Effects of high-fat diets with different carbohydrate-to-protein ratios on energy homeostasis in rats with impaired brain melanocortin receptor activity

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Morens, C., M. Keijzer, K. de Vries, A. Scheurink, and G. van Dijk. Effects of high-fat diets with different carbohydrate-to-protein ratios on energy homeostasis in rats with impaired brain melanocortin receptor activity. Am J Physiol Regul Integr Comp Physiol 289: R156–R163, 2005. First published March 17, 2005; doi:10.1152/ajpregu.00774.2004.—Changes in dietary macronutrient composition and/or central nervous system neuronal activity can underlie obesity and disturbed fuel homeostasis. We examined whether switching rats from a diet with high carbohydrate content (HC; i.e., regular chow) to diets with either high fat (HF) or high fat/high protein content at the expense of carbohydrates (LC-HF-HP) causes differential effects on body weight and glucose homeostasis that depend on the integrity of brain melanocortin (MC) signaling. In vehicle-treated rats, switching from HC to either HF or LC-HF-HP feeding caused similar reductions in food intake without alterations in body weight. A reduced caloric intake (−16% in HF and LC-HF-HP groups) required to maintain or increase body weight underlay these effects. Chronic third cerebroventricular infusion of the MC receptor antagonist SHU9119 (0.5 nmol/day) produced obesity and hyperphagia with an increased food efficiency again observed during HF (+19%) and LC-HF-HP (+33%) feeding. In this case, however, HF feeding exaggerated SHU9119-induced hyperphagia and weight gain relative to HC and LC-HF-HP feeding. Relative to vehicle-treated controls, SHU9119 treatment increased plasma insulin (2.8–4 fold), leptin (7.7–15 fold), and adiponectin levels (2.4–3.7 fold), but diet effects were only observed on plasma adiponectin (HC and LC-HF-HP/<HF). Finally, SHU9119-treated LC-HF-HP-fed rats were less glucose tolerant than others. Relatively low plasma adiponectin levels likely contributed to this effect. Thus HF feeding amplifies obesity induced by impaired MC signaling, provided that the carbohydrate-to-protein (C/P) ratio is high enough. Reduction of the C/P ratio within a HF diet ameliorates hyperphagia and obesity in rats with impaired MC signaling but aggravates associated disturbances in fuel homeostasis.

Obesity has become a dramatic public health problem in most Western countries, and its prevalence is rapidly increasing in developing countries as well (21). It is a major risk factor for life-threatening diseases such as type 2 diabetes and cardiovascular diseases, as well as some types of cancer. Although a general agreement has been reached to point the imbalance between daily energy intake and expenditure as a main cause of weight gain (22), the mechanisms underlying this imbalance are subject to intense investigation. In this respect, many investigators have considered the importance of the macronutrient composition, i.e., the relative amounts of fat, protein, and carbohydrates, of the food consumed. A high dietary fat content, for example, is thought to be a major factor promoting obesity in humans as well as in animals (6, 13). Carbohydrates seem to amplify high-fat diet-induced obesity (16), whereas it has been observed that a high-fat diet with a high protein content induces less weight gain and especially less fat gain (5). Although the mechanisms behind this phenomenon are poorly understood, this so-called ketogenic diet (i.e., rich in lipids and protein and very low in carbohydrates) has become extremely popular among people attempting to lose weight.

Another frequently mentioned factor underlying obesity is that central neuronal systems involved in regulation of energy balance become less sensitive to peripheral feedback signals that relay information regarding the nutritional status. One of these peripheral signals is leptin, a protein product of the ob gene, which is circulating in the bloodstream in proportion to body adiposity (38). Leptin has been proposed as a lipostat signal, which among others activates neurons in the hypothalamus that synthesize melanocortins (MCs), such as α-melanocyte-stimulating hormone (α-MSH) (34). α-MSH acts as an agonist on MC receptors (MC-R), and Vaisse et al. (33) have shown that mutations in the MC4-R are among the most frequent (~4%) monogenetic causes of obesity in humans. Experimental impairment of this signaling pathway in rats by intracerebroventricular infusion of the MC3-R and MC4-R antagonist SHU9119 consistently leads to hyperphagia as, and as a consequence, to the development of obesity (1). These rats also show a major increase in visceral adiposity and become insulin resistant, independently of the effects on food intake (24).

Over the past few years, close associations have been observed between alterations of the MC system and consumption of fat. First, increased dietary fat content has been shown to attenuate the anorexigenic effects of the MC receptor agonist melanotan-II (8). Second, increased activity at the MC4-R has been implicated in the suppression of fat intake (29). This was very much in line with the results of Koegler et al. (17) and Hagan et al. (11), who observed a higher fat consumption and preference, respectively, in Agouti mice and rats intracerebroventrically infused with Agouti-related protein. Thus it is hypothesized that the MC system plays a pivotal role in the interactions between high-fat feeding and regulation of energy homeostasis.

The aim of the present study was to further evaluate whether the integrity of the central nervous system (CNS) MC system is required to mediate the effects of high-fat diets with different carbohydrate-to-protein (C/P) ratios on food intake and food efficiency, body weight homeostasis, and glucose homeostasis. For this purpose, rats with pharmacologically impaired brain melanocortins; ketogenic diet; SHU9119; glucose tolerance
whereas in the other group, the pumps were filled to deliver the injector permanently placed into the guide cannula. In one group, the (Alzet 2002, pumping rate 0.55 ml/h; Alza, Palo Alto, CA) implanted kidneys, gastrointestinal tracts (stomach and caecum) were assessed daily until the end of i3vt treatment at day 10. Animals were killed by decapitation after brief anesthesia with CO2 between 3 and 2 h before the ensuing dark phase. Trunk blood was collected in ice-cooled tubes containing Trasylol and EDTA. Plasma samples, obtained after centrifugation (10 min, 1,500 g, 4°C) were stored at −80°C until analysis. The weights of the livers, epididymal and retroperitoneal fat pads, kidneys, gastrointestinal tracts (stomach + small intestine + caecum + colon), and eviscerated carcasses were determined in subgroups of n = 6 rats for each condition.

Blood glucose levels were assessed using the ferricyanide method of Hoffman. Plasma levels of insulin, leptin, and adiponectin were measured using commercial radioimmunoassay kits (RI-13 K, GL-32 K, and MADP-60 HK, respectively; Linco Research, St. Charles, MO). Plasma concentrations of free fatty acids were measured using a commercial kit (Boehringer Mannheim, Mannheim, Germany). Liver biopsies were cut (25–50 mg) from frozen tissue and boiled for 2 h in 1 M HCl to facilitate glycogen breakdown. After pH neutralization, glucose concentrations were assessed in those samples, indicating the amount of initial glycogen in tissue.

Statistical analysis. Results are expressed as means ± SE. Areas under the curve (AUC) were calculated using the trapezoidal method above a basal line and were reported for the 50 min following the start of the glucose infusion. All statistical analyses were done using SPSS 11.0 (Chicago, IL). A one-way ANOVA was used to compare values obtained at the end of the experimental period, as well as the daily body weight gain, food intake, and food efficiencies. Concerning the IVGTT experiment, the effects of treatment (control vs. SHU9119) and diets (HF, LC-HF-HP, and HC) were evaluated using an ANOVA within each subgroup (n = 6). The start of this time frame was chosen because average food intake of the saline-treated rats switched to the new diets was stable starting from day 5. The end at day 10 was chosen to rule out potential interferences of the intravenous glucose tolerance test (IVGTT) with food efficiencies.

**IVGTT.** On day 10, one-half of the rats were deprived of food after lights on, and they were connected 3 h before lights off to the sampling and infusion cannulas. After two baseline blood samples were taken ~1 h later (respectively 10 and 5 min before the start of the experiment), an intravenous infusion of a 10% glucose solution (in sterile saline) was started at a rate of 0.1 ml/min for 20 min. Blood samples (100 μl) were taken 1, 3, 5, 7, 10, 15, 20, 23, 26, 30, 40, and 50 min after the start of the infusion for glucose and insulin measurements. At the end of the experiment, sterile saline was given back to the rats to compensate disturbances in hemodynamics, and food hoppers were returned to the cages.

### Materials and Methods

Animal preparation. Male Wistar rats (n = 72) were obtained from the breeding colony maintained by the Department of Animal Physiology at the University of Groningen. Their initial weight was 300–350 g. They were individually housed in Plexiglas cages (25 × 25 × 30 cm) on a layer of wood shavings, under controlled temperature and humidity, and maintained on a 12:12-h light-dark cycle (lights on 0300 to 1500). All methods and experiments were approved by the Animal Care Committee of the University of Groningen.

All animals were implanted stereotaxically with a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) into the third cerebral ventricle (i3vt) under N2O-isoflurane anesthesia as previously described (34). Animals were injected with Finadyne (1g/kg body wt; Schering-Plough, Maarssen, The Netherlands) to reduce surgical stress and were allowed to recover for 5–7 days. One-half of the animals (n = 36) were equipped with silicon cannulas inserted into the right and left jugular vein according to techniques described elsewhere (31) to allow blood sampling and infusion, respectively, in a freely moving state. Finally, after they had regained normal growth and body weights almost all rats compensating disturbances in hemodynamics, and food were returned to their cages.

Blood and tissue collection and analysis. After food was removed at the onset of the light phase on day 14, animals were killed by decapitation after brief anesthesia with CO2 between 3 and 2 h before the ensuing dark phase. Trunk blood was collected in ice-cooled tubes containing Trasylol and EDTA. Plasma samples, obtained after centrifugation (10 min, 1,500 g, 4°C) were stored at −80°C until analysis. The weights of the livers, epididymal and retroperitoneal fat pads, kidneys, gastrointestinal tracts (stomach + small intestine + caecum + colon), and eviscerated carcasses were determined in subgroups of n = 6 rats for each condition.

### Table 1. Composition of diets

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>%Energy</th>
<th>HC</th>
<th>%Energy</th>
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<tr>
<td>Protein</td>
<td>228</td>
<td>23</td>
<td>200</td>
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<td>14</td>
<td>260</td>
<td>60.2</td>
<td>260</td>
<td>60.2</td>
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<tr>
<td>Carbohydrates</td>
<td>625</td>
<td>63</td>
<td>190</td>
<td>19.4</td>
<td>50</td>
<td>5.1</td>
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<td>Energy</td>
<td>16.8</td>
<td></td>
<td>16.8</td>
<td></td>
<td>16.8</td>
<td></td>
</tr>
</tbody>
</table>

The high-fat (HF) and low-carbohydrate/high-fat/high-protein (LC-HF-HP) diets were prepared by adding casein (MERCK by Boon), corn oil (Albert Heijn, Zaandam, The Netherlands), animal fat (Ossewit, Vandemoortele Roosendaal, Roosendaal, The Netherlands), cellulose (BUFA, Uitgeest, The Netherlands), guar gum (MERCK by Boon), and vitamins and salt mixes (prepared with components purchased from Sigma Aldrich Chemie, Zwijndrecht, The Netherlands) to the chow diet (RMH-B, Hope Farms, Woerden, The Netherlands) up to the concentrations given.

### Results

Diets and food intake assessment. Food intake and body weights were assessed daily until the end of i3vt treatment at day 14 after the minipump implantation. In subgroups of animals given i3vt SHU9119 or saline, rats were switched from their HC diet (standard laboratory chow) to one of two other diets, or they were maintained on the HC diet until the end of treatment. The experimental diets were 1) a HF diet with adequate carbohydrate and protein contents and 2) a HF-HP diet with a very LC content (LC-HF-HP). The composition of the diets is given in Table 1. Rats were switched to experimental diets one day after the minipump implantation to prevent absorption of potential postanesthesia sickness to the new diet. Animals were fed ad libitum except as mentioned otherwise. Food efficiencies of animals (mg body wt/kcal) were calculated as the ratio of body weight gain (g) from day 5 to day 10 and the total amount of food (kcal) ingested over that period. The start of this time frame was chosen because average food intake of the saline-treated rats switched to the new diets was stable starting from day 5. The end at day 10 was chosen to rule out potential interferences of the intravenous glucose tolerance test (IVGTT) with food efficiencies.
with repeated measures, with one within-subject factor (time) and two between-group factors (treatment = SHU9119 or saline and diet = HF, LC-HF-HP, or HC). Tukey’s test was used for post hoc analysis. A Pearson parametric correlation analysis was performed to test the significance of correlations between body weight changes and associated food intakes in vehicle-treated rats. Multiple linear regression analysis was used to test whether the food intakes at stable body weight differed among vehicle-treated diet groups. The level of significance was set at \( P < 0.05 \).

RESULTS

Food intake and body weight. Food intake and body weight assessed daily are shown in Fig. 1. In the control rats, mean daily food intake decreased after the switch to HF (\( P < 0.001 \)) and LC-HF-HP (\( P < 0.0001 \)) diets relative to the rats maintained on the HC diet. However, this decrease was not associated with alterations in body weight, which increased gradually over the course of i3vt vehicle infusion for all diet groups.

Whatever the diet, the food intake of the i3vt SHU9119-treated rats was significantly increased compared with their respective controls. Mean food intake assessed between day 7 and day 14 was 233% (HF diet, \( P < 0.0001 \)), 202% (LC-HF-HP diet, \( P < 0.0001 \)), and 183% (HC diet, \( P < 0.0001 \)) higher in the SHU9119-treated animals compared with their respective vehicle-treated controls. There was a significant diet effect on the food intake of the SHU9119-treated rats: during the whole experimental period, animals given the LC-HF-HP diet ingested significantly less food (\( P < 0.05 \)) than those fed the HF diet, and their food intake was also reduced compared with the animals fed the HC diet after day 7 (except on day 12).

Whatever the diet, the body weight gain of the SHU9119-treated animals was significantly higher (\( P < 0.0001 \)) than that of the control rats. At the end of the experiment, body weight of the SHU9119-treated rats was 45±3% (HF diet), 32±5% (LC-HF-HP diet), and 33±1% (HC diet) higher than the pretreatment body weight, whereas it was only 8±2% (HF diet), 9±1% (LC-HF-HP diet), and 11±1% (HC diet) higher for the saline-treated animals. There was also a significant diet effect on the body weight gain of the SHU9119-treated animals: after day 5, SHU9119-treated rats fed the HF diet gained significantly more weight than those fed the HC diet and LC-HF-HP (\( P < 0.0001 \)) diets relative to the rats maintained on the HC diet. However, this decrease was not associated with alterations in body weight, which increased gradually over the course of i3vt vehicle infusion for all diet groups.

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Figure 2 shows the food efficiencies of all dietary and treatment groups calculated between days 5 and 10 of treatment. Whatever the diet, food efficiency was significantly increased in the SHU9119-treated animals compared with their respective controls (HC, 212%; HF, 204%; and LC-HF-HP, 192%; \( P < 0.0001 \)) vs. saline-treated rats). Whereas food efficiency was increased in the SHU9119-treated rats fed the HF and LC-HF-HP diets compared with those fed the HC diet,
this effect was not statistically significant in the saline-treated controls. The latter result was due to the fact that the amount of weight gained over this period in saline-treated animals was too small and yielded highly variable effects.

Closer observation of the day-by-day changes in body weight and the associated changes in food intake in the saline-treated individual rats revealed significant correlations (r < 0.001) between the two within each dietary group (data not shown). Multiple regression analysis of daily body weight changes and the associated food intakes revealed significant correlations (r = 0.486). Grouping the caloric input of treatment of the animals according to dietary intakes yields suitable cohorts of 8–12 sets of body weight (changes from 1 to 1 g) over that day or cause moderate or considerable weight gain. Interestingly, food intake of the animals fed a HF diet was also larger compared with rats fed the LC-HF-HP diet at body weight increases but not at stable body weight.

**Body composition.** At the end of the i3vt infusion period, animals were killed by decapitation under CO₂ anesthesia. The organs and tissues were dissected and weighed, and the results are shown in Table 2. Whatever the diet, the liver, epididymal and retroperitoneal fat pads, and full gastrointestinal tract were heavier in the SHU9119-treated animals compared with their respective vehicle-treated controls (P < 0.0001). In the SHU9119-treated animals, diet significantly affected weights of the liver, with LC-HF-HP > HC (21.1 ± 0.6 vs. 18.2 ± 0.7 g, respectively; P < 0.05), of the kidneys, with LC-HF-HP > HC (3.4 ± 0.1 vs. 3.1 ± 0.1 and 2.7 ± 0.1 g, respectively; P < 0.05), and of the epididymal and retroperitoneal fat pads, with HC < HF and LC-HF-HP (24.2 ± 1.3 vs. 33.4 ± 1.2 and 29.6 ± 1.1 g, respectively; P < 0.05). Moreover, the epididymal and retroperitoneal fat pads were slightly heavier in the SHU9119-treated rats given the HF diet compared with those given the LC-HF-HP diet, but the difference was not significant. The weights of the full gastrointestinal tracts and eviscerated carcasses were not significantly different among the SHU9119-treated diet groups.

Concerning the control animals, the epididymal and retroperitoneal fat pads were lighter in the control rats fed the HC diet than in those on the HC or HF diets, with the difference being significant only for LC-HF-HP vs. HC diets (P < 0.05).

**Fuels and hormones.** Table 3 presents the glycogen content of the liver as well as the plasma concentration of fuels and hormones measured on day 14. There was a clear diet effect on the hepatic levels of glycogen, in both saline (P < 0.0001)- and SHU9119-treated (P < 0.0005) rats, with a higher glycogen content in the liver of the rats fed the HC diet relative to the other diets. No treatment effect was detected for this parameter.

### Table 2. Body composition after 14 days of treatment and diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SHU9119</th>
<th>Control</th>
<th>SHU9119</th>
<th>Control</th>
<th>SHU9119</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>369 ± 4a,d</td>
<td>440 ± 22a,e</td>
<td>382 ± 8b</td>
<td>510 ± 6b,e,f</td>
<td>404 ± 6d</td>
<td>473 ± 10f</td>
</tr>
<tr>
<td>Liver, g</td>
<td>13.6 ± 0.8a</td>
<td>18.2 ± 0.7a,d</td>
<td>12.5 ± 0.5b</td>
<td>20.0 ± 0.3b</td>
<td>14.5 ± 0.5a</td>
<td>21.1 ± 0.6a,d</td>
</tr>
<tr>
<td>%BW</td>
<td>3.7 ± 0.2a</td>
<td>4.1 ± 0.1a</td>
<td>3.3 ± 0.1b</td>
<td>3.9 ± 0.1a,e</td>
<td>3.6 ± 0.1c</td>
<td>4.5 ± 0.1a,b,c,d</td>
</tr>
<tr>
<td>Kidneys, g</td>
<td>2.7 ± 0.1a</td>
<td>2.7 ± 0.1a</td>
<td>2.6 ± 0.1b</td>
<td>3.1 ± 0.1a,d</td>
<td>2.8 ± 0.1c</td>
<td>3.4 ± 0.1a,c</td>
</tr>
<tr>
<td>%BW</td>
<td>0.7 ± 0.1a</td>
<td>0.6 ± 0.1a</td>
<td>0.7 ± 0.1b</td>
<td>0.6 ± 0.1a</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Epididymal + retroperitoneal fat, g</td>
<td>8.3 ± 0.7a,d</td>
<td>24.2 ± 1.3a,e,f</td>
<td>10.9 ± 0.9b</td>
<td>33.4 ± 1.2a,e</td>
<td>12.7 ± 0.9a,d</td>
<td>29.6 ± 1.1a,e,f</td>
</tr>
<tr>
<td>%BW</td>
<td>2.2 ± 0.2a,d</td>
<td>5.5 ± 0.2a,e</td>
<td>2.3 ± 0.2b</td>
<td>6.0 ± 0.3b</td>
<td>3.1 ± 0.2a,d</td>
<td>6.3 ± 0.2a,e,f</td>
</tr>
<tr>
<td>Gastrointestinal tract (full), g</td>
<td>27.9 ± 0.7a</td>
<td>51.9 ± 2.1a</td>
<td>24.8 ± 2.7b</td>
<td>54.3 ± 2.7a,b</td>
<td>26.1 ± 0.9a</td>
<td>47.4 ± 2.1a,c</td>
</tr>
<tr>
<td>%BW</td>
<td>7.6 ± 0.2a</td>
<td>11.8 ± 0.5a</td>
<td>6.5 ± 0.6b</td>
<td>10.7 ± 0.4a,b</td>
<td>6.5 ± 0.5a,b</td>
<td>10.0 ± 0.3a,b</td>
</tr>
<tr>
<td>Eviscerated carcass, g</td>
<td>289.3 ± 4.6a</td>
<td>318.1 ± 17.3a</td>
<td>320.5 ± 2.7b</td>
<td>355.7 ± 4.5a</td>
<td>321.5 ± 12a</td>
<td>338.6 ± 10.0a</td>
</tr>
<tr>
<td>%BW</td>
<td>78.4 ± 0.3a</td>
<td>72.2 ± 0.6a</td>
<td>78.9 ± 0.5b</td>
<td>71.2 ± 0.7a</td>
<td>78.2 ± 0.8a</td>
<td>71.3 ± 0.4a</td>
</tr>
</tbody>
</table>

Values are means ± SE. BW, body weight. a,b,c,d,e,f Values with similar superscript letters within a row are significantly different (P < 0.01).
There was neither a treatment effect nor a diet effect on the plasma glucose level. Whatever the diet, the plasma level of total free fatty acids (FFA) was not different between the saline- and the SHU9119-treated animals. There was a significant effect of the diet in the SHU9119-treated animals only, with a lower plasma FFA level assessed in the SHU9119-treated rats fed the HC diet compared with the SHU9119-treated rats fed the HF or LC-HF-HP diet (0.18 ± 0.02 vs. 0.32 ± 0.02 and 0.28 ± 0.03 mmol/l, respectively). The plasma FFA level was slightly lower in the SHU9119-treated rats fed the LC-HF-HP diet compared with those given the HF diet, but the difference was not significant. Whatever the diet, the difference between the SHU9119-treated rats and their respective controls regarding plasma insulin level was highly significant (P < 0.005). The insulin level was slightly higher in the SHU9119-treated rats fed the HF diet, but this difference was not significant.

Plasma leptin levels were dramatically increased in the SHU9119-treated animals (almost 10 times higher, whatever the diet; P < 0.0001). No diet effect was detected in either the controls or the SHU9119-treated rats. Plasma adiponectin levels were significantly higher, whatever the diet, in the SHU9119-treated rats compared with their respective controls, with 3.7-, 2.9-, and 2.4-fold increases in the HC, HF, and LC-HF-HP fed rats, respectively. Moreover, there was a diet effect in both SHU9119- and saline-treated rats (in controls: HF > LC-HF-HP > HC, and for SHU9119-treated rats: HF > LC-HF-HP = HC; P < 0.05). Finally, plasma resistin levels remained unaffected (no significant differences) whatever the condition (treatment or diet).

**DISCUSSION**

This study was aimed at evaluating whether the integrity of the brain MC system is required for the effects of HF feeding with varying carbohydrate and protein contents on energy balance and fuel homeostasis. Therefore, we investigated whether shifting from a HC diet to a HF diet with either a relatively high or low C/P ratio interfered with food intake, body weight maintenance, and body composition, as well as fuel homeostasis in normal vehicle-treated rats and in rats chronically i3vt treated with the MC3/MC4-R antagonist, SHU9119.

In vehicle-treated controls, switching from a HC diet to a HF diet caused a reduction in food intake, but this reduction was not associated with a change in body weight. This phenomenon of reduced food intake in the face of similar increases in body weight was the result of an increase in food efficiency; i.e., a response of HF feeding that has been previously reported by others (12, 32). Indeed, careful analysis of the day-by-day amount of energy required to maintain stable or increase body
weight clearly revealed lower values in rats fed the HF diet than in rats fed the HC diet. A reduction in food intake without alterations in body weight was also observed when rats were switched to a HF diet that was also high in proteins (LC-HF-HP, P elevated at the expense of C). This effect was again explained by an increased food efficiency relative to that observed in the HC feeding condition. Thus, whereas LC-HF-HP diets are used to cause weight loss in obese humans (10), such an effect was not observed in lean rats in the present study, despite the fact that they reduced food intake.

Relative to vehicle-treated controls, rats chronically i3vt infused with SHU9119 became hyperphagic irrespective of diet composition, and this resulted in dramatic increases in body weight and body fat content. These data are in agreement with previous reports (1, 14, 28, 36) and once again demonstrate the importance of a functional CNS MC system in the control of energy balance and body weight homeostasis (30). In contrast to vehicle-treated controls, however, rats switched to a HF diet and treated with SHU9119 displayed hyperphagia that was clearly more pronounced than that observed in SHU9119-treated HC rats during the first 3 days of treatment. Whereas this elevated food intake fell back after a few days to the same level observed in HC-fed SHU9119-treated rats, body weight gain in the HF group remained enhanced relative to that in the HC group. As in vehicle-treated HF feeding rats, this effect appeared to be caused by an increase in food efficiency. One implication of these findings is that intact MC signaling is required for the reducing effect of a HF diet on food intake. Thus it can be concluded that impairment of brain MC signaling not only is disruptive for regulation of energy balance but also allows an increase in dietary fat content to be additive to this effect. The finding that elevation of dietary fat content augments obesity induced by an impairment of brain MC signaling is consistent with the data of Butler et al. (7), who performed comparable studies in MC4-R knockout mice.

A different picture emerged when viewing SHU9119-treated rats switched to the LC-HF-HP diet. In this event, the hyperphagia was clearly less pronounced than that observed in SHU9119-treated rats switched to the HF diet or left on the HC diet. The mechanisms through which caloric intake in the LC-HF-HP-fed rats differed from those in the other diet groups are largely unknown, but the data in the present study demonstrate that they do not rely on the integrity of the brain MC system. Because anorexigenic effects of high dietary protein levels have been documented (4, 15, 23, 25), one possibility might be that the elevated absolute amount of ingested proteins as a result of i3vt SHU9119 treatment reduced appetite in the LC-HF-HP group. The observation that the vehicle-treated rats fed the LC-HF-HP diet did not show this effect might be explained by the notion that the protein content (35% of energy content) of our LC-HF-HP diet was at the threshold to induce reductions in food intake in other studies (2, 26). Despite the fact the LC-HF-HP diet reduced caloric intake in SHU9119-treated rats, the temporal changes in body weight of those rats were similar to what was observed in SHU9119-treated rats fed the HC diet. Because increased food efficiency also was observed in SHU9119-treated rats fed a HF diet, we concluded that switching from a HC diet to any diet with a HF content

![Fig. 4. Plasma levels of glucose (A–C) and insulin (D–F) before, during, and after an intravenous glucose tolerance test made 10 days after the start of i3vt SHU9119 or saline infusion. ■, SHU9119 + HC diet; ○, control + HC diet; ◆, SHU9119 + HF diet; □, control + HF diet; ▲, SHU9119 + LC-HF-HP diet; δ, control + LC-HF-HP diet. Values are means ± SE. *P < 0.05, SHU9119 vs. control. #P < 0.05; LC-HF-HP diet vs. HF and HC diets.](http://ajpregu.physiology.org/Download)
causes an increase in food efficiency, and this effect appears to be independent of MC3/MC4-R signaling. This increased food efficiency, however, can only explain in part the HF diet-induced amplification of obesity in rats with an impaired brain MC signaling. Despite similar food efficiencies in SHU9119-treated rats fed the HF and LC-HF-HP diets, body weight gain was increased in the HF group. This may be explained by a reduced MC-mediated thermogenesis in that group, implying then that a HF diet (with a high C/P ratio) may cause more MC-mediated thermogenesis than a LC-HF-HP (with a low C/P ratio).

To investigate diet and/or treatment effects on blood glucose homeostasis, we subjected vehicle- and SHU9119-treated rats to IVGTTs on one of the final days of i3vt treatment. In vehicle-treated rats, intravenously infused glucose resulted in similar plasma glucose and insulin responses in the three diet groups, indicating that glucose tolerance was not affected by diet per se within the time course of this experiment. In the SHU9119-treated groups, the IVGTTs led to markedly enhanced insulin responses that were not different among diet groups either. This hypersecretion of insulin was probably necessary to counter the insulin resistance in these animals, which occurred with the development of obesity (9) and/or with the blockade of MC signaling (24). However, although the glucose responses (assessed by AUC) of SHU9119-treated rats fed the HC and HF diets were similar to those of their respective vehicle-treated controls, the SHU9119-treated rats switched to the LC-HF-HP diet had markedly elevated plasma glucose responses relative to other groups. This shows first that an intact central MC system appears to be necessary for normal glucose homeostasis with a LC-HF-HP diet. Furthermore, the elevated plasma adiponectin level in the SHU9119-treated rats fed the HF diet relative to those fed the HC diet is of interest. It might be hypothesized that a rise in adiponectin secretion is necessary to prevent a further derangement of glucose homeostasis in SHU9119-treated animals fed any HF diet (for example, due to elevated triglyceride loading into several insulin-sensitive tissues, including muscle and liver, see Ref. 35). For some unknown reason, an elevation of the plasma adiponectin level was absent in the SHU9119-treated rats fed the LC-HF-HP diet, which potentially could have caused glucose intolerance in these animals (19, 37). Indeed, adiponectin is known to enhance glucose tolerance and insulin sensitivity, and genetically modified mice lacking adiponectin present a mild insulin resistance associated with glucose intolerance (18). Other studies have indicated a fall in the plasma adiponectin levels as a strong marker of the development of type 2 diabetes mellitus (see e.g., Ref. 3). We did not find any significant effect of the SHU9119 treatment or of the diet on the plasma resistin and leptin levels, whereas these hormones also produced by white adipose tissue are thought to play a role in glucose homeostasis as well (27, 35). In this respect, the location of elevated fat storage is of interest, because adipocyte hormone production appears to be depot-specific (see e.g., Ref. 19). Thus, although vehicle-treated and SHU9119-treated rats fed the LC-HF-HP and HC diets had the same body weight, the rats fed the LC-HF-HP diet had heavier epididymal and retroperitoneal fat pads in either treatment group. Because a high visceral fat accumulation has been linked to the development of a variety of metabolic and circulatory diseases (9, 19), this finding might lead to further questions regarding the beneficial health effects of a ketogenic diet.

Another part of the underlying mechanisms leading to glucose intolerance might be revealed when viewing the associated plasma insulin responses during the IVGTTs in the SHU9119-treated rats fed the LC-HF-HP diet. Thus, compared with the other SHU9119-treated groups, it appeared that the individual insulin responses induced by the IVGTTs differed greatly among the SHU9119-treated rats fed the LC-HF-HP diet. Correlation analysis revealed that the height of the peak plasma insulin level during the IVGTT was highly inversely related to the glucose response assessed by AUC ($R^2 = -0.932$). One interpretation of these interactions might be that the insulin hypersecretion required to maintain normal glucose tolerance exceeded maximal B-cell capacity in some of the LC-HF-HP-fed rats with impaired MC signaling. Although we have not followed up on this in the present study (e.g., by investigating histological preparations of the pancreas), such a condition has been suggested to preclude the development of type 2 diabetes mellitus (20).

There are a limited number of studies addressing the question of how dietary macronutrient composition can affect glucose tolerance and insulin sensitivity, especially in obese humans. Even fewer studies are available that assess the effects of LC diets on these parameters. In a recently published study, however, Foster et al. (10) assessed, in obese subjects, the effects of weight loss due to a conventional diet or a LC-HF-HP diet on insulin sensitivity. They demonstrated that either dietary manipulation was associated with an improvement of insulin sensitivity as determined by an oral glucose tolerance test. Those data do not allow any conclusion regarding the effects of the macronutrient composition of the diets independently of lost body weight. Additional studies addressing more specifically the long-term consequences of LC-HF-HP diets on glucose tolerance and insulin sensitivity (perhaps as a result of alterations in adipocyte hormone secretion, insulin signaling cascades in effector tissues, and/or B-cell responsiveness) are therefore warranted in rats with impaired MC signaling as well as in normal rats.

In conclusion, this work shows that a HF diet amplifies obesity induced by impaired brain MC signaling, provided that the ratio of dietary C/P is high enough. Part of the underlying mechanism is explained by dietary fat having a stimulatory effect on food efficiency, an effect that does not seem to rely on the integrity of the MC system. Furthermore, the integrity of the brain MC system is not required for a LC-HF-HP diet to induce a reduction in food intake and a lower body weight gain compared with a HF diet. In any event, animals with impaired brain MC signaling do become massively obese and develop hyperinsulinemia, whatever the composition of the diet they are eating. The consumption of a LC-HF-HP diet leads to an impaired glucose tolerance in the SHU9119-treated rats, which may be due to relatively low adiponectin levels and/or a limitation of B-cell responsiveness in some of these animals. Finally, these data question the potential beneficial effects of a ketogenic diet for fuel homeostasis, at least when its consumption is not associated with a significant weight loss.

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