Increasing the Protein:Carbohydrate Ratio in a High-Fat Diet Delays the Development of Adiposity and Improves Glucose Homeostasis in Mice

Susanne Klaus

German Institute of Human Nutrition, Potsdam-Rehbruecke, Group of Energy Metabolism, D-14558 Nuthetal, Germany

ABSTRACT Dietary fat is considered an important contributing factor in the obesity epidemic, and high-fat diets are usually paid to the interaction of fat with the other macronutrients. The aim of this study, therefore, was to investigate the effects of high-fat, isoenergetic diets with different protein:carbohydrate (CHO) ratios on obesity, energy metabolism, and glucose homeostasis in mice. Male adult C57BL/6J mice consumed ad libitum for 10 wk a control diet (41:42:17 ratio of CHO:protein:fat, 15.5 kJ/g) or 2 different high-fat diets: high carbohydrate (HC; 41:16:43, 17.7 kJ/g) or low carbohydrate (LC; 11:45:44, 17.5 kJ/g). Body weight and fat gains were rapid and were greater in HC mice than in other groups due to an initial pronounced hyperphagia and subsequent passive overconsumption. Weight and fat gains were less in LC mice but still greater than in controls. Energy expenditure was not affected by the diets, and total energy intake explained 84% of the variation in final body weight. The respiratory quotient was lower in LC mice than in other groups, indicating high fat oxidation rates due to the LC diet. Blood glucose was lower and insulin sensitivity greater in LC mice than in HC mice. We conclude that increasing the protein:CHO ratio in a high-fat diet delays but does not prevent the development of adiposity. However, glucose homeostasis was improved in LC mice, indicating that a combination of high fat and high CHO is responsible for the development of metabolic syndrome–related traits in mice. J. Nutr. 135: 1854–1858, 2005.

KEY WORDS: • low carbohydrate diets • energy metabolism • macronutrients • indirect calorimetry

Obesity is increasing worldwide and dietary fat is considered to be one of the important environmental factors contributing to the obesity epidemic (1,2). Fat content is one of the main factors influencing the energy density of diets, and an increase in energy density was shown to result in passive overconsumption in humans, which in turn promotes the development of obesity (3,4). However, the role of dietary fat in human obesity is also subject to debate (5). Animal studies demonstrated the development of obesity and diabetes-related traits in certain strains of rats and mice consuming high-fat diets ad libitum. One widely used model for obesity studies is the C57BL/6 mouse, especially in an exploration of the interplay between genetic background and environmental factors (6). C57BL/6 mice are highly susceptible to the development of diet-induced obesity (DIO);1 they are also prone to develop diabetes-related traits when DIO is manifest (7,8). When weaned onto a high-fat, high-sucrose diet, they develop hyperglycemia and hyperinsulinemia, for which fat was found to be the important stimulus, independent of energy intake (9–11). Although it is clear from these studies that dietary fat is an important factor, little attention has been paid to the role of the protein:CHO ratio and its interaction with dietary fat in the development of obesity and diabetes-related traits. In addition, it is still not well established whether hyperphagia alone or also reduced energy expenditure (EE) causes the development of obesity in rats and mice fed high fat diets. Many experimental approaches suffer from the problem that not only is dietary fat content changed, but also other dietary components compared with standard diets. Most commercial suppliers use different sources of macronutrients for standard and high-fat diets; this affects diet digestibility and also alters the macronutrient quality in addition to quantity. A further problem is that many commercial high-fat diets have a reduced proportion of energy as protein. This could result in increased food intake to ensure appropriate amino acid supply. The occurrence of hyperphagia under these conditions could thus be related to the protein rather than to the fat content of the diet. To examine in detail the role of the different macronutrients in the development of obesity, it is therefore necessary to employ diets with identical macronutrient sources but varied macronutrient ratios. Therefore, the aim of this study was to investigate the effect of 2 defined, semisynthetic, high-fat, isoenergetic diets with different protein:CHO ratios on the development of obesity, energy metabolism, and glucose homeostasis in C57BL/6 mice compared with a low-fat diet composed of the same ingredients.

1 This study was supported by the Deutsches Institut für Ernährungsforschung.
2 To whom correspondence should be addressed. E-mail: klaus@mail.dife.de.
3 Abbreviations used: CHO, carbohydrates; DIO, diet induced obesity; HC, high carbohydrate; LC, low carbohydrate; EE, energy expenditure; REE, resting energy expenditure; QMR, quantitative magnetic resonance; RQ, respiratory quotient; TEE, total energy expenditure.
TABLE 1  Composition of semisynthetic macronutrient diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Control diet</th>
<th>HC diet</th>
<th>LC diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>30</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Metabolizable energy, kJ/g</td>
<td>15.5</td>
<td>17.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Protein, energy %</td>
<td>41.7</td>
<td>16.0</td>
<td>45.0</td>
</tr>
<tr>
<td>CHO, energy %</td>
<td>41.1</td>
<td>41.0</td>
<td>11.5</td>
</tr>
<tr>
<td>Fat, energy %</td>
<td>17.2</td>
<td>43.0</td>
<td>43.6</td>
</tr>
</tbody>
</table>

1 Diet components as described in Daenzer et al. (12).

MATERIALS AND METHODS

Animal maintenance and experimental setup. Experiments were performed in 24 adult (9- to 10-mo-old) male C57Bl/6 mice obtained from an in house breeding colony derived from C57Bl/6NCrl mice purchased at Charles River Wiga. Mice were housed individually at 22°C with a 12 h:12 h dark-light cycle; food and water were freely available. Animal maintenance and experiments were in accordance with the guidelines of the ethics committee of the Ministry of Agriculture and Environment (State of Brandenburg, Germany). Before the experiments, all mice consumed ad libitum a standard pelleted rodent diet containing (wt:wt) 19% protein, 4% fat, and 50.5% carbohydrates (Altromin 1321). At the start of the experiment, mice were distributed randomly into 3 experimental groups (n = 8) and assigned to the different macronutrient diets as described previously (16). Measurements were performed at 6-min intervals over a 23-h period. For resting energy expenditure (REE), the mean of the 10 lowest individual measurements for each mouse was used.

Statistical analysis. Results are given as means ± SEM. Statistical significance was assessed by ANOVA (factorial or repeated measurements when appropriate) followed by least significant difference post-hoc test (Statview 4.5 for Apple Macintosh, Abacus Concepts). Differences were considered significant at P < 0.05.

RESULTS

Mice weighed 38.4 ± 0.6 g at the beginning of the experiment, with no differences among the groups. HC mice rapidly gained body weight and body fat, and final body weight was highest in the HC group, intermediate in the LC group, and lowest in the control group (Fig. 1A). Lean body mass did not differ among the groups (data not shown). Therefore, differences in body weight were due to changes in body fat. Body-fat gain was greater in the HC group than in the other groups (Fig. 1B).

Total food intake was greater in HC mice than in the control and LC mice, which did not differ from one another (Table 2). Due to the lower energy content of the control diet compared with the high-fat diets, daily energy intake differed among all groups, resulting in the greatest total energy intake in the HC group, intermediate intake in the LC group, and the respective diets and water during the measurement. The respiratory quotient (RQ = \( V_{CO2}/V_{O2} \)) and EE (kJ/d) were calculated as described previously (16). Measurements were performed at 6-min intervals over a 23-h period. For resting energy expenditure (REE), the mean of the 10 lowest individual measurements for each mouse was used.

Insulin tolerance test. Insulin sensitivity was tested after 70 d of dietary intervention by i.p. injection of insulin (Actrapid, 1.5 U/g body wt), as described previously (14). Glucose concentration was determined in blood from the tail at 0, 15, 30, and 60 min after insulin injection.

Energy expenditure. EE was measured by indirect calorimetry in individual mice, as described earlier (13–15), using an open respirometric system (gas analyzers: Magnus 16 and Uras 14, Hartmann & Braun). Measurements were performed during wk 8 or 9 of the intervention. Mice were unrestrained and had free access to their

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lowest energy intake in the control group. Because eating behavior changed during the experiment, daily food and energy intakes for the first 2 wk and for wk 3–10 were calculated separately (Table 2). During the first 2 wk, total food intake and energy intake were 24 and 41% greater, respectively, in the HC group compared with controls. This early hyperphagia was lessened during the following 8 wk of feeding; i.e., daily food intake during this time did not differ between HC and control mice, and daily energy intake was only 20% greater in HC than in control mice. LC mice exhibited a different feeding behavior; i.e., they consistently consumed the same amount of food as control mice, resulting in a persistent energy overconsumption of 11–14% relative to controls (Table 2).

Total energy intake over 70 d reflected very closely the final body weight (P < 0.0001, R = 0.91, Fig. 2). Cumulative energy intake throughout the experimental period thus explained 84% of the variation in final body weight.

Total macronutrient intake differed among the groups. Total fat intake was lower in the control group, protein intake was lower in the HC group, and CHO intake was lower in the LC group than in the other 2 groups. Total fat intake was higher in the HC group than in the other groups, due to the greater total food intake (Table 2).

EE was measured after 8–9 wk of dietary intervention when body-weight differences were already established. Weight-specific EE did not differ among the groups (Table 2). The daily pattern of EE, which reflects activity patterns, was also similar among the groups (Fig. 3A). Total energy expenditure (TEE) over 24 h was greater in the HC group than in the other groups, due exclusively to greater body weight. When normalized for body weight, TEE as well as REE did not differ among the groups (Table 2). The respiratory quotient (RQ) is indicative of the overall substrate oxidation; i.e., lipid oxidation results in an RQ close to 0.7, whereas carbohydrate oxidation has an RQ of 1.0 (17). The RQ was similar in all groups during daytime (i.e., inactivity period) but differed among the groups at night, i.e., during the activity and feeding period (Fig. 3B). In the control group, nighttime RQ values were close to 1, reflecting almost exclusive carbohydrate oxidation (Table 2). The lowest RQ values (i.e., highest fat oxidation rates) were in the LC group. They were greater in the HC group, and in this group, the nighttime RQ was less than that of controls (Table 2).

Postabsorptive blood glucose levels during wk 10 were lower in the LC mice (5.05 ± 0.35 mmol/L) than in the HC group (6.75 ± 0.35 mmol/L). Concentrations in the control group were intermediate and not different from the other groups (5.80 ± 0.45 mmol/L). Interestingly, insulin sensitivity was higher in the LC group than in the other 2 groups, which did not differ from one another (Fig. 4).

**DISCUSSION**

Due to recent renewed interest in low-carbohydrate diets, the role of the different macronutrients in the development of obesity and diabetes-related traits continues to be debated. It is very difficult to conduct long-term human intervention trials with tightly controlled macronutrient intake because of problems with compliance and adherence to such diets. ani-

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**TABLE 2**

Energy homeostasis in adult mice fed semisynthetic diets differing in macronutrient contents for 10 wk

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control diet</th>
<th>HC diet</th>
<th>LC diet</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total food intake, g</td>
<td>205.1 ± 4.6b</td>
<td>227.2 ± 5.3b</td>
<td>200.4 ± 4.4b</td>
<td>0.0015</td>
</tr>
<tr>
<td>Total energy intake, kJ</td>
<td>3168 ± 71a</td>
<td>3665 ± 93c</td>
<td>3542 ± 77a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total CHO intake, g</td>
<td>86.13 ± 1.92b</td>
<td>109.1 ± 2.56c</td>
<td>26.05 ± 0.57a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total protein intake, g</td>
<td>84.08 ± 1.88b</td>
<td>40.90 ± 0.96a</td>
<td>100.2 ± 2.19c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total fat intake, g</td>
<td>14.35 ± 0.32a</td>
<td>45.44 ± 1.07c</td>
<td>40.07 ± 0.87b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Food intake (wk 1–2), g/d</td>
<td>2.90 ± 0.09a</td>
<td>3.61 ± 0.08b</td>
<td>2.91 ± 0.09a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Energy intake (wk 1–2), kJ/d</td>
<td>44.8 ± 1.4a</td>
<td>63.0 ± 1.4a</td>
<td>51.3 ± 1.6a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Food intake (wk 3–10), g/d</td>
<td>2.94 ± 0.07ab</td>
<td>3.13 ± 0.08a</td>
<td>2.85 ± 0.05a</td>
<td>0.026</td>
</tr>
<tr>
<td>Energy intake (wk 3–10), kJ/d</td>
<td>45.4 ± 1.0a</td>
<td>54.6 ± 1.4a</td>
<td>50.4 ± 1.0a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TEE, kJ/d</td>
<td>47.4 ± 1.16a</td>
<td>54.3 ± 1.42a</td>
<td>48.3 ± 1.35a</td>
<td>0.003</td>
</tr>
<tr>
<td>TEE, kJ/(d · g)</td>
<td>1.16 ± 0.023</td>
<td>1.16 ± 0.026</td>
<td>1.11 ± 0.021</td>
<td>NS</td>
</tr>
<tr>
<td>REE, kJ/d</td>
<td>33.8 ± 2.18a</td>
<td>41.9 ± 2.95a</td>
<td>35.5 ± 1.38a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>REE, kJ/(d · g)</td>
<td>0.83 ± 0.014</td>
<td>0.90 ± 0.030</td>
<td>0.82 ± 0.028</td>
<td>NS</td>
</tr>
<tr>
<td>RQ</td>
<td>0.957 ± 0.009b</td>
<td>0.933 ± 0.013a</td>
<td>0.900 ± 0.01a</td>
<td>0.0003</td>
</tr>
<tr>
<td>RQ night only</td>
<td>0.999 ± 0.007²</td>
<td>0.945 ± 0.006b</td>
<td>0.902 ± 0.01a</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹ Data are means ± SEM, n = 8. Means in a row without a common letter differ, P ≤ 0.05.
² Energy expenditure and RQ were measured during wk 8–9.
³ NS, not significant; P > 0.05.
mal studies using well-established models allow precise control and recording of dietary components and intake levels. In this study, defined semisynthetic diets were used to explore not only the role of fat but also the interaction of macronutrients in the development of obesity and glucose homeostasis in a mouse model. Two high-fat diets differing only in the protein:CHO ratio were prepared and compared with a low-fat diet composed of the same ingredients. It became evident that an increase in fat and thus energy density led to continuous passive overconsumption, which in turn increased adiposity. An increase in the protein:CHO ratio, on the other hand, prevented the initial active hyperphagia that occurred with a high-fat, high-CHO diet. Furthermore, it beneficially affected diabetes-related traits.

Certain diets promote dietary hyperphagia in humans and also in rodent models (18,19). In human studies, it was reported that an increase in the dietary fat content (i.e., increased energy density) led to so-called passive overconsumption because the amount of food eaten did not change (4,20). However, it was shown in rats that high-fat diets can also enhance daily energy intake and weight gain at least in part via a mechanism that is unrelated to energy density (21). In the present study, passive overconsumption was apparent in the LC group, which consumed the same amount of food as the control group. In the HC group, on the other hand, early active hyperphagia was evident in addition to the continuous passive overconsumption. Interestingly, this active hyperphagia was transient and disappeared almost completely after ~3 wk, confirming earlier studies in rats (22,23). Thus, it is clear that it is not high fat alone but rather the combination of high fat with high CHO that leads to this transient overfeeding. Human studies also suggest that energy density affects food intake only in the short term, whereas in the long term, these effects are modulated and compensation occurs (3). The mechanisms leading to the observed initial hyperphagia with consumption of a high-fat, high-CHO diet are not clear. This hyperphagia can be attributed to increased palatability, possibly related to distinct sensory properties of fat (4). Because this has been observed in humans as well as in rats and mice, it is reasonable to assume the existence of common physiological mechanisms that underlie this active overfeeding.

It is interesting to note that even in the long term, the mice did not compensate for the changes in dietary energy density irrespective of the diet composition. It remains to be established whether this is unique to the strain of mice used or a general phenomenon. Using another mouse strain (FVB/N mice), we also found no compensation in food intake when mice were fed a macronutrient choice diet compared with a standard rodent diet. However, unlike the C57BL/6J mice used here, FVB/N mice did not become obese despite an increased energy intake, thus indicating a decreased energy efficiency (14).

Low-CHO (high-fat) diets have recently gained attention in the treatment of obesity (24,25). Although such diets have been popular for a long time, only in the past few years have randomized trials evaluated the long-term efficacy of such diets. The weight-reducing properties of such diets were similar to those of conventional studies (26–28); i.e., the weight-reducing effect of low-CHO diets is due mainly to a decreased energy intake, possibly caused by the higher satiating capacity of protein (25). Nevertheless, it was proposed that part of the weight-reducing effect of a high-fat, high-protein diet could be due to increased diet-induced thermogenesis caused by the high protein content (29). The present study does not support this hypothesis, at least not in the long term. After several weeks of feeding, EE did not differ among the groups and overall energy intake could explain >80% of the differences in final body weight. This strongly suggests that long-term body-weight gain is determined mainly by energy intake, irrespective of the macronutrient composition. In a previous study, we observed an increase in nighttime oxygen consumption in rats fed high-protein diets for 8 wk, but there was no effect on TEE.

![FIGURE 3 TEE (A) and RQ (B) in mice measured over 23 h after 8–9 wk of consuming semisynthetic diets differing in macronutrient content. Gray bar indicates nighttime (lights off). Values are means ± SEM, n = 8. For statistical analysis see Table 2.](https://academic.oup.com/jn/article-abstract/135/8/1854/4663954/FIGURE-3-TEE-and-RQ-in-mice-measured-over-23-h-after-8-9-wk-of-consuming-semisynthetic-diets-differing-in-macronutrient-content-gray-bar-indicates-nighttime-lights-off-values-are-means-%2B-SEM-n-8-for-statistical-analysis-see-table-2)

![FIGURE 4 Glucose sensitivity of mice after 10 wk of consumption of semisynthetic diets differing in macronutrient content. Insulin (1.5 U/g body wt) was injected i.p., and whole-blood glucose concentration was measured at the indicated times. Values are means ± SEM, n = 8. Means at a time without a common letter differ, P ≤ 0.05.](https://academic.oup.com/jn/article-abstract/135/8/1854/4663954/FIGURE-4-Glucose-sensitivity-of-mice-after-10-wk-of-consumption-of-semisynthetic-diets-differing-in-macronutrient-content-insulin-1-5-U-g-body-wt-was-injected-i-p-and-whole-blood-glucose-concentration-was-measured-at-the-indicated-times-values-are-means-%2B-SEM-n-8-means-at-a-time-without-a-common-letter-differ-P-%2C-le-0-05)
Interestingly, net fat oxidation increased with higher amounts of dietary protein although the dietary fat content was similar (16). In the present study, RQ was significantly reduced in the LC group compared with the HC group, which is also suggestive of an increased fat oxidation rate. Human intervention trials suggest that weight-reducing diets with increased protein content may have a more favorable health outcome than similar low-protein diets in the long term (30–32). Layman and colleagues (31) demonstrated that consumption of a diet with increased protein and a reduced CHO:protein ratio stabilized blood glucose during nonabsorptive periods and reduced the postprandial insulin response. It was suggested that weight-loss diets with decreased CHO and increased protein levels provide metabolic advantages, possibly due to the unique effects of leucine on muscle protein synthesis and insulin signaling (33). From the present study, it becomes clear that even with ad libitum consumption, an increase in protein:CHO ratio in a high-fat diet beneficially affects glucose homeostasis; i.e., mice fed high-protein diets (LC group) had lower nonfasting glucose levels and improved insulin sensitivity compared with the HC group. It is somewhat surprising that control mice also were insulin resistant; however, it should be noted that the mice were ~1 y old at the end of the experiments, making it possible that this was age related. Because the control and HC diets had similar high protein contents, the improvement in insulin sensitivity in mice consuming the HC diet is most probably due to the decreased CHO content.

This study shows that increasing the protein:CHO ratio in a high-fat diet delayed but did not prevent development of adiposity in C57BL/6 mice. Obesity development was due to passive overconsumption of energy and was further worsened by an initial active hyperphagia when a high-fat, high-CHO diet was fed. However, an increase in the protein:CHO ratio in a high-fat diet improved glucose homeostasis, indicating that a combination of high fat and high CHO is responsible for the development of some of the traits related to metabolic syndrome in mice. Thus, the detrimental health effects of a high-fat diet cannot be attributed to fat alone; rather, they result from an interaction of the macronutrients. Further studies are warranted to assess health effects that relate not only to macronutrient quantities but also to the quality of the different macronutrients.

Acknowledgment

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Literature Cited