appetite (2, 4, 8), and may therefore be postingestive effects on energy balance, for instance, by influencing subsequent appetite (2, 4, 8), and may therefore be important in trying to understand the overall homeostatic mechanisms controlling energy balance.

In this study we examined the effect of energy excess and deficit on both the short-term and long-term changes in macronutrient stores in healthy subjects by using continuous whole-body calorimetry to obtain complete substrate balance measurements in lean men during 12 d of over- and underfeeding.

SUBJECTS AND METHODS

Study design

Five healthy male volunteers were recruited by advertisement; one volunteer participated in both over- and underfeeding phases of the study. The study was approved by the Dunn Nutrition Centre Ethical Committee and written informed consent was obtained from all the subjects.

For 7 d before the start of the study subjects lived in a metabolic suite, were weighed daily, and were fed to maintain energy balance. Subjects entered the calorimeter at 2000 on day 0 and after an overnight equilibration period, measurements commenced at 0900 on day 1 and continued uninterrupted for 12 d until 0900 on day 13. Body weight was measured at 0900 each morning. While in the calorimeter, all subjects followed the same protocol, which comprised 8 h of sleeping, a 1-h measurement of basal metabolic rate (BMR), three 40-min periods on a cycle ergometer at a work rate of 50 W (1 kp and 50 rpm), 190 min standing (including dressing and undressing), and the remainder in discretionary sedentary activities that varied between subjects, including watching television and videos, reading, writing, and making jigsaw puzzles.

Whole-body calorimetry

The calorimeter had a volume of 29.4 m³ and was maintained at a temperature of 24 ± 0.25 °C. It was ventilated at a rate selected individually for each subject to produce a range of carbon dioxide concentrations that corresponded to the most linear region of the carbon dioxide analyzer scale. Ventilation rates ranged from 140 to 200 L/min and were monitored by rotameter (type 2100; KDG Flowmeters, Burgess Hill, United Kingdom). Oxygen concentrations were measured by using a paramagnetic analyzer (model OA184; Servomex, Crowborough, United Kingdom), carbon dioxide by a single-beam infrared analyzer (model SB300; ADC, Hoddesdon, United Kingdom), and water vapor by an optical condensing dew point meter (model DP5; MBW Electronik, Wettingen, Switzerland). Data were collected via an integrated measurement system (type 3510; Solartron, Farnborough, United Kingdom) into a minicomputer (Hewlett Packard, Winnersh, United Kingdom).

After initial manual calibration, automatic measurement recording began with a computer-controlled calibration sequence during which the analyzers sampled nitrogen to record the zero readings, 1% carbon dioxide in air to record the carbon dioxide analyzer span, and fresh air to record the oxygen analyzer span. This sequence was repeated every 4 h. Between calibrations, calorimeter air was sampled at 400-s intervals with a record of ventilating air composition every 40 min. Exchange rates for oxygen and carbon dioxide were calculated by using the expressions of Brown et al (17) for suction-ventilated systems. These expressions produce gas-exchange measurements that immediately reflect changes in the subject’s metabolism by accounting for the rate of change of the oxygen and carbon dioxide contents of the chamber as well as the rates of influx and efflux of these gases.

Substrate oxidation calculations

All urine samples were collected individually and aliquots were frozen for the determination of nitrogen excretion (Kjeltec System 1, model DS200; Tecator, Bristol, United Kingdom) from which net protein oxidation was calculated. The net oxidation of fat and carbohydrate was calculated from oxygen consumption, carbon dioxide production, and nitrogen excretion by assuming that the ratio of oxygen consumption to carbon dioxide production to oxygen consumption for carbohydrate, fat, and protein, respectively, is 1.0, 0.71, and 0.835 and the volume of oxygen consumed per gram of substrate oxidized to be 0.746, 2.01, and 0.952 L/g (18). The energy equivalence of protein, carbohydrate, and fat was assumed to be 18.56, 39.4 kJ/g (18). Further details of the calculation are presented elsewhere (19, 20). The accuracy of oxidation estimates for fat and carbohydrate is limited by the accuracy with which the gas exchange can be measured. In this chamber the limit of detectable errors was ±1%, which limits the accuracy of estimates of fat and carbohydrate oxidation to ±9.5 and ±20 g/d, respectively (19, 20). Substrate balance was calculated as the difference between measured metabolizable intake and oxidation.

![Diagram of nutrient energy balance](image-url)

**Figure 1.** The energy balance equation expressed in terms of the individual energy-yielding macronutrients. Reproduced with permission from reference 5.
Macronutrient intake

While in the calorimeter, subjects were fed a 3-d rotating diet designed to produce either an energy deficit of approximately two-thirds or an energy surplus of one-third, based on predicted total energy expenditure (TEE). Overfeeding and underfeeding were initiated on the first day in the calorimeter. The overfeeding diets provided 15% of energy as protein, 35% as fat, and 50% as carbohydrate, although the precise selection of food varied to suit individual preferences. Two subjects received 15 MJ/d and one subject 19.5 MJ/d. The underfeeding diet provided 3.5 MJ/d for all subjects. This was supplied partly in the form of a commercial very-low-energy diet (Modifast; Kent Pharmaceuticals, Ashford, United Kingdom), which provided 1.86 MJ/d, comprising 50 g protein, 7 g fat, and 45 g carbohydrate and was nutritionally complete with respect to micronutrients. The remainder of the diet was supplied as normal foodstuffs, giving a final macronutrient composition of 31% of energy as protein, 24% as fat, and 45% as carbohydrate. Drinking water was allowed ad libitum.

Duplicate samples of the diet were prepared and analyzed for gross energy (adiabatic bomb calorimeter; Gallenkamp, Loughborough, United Kingdom) and nitrogen contents (Tecator DS200 and Kjeltec System 1). Fat and carbohydrate contents were then calculated. Radioopaque markers were given with the first and last meal in the calorimeter and all fecal samples produced in the calorimeter and for several days thereafter were weighed, frozen, and X-rayed to identify the presence of the markers. Feces produced between the appearance of the first and last markers were analyzed for gross energy and nitrogen contents as described above. There was no significant trend in nitrogen excretion in feces, so the total fecal losses of protein were summed for the 12-d period and assumed to be equally divided across the entire measurement period to overcome day-to-day variations. The fat and carbohydrate contents were calculated by assuming that ash represents 12% of the dry fecal weight. Skin losses of nitrogen were assumed to remain unchanged during energy imbalance relative to habitual losses, which were estimated as 4% of habitual dietary nitrogen intake (21). Measured metabolizable energy was calculated as gross energy intake minus fecal and skin losses. Dietary intake, fecal losses, energy expenditure, and substrate oxidation were all computed for 12 24-h periods from 0900 on day 1 to 0900 on day 13.

RESULTS

The physical characteristics of the subjects at the start of the study are shown in Table 1, along with the prescribed mean energy intake, measured energy expenditure during a baseline measurement of energy expenditure before the main study, and calculated energy balance. In most cases the percentage energy surplus or deficit was close to that prescribed, (with the exception of subject 2 who was overfed by only 25% and subject 6 who was overfed by 39%).

The metabolic response of the subjects to overfeeding is shown in Figure 2 and to underfeeding in Figure 3. During overfeeding, BMR increased by 0.42 MJ (5.7%) and TEE increased by 0.75 MJ (6.2%). During underfeeding BMR decreased by 0.82 MJ (8.3%) and TEE decreased by 1.20 MJ (10.5%). These changes were small in relation to the energy deficit or surplus, and energy imbalance was principally accommodated by progressive changes in body weight that were essentially linear over time. Weight increased by 2.90 kg (+4.0%) during overfeeding and decreased by 3.18 kg (−4.3%) during underfeeding. The change in fat stores measured by substrate balance accounted for 31.3% of the weight change during overfeeding and 65.9% during underfeeding. Further details of the change in body composition and the comparison with estimates from in vivo body composition studies were reported previously (19).

Marked changes in the respiratory quotient (RQ) occurred in all subjects, reflecting changes in the proportion of substrates contributing to oxidation. On the baseline diet, assuming RQ equals the food quotient (FQ), the RQ would be expected to be 0.874. During overfeeding, RQ increased from 0.875 on day 1 to 0.92 on day 12 and during underfeeding decreased from 0.803 to 0.764. This illustrates the rapidly induced change in substrate oxidation, even on the first day of energy imbalance, relative to the baseline diet.

The changes in metabolic fuel selection are shown in Figure 4 and changes in macronutrient balance in relation to intake and oxidation are shown in more detail for carbohydrate, protein, and fat in Figures 5 and 6. During both over- and underfeeding the contribution of protein to the fuel mixture remained remarkably constant, whereas major changes occurred in the relative contribution of carbohydrate and fat.

Over the first few days of underfeeding there was a sharp decrease in carbohydrate oxidation and by day 4 onward carbohydrate intake and oxidation were closely matched, although there remained a small, persistent daily negative carbohydrate balance with intakes of 83 g/d compared with oxidation of 106 g/d (1.67 MJ/d) on day 12, which presumably reflects a gradual but progressive decrease in muscle glycogen. To meet the body’s energy requirement endogenous fat oxidation increased to ~177 g/d (6.96 MJ/d) relative to an intake of 20 g/d. By day 12 the proportion of the subjects’ energy requirement derived

<table>
<thead>
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<th>TABLE 1 Subject characteristics</th>
<th>Underfeeding study</th>
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<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 3*</td>
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<tr>
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<td>Height (m)</td>
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<tr>
<td>Energy balance (%)</td>
<td>−67</td>
<td>−66</td>
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* Same person.
FIGURE 2. Cumulative changes in components of energy balance during overfeeding relative to day 1. By day 12 the change in BMR ranged from +0.37 to +0.83 MJ/d and the change in TEE ranged from +0.33 to +1.16 MJ/d. BMR, basal metabolic rate; TEE, total energy expenditure.

from protein, carbohydrate, and fat was 14.8%, 16.6%, and 68.6%, respectively.

During overfeeding, carbohydrate balance was again achieved after the first few days; by day 12 carbohydrate oxidation was 551 g/d compared with an intake of 539 g/d. This rate of carbohydrate oxidation was about five times greater than at the end of the underfeeding period, providing evidence of the striking autoregulatory control of carbohydrate intake on its own oxidation. Because carbohydrate oxidation was providing \( \approx 8.68 \) MJ/d, there was a greatly reduced need for fat as an energy source; fat oxidation was suppressed to \( \approx 59 \) g/d or 2.32 MJ/d and the bulk of the 150 g dietary fat/d was stored. Hence, by day 12 the proportion of the subjects’ energy requirement derived from protein, carbohydrate, and fat was 13.3%, 68.3%, and 18.4%, respectively.

The contribution of individual macronutrients to net cumulative energy balance is shown in Figure 7. Overall, during underfeeding, protein, carbohydrate, and fat represent 9.5%, 6.2%, and 84.3% of the endogenous energy oxidized and during overfeeding 8.7%, 17.8%, and 73.5% of the energy stored, respectively. Expressed in grams these changes repro-
they have exceptionally good performance specifications with limits of detectable errors at ± 10 g/d for fat and ± 20 g/d for carbohydrate. This was maintained during the long calorimeter runs by recalibration of the gas analyzers every 4 h and appropriate interpolation to account for any instrument drift. The close agreement by day 12 between the known carbohydrate intakes and the computed oxidation rates provides good evidence that the macronutrient measurements are robust.

The measurements of energy expenditure confirm the well known inability of the body to invoke sufficient metabolic changes to prevent alterations in body weight in response to energy excess or deficiency (22, 23). Of greater interest are the details of the changes in macronutrient stores and the mechanisms by which these occur. This study extends observations on the oxidative hierarchy of macronutrient regulation made previously by ourselves and others under a variety of conditions, including subjects in energy balance or fed ad libitum (3, 9, 24–26), subjects overfed for short periods of time (6, 7, 14), subjects overfed carbohydrate for long periods (24, 27, 28), and subjects in balance, overfed and underfed for long periods but with only spot measurements of fuel utilization at the end of the treatments (10, 22). In common with these studies, ours shows that fuel selection is dominated by carbohydrate intake, and that when carbohydrate oxidation rises in response to overfeeding there is a profound counterregulatory suppression of fat oxidation even in the presence of large quantities of exogenous fat. The converse holds true during underfeeding. There is no evidence of fat-driven autoregulation, rather, fat oxidation simply reflects the difference between the rates of carbohydrate and protein oxidation and TEE.

The changes in carbohydrate utilization rates were sufficient to accommodate a difference in intake from 83 to 539 g/d, and to virtually reestablish daily balances (at new glycogen concentrations) after only 2–4 d of energy imbalance. This is consistent with our previous studies, including extreme carbohydrate manipulations, which have shown rapidly inducible autoregulatory adjustments in carbohydrate oxidation rates (9, 26) over short periods of time. Furthermore, it was shown that this effect persists after normalization of the diet because of the influence of the perturbed glycogen stores (9).

During overfeeding with a mean energy surplus of 4.1 MJ/d, carbohydrate oxidation predominated. However, the RQ did not exceed 1 during any 24-h period, suggesting that despite this large energy surplus, there was no net lipogenesis. This confirms previous observations that de novo lipogenesis is quantitatively unimportant in human subjects consuming typical oral diets (7, 11, 12, 27, 29). Indeed, studies that have shown net lipogenesis are limited to those in patients receiving hyperenergetic artificial nutrition, where carbohydrate intake alone is in excess of energy requirements (30). In such cases, maximum carbohydrate oxidation is insufficient and carbohydrate balance can only be achieved by the net conversion of carbohydrate to fat.

Other studies have examined the mechanism of the increase in carbohydrate oxidation in response to overfeeding in more detail. In the study of Clore et al (31), subjects were overfed by 4.2 MJ/d for 4 d on a mixed diet and an increase in hepatic glucose production and decreased gluconeogenesis was observed. Schwarz et al (32) examined 25% and 50% increases and decreases in carbohydrate intake and showed a graded dose response in terms of carbohydrate oxidation. This effect was
mediated by increased hepatic glucose production, which stimulated moderate hyperinsulinemia. This decreased lipolysis and fatty acid availability. The net effect was to increase glycogen stores and enhance the delivery of extracellular glucose, thus favoring increased carbohydrate oxidation and a reciprocal decrease in fat oxidation.

Conversely, during underfeeding fat is the major oxidative fuel. Here, with absolute compliance with a 3.5-MJ/d diet and a mean energy deficit of 7.25 MJ/d, the net loss of body fat was only 159 g/d and weight loss averaged 242 g/d. This gives an indication of the likely rates of weight loss during voluntary dieting.

FIGURE 5. Changes in macronutrient balance during overfeeding. ○, intake; ●, oxidation; hatched bars, daily balance. On days 9-12 the intersubject range of nutrient balance was -14 to -44 g/d for carbohydrate, -24 to -51 g/d for protein, and -147 to -160 g/d for fat.

FIGURE 6. Changes in macronutrient balance during underfeeding. ○, intake; ●, oxidation; hatched bars, daily balance. On days 9-12 the intersubject range of nutrient balance was -49 to +3 g/d for carbohydrate, 15 to 30 g/d for protein, and 55 to 128 g/d for fat.
The current data provide clear evidence of the relative position of protein in the oxidative hierarchy model. It has long been assumed that there is autoregulatory control of protein oxidation about as efficient as that of carbohydrate (33). More recently, it has been suggested that the autoregulatory control of protein oxidation is greater than that of carbohydrate (4). This claim is based largely on the fact that protein appears to have a greater satiating power than carbohydrate, and it has been inferred that satiating power correlates directly with "obligatory oxidative disposal" (4). Our data show that a 160% increase in protein intake (from 47 g/d during underfeeding to 93 g/d during overfeeding) was almost matched by a 420% increase in protein oxidation (from 83 g/d during underfeeding to 93 g/d during overfeeding), and protein balance was significantly different from zero throughout both the underfeeding and overfeeding experiments. In contrast, a 550% increase in carbohydrate intake (from 83 g/d during underfeeding to 539 g/d during overfeeding) only caused a 12% increase in protein oxidation (from 83 g/d during underfeeding to 122 g/d during overfeeding) only caused a 12% increase in protein oxidation (from 83 g/d during underfeeding to 93 g/d during overfeeding), and protein balance was significantly different from zero throughout both the underfeeding and overfeeding experiments. In contrast, a 550% increase in carbohydrate intake (from 83 g/d during underfeeding to 539 g/d during overfeeding) was almost matched by a 420% increase in protein oxidation (from 106 g/d during underfeeding to 551 g/d during overfeeding). Clearly, carbohydrate was much more responsive, and it certainly exerted a much greater influence on the reciprocal changes in fat utilization than did protein. The small increase seen in protein oxidation during overfeeding results in a greater than expected deposition of protein. The work of Forbes, on the relation of lean and fat tissue, would suggest that in these nonobese men lean tissue would comprise ~40% of the total weight change. If lean tissue is 20% protein, this would imply an increase in protein of 232 g. In this study, ~50% more protein was deposited than this theoretical calculation predicts. Although we have no data to indicate the mechanism of this effect, protein intakes were high and the subjects were also engaged in moderate activity, which may promote nitrogen retention. Furthermore, we might speculate that the major increases in carbohydrate oxidation, commensurate with the carbohydrate overfeeding, limited the potential for significant increases in protein oxidation and thus, the excess protein intake had to be deposited. The failure to achieve the expected increase in protein oxidation is further evidence for the superiority of carbohydrate to protein in the oxidative hierarchy of nutrients.

The present study did not address post-ingestive effects on subsequent food intake but it is apparent that, strictly in terms of the oxidative hierarchy, protein takes a subordinate position to carbohydrate. Indeed, in the long term this must be so because obese individuals have a marked increased in protein mass but only small increases in carbohydrate.

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