Behavioral and endocrine traits of obesity-prone and obesity-resistant rats on macronutrient diets

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Wang, J ian, J esline T. Alexander, Ping Zheng, Hi J oon Yu, Jordan Dourmashkin, and Sarah F. Leibowitz. Behavioral and endocrine traits of obesity-prone and obesity-resistant rats on macronutrient diets. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E1057–E1066, 1998.—Patterns of eating behavior, body weight gain, and hormone changes were examined in normal-weight albino Sprague-Dawley rats on macronutrient diets. These diets consisted of either three separate jars with pure macronutrients, fat, carbohydrate, and protein, from which to choose, or a single diet with different concentrations of fat and carbohydrate. Similar patterns on the choice-diet and single-diet paradigms were observed. During the first 7–10 days on these diets but not subsequently, the rats consuming a fat-rich diet exhibit significant hyperphagia, an increase in both total and fat intake that produces higher body weight gain. Compared with a 10% fat diet, a 30% fat diet is associated with a decline in insulin and corticosterone (CORT) levels, whereas a 60% fat diet produces an increase in circulating glucose. Levels of glucose are positively correlated with fat intake, and together these measures are consistently related to body fat. These relationships are most strongly expressed in rats that consume a fat-rich diet with >30% fat. Whereas insulin levels are also positively related to body fat, CORT is inversely related in these normal-weight subjects. In animals consuming a high-fat diet, a clear separation can be seen between “obesity-prone” (OP) rats with 100% greater body fat than “obesity-resistant” (OR) rats. The OP rats, which consume 15% more total calories, have significantly higher insulin and glucose levels. In animals that consume a diet with >30% fat, it is the OP but not the OR rats that exhibit a positive relation between fat intake, glucose levels, and body fat and reveal an additional association between carbohydrate intake, insulin, and body fat. Thus these rats on macronutrient diets exhibit distinct traits that relate behavior to hormone disturbances and adiposity and distinguish subjects that are prone vs. resistant to obesity.

insulin; glucose; fat intake; corticosterone; adiposity

OBESITY is an increasing health problem, with approximately one-third of the American population overweight (6). Increased ingestion of fat-rich foods in many affluent countries over the century has been implicated in the rise of obesity (6). Dietary fat is a significant contributor to hyperphagia, weight gain, and fat deposition in many species, including rodents, dogs, and humans (3, 6, 34, 42, 43, 45). Furthermore, body fat has been shown to increase in direct proportion to the fat content in the diet (4, 34). This higher fat content has been associated with endocrine and metabolic changes that promote fat storage (3, 6, 17). These hormone changes include hyperinsulinemia, hyperleptinemia, hyperglycemia, and, in some cases, hypercortisolemia (3, 22, 32, 35, 39, 43). Genetic models of obesity, both rats and mice, exhibit similar disturbances in caloric intake, adiposity, and circulating hormones (6, 15, 18). Moreover, there are certain outbred strains of rats, such as the Osborne-Mendel and Wistar-Lewis, and inbred strains of mice that are susceptible to the weight-promoting properties of a high-fat diet (7, 12, 21, 45).

The focus of this report is on the genetically heterogeneous Sprague-Dawley rat and its responsiveness to diets that allow free selection of the macronutrients, fat, carbohydrate, and protein, from separate food jars. This feeding paradigm, which is similar to the free selection experienced in the human population, has been used in behavioral studies to examine meal patterns of animals showing different preferences for the specific nutrients (37, 38). However, there is little information on the endocrine profile of these animals and their patterns of body weight regulation as they relate to the circulating hormones as well as their behavioral preferences. Studies of these parameters should provide a unique opportunity for examining both behavioral and physiological disturbances that develop in human dietary obesity (2, 6, 42, 43). The behavioral analyses performed to date (10, 37, 38) demonstrate that rats on these macronutrient diets vary greatly in their preferences for the fat source, which across individuals may range from 10 to 60% of the total diet (37, 38). Moreover, the meal patterns of these rats clearly differ, with fat-preferring animals consuming their nutrients in larger and less frequent meals. Studies using these diets have also revealed differential effects of brain neurochemicals on patterns of nutrient ingestion (6, 25, 26).

This investigation sought to characterize rats on the nutrient diets and provide an integrated analysis of their behavioral and neuroendocrine profiles, specifically as they relate to the animals’ propensity to gain weight and deposit fat. Additionally, the experiments were designed to compare the characteristics of rats with a free choice of the macronutrients to those with a single mixed diet having different amounts of fat and carbohydrate. From these tests, it has become clear that, within a few weeks on the diets, rats can exhibit distinct traits reflective of disturbances in weight regulation. These traits, linking the behavioral process of fat and carbohydrate ingestion to circulating insulin, glucose, and body fat, are similar to those described in human studies of dietary obesity (1, 6, 43). These systematic studies in more natural dietary conditions constitute the first step toward characterizing the mechanisms that determine the propensity of individual rats to overeat and gain weight in a feeding paradigm allowing free nutrient selection.

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Methods

Animals

Adult male Sprague-Dawley rats (275–325 g; Charles River Breeding Labs, Kingston, NY) were individually housed in a fully accredited AAALAC facility (22°C, with lights off at 3:30 PM for 12 h), according to institutionally approved protocols as specified in the National Research Council Guide to the Care and Use of Laboratory Animals.

Diet

All rats were maintained ad libitum on food and water for a period of 4–5 wk. Two feeding paradigms were used, one with single mixed diets and one with a choice of the three macronutrient diets as described elsewhere (37, 38). In the single-diet paradigm, rats were maintained on either a high-carbohydrate/low-fat diet (HCD: 10% fat, 65% carbohydrate, 25% protein; 3.75 kcal/g), a high-fat/low-carbohydrate diet (HFD: 60% fat, 15% carbohydrate, 25% protein; 5.10 kcal/g, n = 84), or a control diet (COND: 30% fat, 45% carbohydrate, 25% protein; 3.98 kcal/g/n = 28) with a more balanced proportion of different macronutrients. Protein consisted of 97% casein (Bioserv) mixed with 4% minerals (USP XV Salt Mixture Briggs, ICN Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals), and 0.03% cysteine (L-cysteine hydrochloride, ICN Pharmaceuticals). Carbohydrate was composed of 28% dextrin, 28% corn starch (ICN Pharmaceuticals), and 37% sucrose (Domino) mixed with 4% minerals and 3% vitamins. Fat (7.7 kcal/g) consisted of 86% lard (Armour) mixed with 8% minerals and 6% vitamins. In the choice-diet experiment, additional groups of rats (n = 248) were allowed free access to separate sources of protein (3.7 kcal/g), carbohydrate (3.7 kcal/g), and fat (7.7 kcal/g).

Test Procedures

Food intake was measured four times a week and body weight twice a week for a 4- to 5-wk test period, after which rats were killed. For both experiments, rats were killed ~1–2 h before dark onset, and blood was collected for serum analysis of corticosterone (CORT), insulin, and glucose. Rats were killed before the onset of the natural feeding cycle to enable us to detect both an increase and a decrease in circulating CORT and to observe changes in insulin and glucose that are generally unaffected by prior feeding episodes. Plasma CORT and insulin levels were assayed by RIA with methods similar to those of Krey et al. (24) and Herbert et al. (19), respectively. Plasma glucose levels were analyzed with a Beckman Glucose Analyzer No. 2. Unilateral body fat pads (inguinal, retroperitoneal, and epididymal) were dissected and weighed, and total weight of these three fat pads was recorded.

Data Analysis

Each animal’s kilocaloric intake was averaged for a given week, and data are presented as kilocalories per 24 h and as nutrient preference (% of total kcal intake). Body weight, body weight gain, and body fat are calculated for the final week of measurements. Comparisons across multiple groups were performed using one-way ANOVA, followed by a Duncan’s new multiple range test or Student’s t-test when appropriate. All values are expressed as means ± SE. The criterion for use of the term “significant” in the text is that the probability value for a given test is P < 0.05.

Results

Behavioral and Hormonal Patterns of Rats in a Single-Diet Paradigm

Food intake and body weight measures. In this experiment, the rats (n = 197) are given one of three single diets with varying fat and carbohydrate content; they yield behavioral patterns illustrated in Fig. 1. During the 1st wk on these mixed diets, the rats consuming the HFD exhibit significant hyperphagia, a 7% higher caloric intake (P < 0.01) compared with the rats on the HCD or COND. This overeating lasts ~10 days, after which the HFD rats show a gradual decrease in total intake and by week 4 return to a level comparable to that of the other two groups. This amount of food intake, ~108 kcal/day, generates daily fat consumption ranging from 14 kcal on the HCD to 74 kcal on the HFD. In rats of similar body weight at the beginning of the experiment (275–325 g), this greater fat intake on the HFD produces a significant increase in body weight gain, which is highest during week 1, when the rats show significant hyperphagia on this diet (Fig. 1). At the end of a 4-wk period, this weight gain yields significantly higher body weight in the HFD rats compared with the COND (+9%, P < 0.05) or HCD (+14%, P < 0.05) subjects and significantly heavier fat pad weights (+22% and +69%, P < 0.05, respectively).

Hormone measures. With increased dietary fat, there is a decline in circulating insulin and CORT (Fig. 2). A significant decrease in insulin (~19%, P < 0.05) and CORT (~13%, P < 0.05) is evident as fat rises from 10% (HCD) to 30% (COND), whereas CORT declines further on the HFD containing 60% fat (~11%, P < 0.05). This drop in insulin and CORT is accompanied by an increase in blood glucose in subjects on the HFD (+10%, P < 0.01) compared with the COND or HCD rats. A close relationship between this change in circulating glucose and the ingestion of fat is suggested by a significant positive correlation between glucose levels and total kilocaloric intake on the HFD (r = 0.34, P < 0.05) as opposed to the COND [r = −0.08, not significant (NS)] or HCD (r = 0.18, NS). In contrast, total intake is inversely related to CORT levels on all diets, ranging from r = −0.29 to r = −0.37 (P < 0.05), but shows no relation to circulating insulin levels.

Behavioral and Hormonal Patterns of Rats in a Choice-Diet Paradigm

Food intake and body weight measures. The second group of rats (n = 248), given a choice of the macronutri-
ents, shows a wide variation in their preference for fat, ranging from 10 to 70% (averaging 35%) of the total daily calories consumed (107 kcal). This range of fat preference in this choice-diet paradigm has permitted the animals to be subgrouped as HC eaters (10–25% fat), CON eaters (25–45% fat), or HF eaters (45–60% fat). Analyses of these subgroups reveal behavioral and hormonal patterns that are very similar to those described above for the single-diet groups and that can be linked to the amount of fat consumed, as opposed to carbohydrate or protein.

As for the single HFD rats, the HF eaters compared with the HC eaters (Fig. 3) exhibit a significant overeating of total calories (+8%, P < 0.01) during the first 10 days on the diets. In subsequent weeks, this initial hyperphagia of the HF eaters is followed by a decline in total daily intake to a level (106 kcal) comparable to the HC or CON eaters as well as to the daily intake of the single-diet groups (108 kcal). As can be seen in Fig. 3, the higher fat diet selected by the HF eaters is associated with a significant decline in carbohydrate and protein intake, producing a diet with 55% fat and 16% carbohydrate compared with only 16% fat and 40% carbohydrate selected by the HC eaters. As expected, the weight gain and body weight of the HF eaters are significantly greater than those of the other two groups, resulting in a 50% increase (P < 0.01) in body fat pad weights.

Hormone measures. Similar to the pattern exhibited by the rats on a single diet with high dietary fat (Fig. 2), the HF and CON eaters that naturally choose a diet with 30% or greater fat content show reduced circulating levels of insulin and CORT compared with the HC eaters (Fig. 4). The HF eaters also have higher blood glucose levels (+10%, P < 0.05) compared with HC eaters. This rise in glucose appears related specifically to the amount of fat consumed, as reflected by a strong, significant positive correlation between glucose levels and fat intake in the HF eaters (n = 70, r = +0.53, P < 0.01), a weaker but significant correlation across the entire group of choice-diet subjects (n = 248, r = +0.28, P < 0.01), and loss of this association in the HC eaters (r = −0.11, NS) or CON subjects (r = −0.10, NS). Furthermore, glucose is unrelated to the amount of total calories, carbohydrate, or protein consumed in the different groups, and levels of insulin or CORT are not
correlated with the ingestion of any specific macronutrient.

Behavioral and Hormonal Factors Related to Body Fat

In both the single-diet and choice-diet paradigms, body weight and fat pad weight rise with the amount of fat consumed, and this occurs in association with a decline in insulin and CORT and an increase in glucose levels as dietary fat rises above 30% (Figs. 1–4). This rise in fat intake is associated with an increase in body weight, and the amount of fat consumed is strongly related to body fat, more consistently than body weight. This is evident in the single-diet condition, in which body fat and total kilocaloric intake are positively correlated in HFD subjects ($r = 0.71$, $P < 0.01$) more strongly than in COND ($r = 0.45$, $P < 0.05$) or HCD ($r = 0.13$, NS) subjects. It is also seen in the choice-diet condition, which reveals a strong, positive correlation between body fat and fat intake in the total group of subjects ($r = 0.60$, $P < 0.01$) and a similar association in the HF eaters ($r = 0.58$, $P < 0.01$) that is not evident in the CON ($r = 0.10$, NS) or HC ($r = 0.20$, NS) eaters. This is in contrast to a weaker or inverse relation between body fat and carbohydrate, protein, or total caloric intake, as illustrated in Fig. 5 for the total group.

Similar to dietary fat, body fat is also positively related to circulating levels of glucose and insulin, specifically under conditions when fat intake rises above 30%. This association between body fat and glucose levels is detected in the HFD group ($r = 0.34$, $P < 0.01$) but not in the COND ($r = 0.02$, NS) and HCD ($r = 0.19$, NS) groups and is also seen in the HF eaters ($r = 0.34$, $P < 0.01$) but not the CON ($r = 0.18$, NS) or HC ($r = 0.10$, NS) eaters. Similarly, insulin levels and fat pad weights are positively related in both the HFD group ($r = 0.37$, $P < 0.01$) and HF eaters ($r = 0.28$, $P < 0.05$) but not in the HCD group or HC eaters. In contrast, circulating CORT, which is highest in rats consuming a high-carbohydrate diet and declines with increased fat intake (Figs. 2 and 4), shows a small but consistent inverse relationship with body fat in most dietary groups ($r = 0.31$ to $0.38$, $P < 0.05$). The ratio of insulin to CORT is also a correlate of body fat in the different groups ($r = 0.42$ to $0.52$, $P < 0.05$), sometimes more strongly than insulin alone.
In contrast to fat, dietary protein and carbohydrate, which are inversely correlated with body fat (Fig. 5), show few consistent relationships with the circulating hormones. In two instances, however, associations observed in the choice-diet paradigm are worth noting. In the HF eaters, dietary carbohydrate, which ranges from 15 to 25% of the total diet, is a positive correlate of insulin levels ($r = 0.44$, $P < 0.01$), a relationship not detected in the HC eaters. Rather, these HC eaters show dietary protein, a major component (>33%) of their overall diet, to be positively related to CORT ($r = 0.47$, $P < 0.01$) and inversely related to insulin ($r = -0.35$, $P < 0.01$).

Obesity-Prone and Obesity-Resistant Rats Consuming Specific Diets

To further examine the positive associations among dietary fat, body fat, and circulating levels of glucose or insulin, the rats in the single-diet and choice-diet experiments were further categorized on the basis of their propensity to gain weight and deposit fat on a given diet. The results of this analysis of obesity-prone and obesity-resistant rats are presented in Table 1 (single-diet paradigm) and Table 2 (choice-diet paradigm), with each having a high-carbohydrate and high-fat diet group. Whereas certain differences between the high and low body fat subgroups are evident in the high-carbohydrate diet conditions (see Tables 1 and 2), the high dietary fat clearly causes all subjects to reach a higher body fat range and reveals in the obesity-prone subjects exaggerated behavioral and endocrine traits associated with disturbances in glucose homeostasis. These distinguishing traits are illustrated in Fig. 6 for the subjects that, when given a choice of nutrients, show a natural preference for a fat-rich diet.

Behavioral measures. In the choice-diet paradigm, ~30% ($n = 70$) of the total group selects a diet rich in fat (>40% of total diet). Within this group of HF eaters, the obesity-prone subjects, with 13% higher body weight and 80% more body fat compared with the obesity-resistant subjects (Fig. 6 and Table 2), have behavioral patterns very similar to those in the single HFD paradigm (Table 1). These include a 13% higher daily caloric intake because of more fat calories but no

<table>
<thead>
<tr>
<th>Table 1. Nutrient intake, body weight, and hormones in animals of low and high body fat in a single-diet paradigm</th>
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<tr>
<td></td>
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<tr>
<td>Total intake, kcal/day</td>
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<tr>
<td>Body fat pad weight, g</td>
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<tr>
<td>Body weight, g</td>
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<td>Body weight gain, g/day</td>
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<tr>
<td>Insulin, µU/ml</td>
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<td>Glucose, mg/dl</td>
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<tr>
<td>Insulin/glucose ratio</td>
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<tr>
<td>Corticosterone, ng/ml</td>
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Values are means ± SE. *$P < 0.05$, †$P < 0.01$, high body fat vs. low body fat subgroups.
Table 2. Nutrient intake, body weight, and hormones in animals of low and high body fat in a choice-diet paradigm

<table>
<thead>
<tr>
<th></th>
<th>High-Carbohydrate Eaters (n=74)</th>
<th>High-Fat Eaters (n=70)</th>
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<tbody>
<tr>
<td></td>
<td>Low body fat</td>
<td>High body fat</td>
</tr>
<tr>
<td>Total intake, kcal/day</td>
<td>103 ± 1.9</td>
<td>110 ± 5.2*</td>
</tr>
<tr>
<td>Fat intake, kcal/day</td>
<td>12 ± 2.0</td>
<td>18 ± 2.0*</td>
</tr>
<tr>
<td>Carb intake, kcal/day</td>
<td>44 ± 6.0</td>
<td>44 ± 3.0</td>
</tr>
<tr>
<td>Protein intake, kcal/day</td>
<td>47 ± 3.0</td>
<td>48 ± 4.0</td>
</tr>
<tr>
<td>%Fat intake</td>
<td>12 ± 3.0</td>
<td>16 ± 4.0</td>
</tr>
<tr>
<td>%Carbohydrate intake</td>
<td>43 ± 3.0</td>
<td>40 ± 3.0</td>
</tr>
<tr>
<td>%Protein intake</td>
<td>46 ± 3.0</td>
<td>44 ± 3.0</td>
</tr>
<tr>
<td>Total body fat, g</td>
<td>12.6 ± 0.4</td>
<td>19.0 ± 0.8†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>447 ± 9.5</td>
<td>497 ± 8.0†</td>
</tr>
<tr>
<td>Body weight gain, g/day</td>
<td>3.6 ± 0.4</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>67 ± 7.0</td>
<td>85 ± 7.0*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>138 ± 4.0</td>
<td>142 ± 4.0</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>0.47 ± 0.0</td>
<td>0.52 ± 0.1</td>
</tr>
<tr>
<td>Insulin/glucose ratio</td>
<td>0.02 ± 0.0</td>
<td>0.04 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Carb, carbohydrate. *P < 0.05, †P < 0.01, high body fat vs. low body fat subgroups.

difference in the amount of carbohydrate or protein consumed. This caloric shift increases the rats’ fat preference from 51 to 58% of the total diet (P < 0.05) and, consequently, their body weight gain (Fig. 6). The fat pad weights of the obesity-prone subjects are positively correlated with these behavioral measures of total kilocaloric intake (r = +0.52, P < 0.01) and specifically fat intake (r = +0.60, P < 0.01) but not carbohydrate (r = +0.06, NS) or protein (r = −0.02, NS) intake. In the single-diet paradigm, a similar association is evident between total caloric intake on a high-fat diet and body fat (r = +0.42, P < 0.05) in the obesity-prone subjects. This relationship between fat intake and adiposity is not detected in the obesity-resistant groups in either diet paradigm. It is also absent in the subgroups consuming a high-carbohydrate diet that have <15% fat in their diet and considerably smaller body fat pads (Tables 1 and 2).

Hormone measures. In the choice-diet condition, the hormonal profile of obesity-prone HF eaters also differs from that of obesity-resistant HF eaters. Whereas insulin is generally suppressed by a high-fat diet across the total population (Figs. 2 and 4), the obesity-prone HF eaters have significantly higher levels (+65%, P < 0.05) of this hormone compared with the obesity-resistant HF eaters, even with 12 more kilocalories of fat in their diet (Fig. 6). They also exhibit a 12% increase (P < 0.05) in their glucose levels and a significantly higher insulin-to-glucose ratio, with no difference seen in their CORT levels. As for the positive relationship between fat intake and body fat, circulating glucose levels are a distinguishing trait and a consistent correlate of body fat only in obesity-prone subjects, rather than obesity-resistant subjects, and most consistently in subjects consuming a high-fat diet. In both the single- and choice-diet paradigms, positive relationships between glucose and body fat, ranging from r = +0.38 to +0.55 (P < 0.05), are evident in the subgroup of fat-preferring, obesity-prone rats. These relationships are less consistent or absent in the high-fat-eating, obesity-resistant rats, as well as in the
subgroups of rats that consume a high-carbohydrate/low-fat diet and have generally smaller body fat pads (Tables 1 and 2).

Whereas this evidence demonstrates that dietary fat is a primary factor related to body fat deposition, it is likely that dietary carbohydrate is also a contributing factor under certain conditions. By itself and in generally normal-weight subjects, carbohydrate is inversely related to body fat, as illustrated in Fig. 5. However, as indicated above, carbohydrate is a positive correlate of insulin secretion specifically in the HF eaters \( r = +0.44, P < 0.01 \). Moreover, in the present analysis of rats with differential weight gain, it is in the obesity-prone HF eaters that carbohydrate as well as insulin is related to body fat. In these heavier rats that are hyperinsulinemic (Fig. 6), carbohydrate constitutes 10–25% of their diet, which also contains 47–68% fat. The amount of carbohydrate ingested is strongly and positively related to circulating insulin \( r = +0.64, P < 0.01 \), which in turn is positively correlated with circulating glucose \( r = +0.58, P < 0.05 \). These relationships are not detected in obesity-resistant HF eaters, which have lower basal levels of insulin.

DISCUSSION

These results, obtained in rats given a single diet or a choice of macronutrient diets, reveal distinct traits relating to dietary nutrients, hormones, and body fat. The main findings are that dietary fat, but not carbohydrate or protein, is positively correlated with adiposity. It reduces levels of insulin and CORT, whereas it increases and is positively related to glucose levels. The full impact of dietary fat is observed when it accounts for >30% of the total diet and particularly in obesity-prone subjects. Obesity-prone subjects consuming a high-fat diet consistently exhibit a positive relationship among dietary fat, body fat, and glucose and insulin levels, with dietary carbohydrate becoming a contributing factor as dietary fat and body fat rise.

Dietary Fat, Hyperphagia, and Adiposity

There has been considerable discussion as to whether the ingestion specifically of fat, rather than the intake of total calories including carbohydrate and protein, has a particular role in obesity (2, 4, 6, 14, 30). The evidence from the present report argues for a specific effect of dietary fat that, at levels >30%, has a primary impact on body adiposity. This is evident in animals that exhibit a natural preference for dietary fat when given a choice, and it can be induced by the presentation of a single diet rich in fat, consistent with previous studies (4, 21, 34). In the choice paradigm, it is the fat content that exhibits the strongest relationship to body fat pad weights, although total calories also play a role. A high-fat diet produces both adipocyte hypertrophy and hyperplasia (21).

Also debated is the issue as to whether behavior, involving the overconsumption of fat, is necessary for obesity to develop (29, 36). The evidence reported here supports the importance of behavior, with a strong natural preference for fat associated with a significant increase in body fat. During the initial 1–2 wk when the diets are first presented, hyperphagia along with more rapid weight gain is evident when rats consume a high-fat diet, as reported previously (29, 43). Rats given or choosing a high-fat diet do not compensate for the increased caloric content of the diet, possibly because of the increase in palatability (33) and reduced satiating quality of dietary fat (2, 29, 30). Subsequently, increased weight gain and fat deposition on a high-fat diet may continue in the absence of overeating, possibly due to the animal’s ability to convert dietary fat to body fat with greater efficiency (14, 29).

Dietary Fat and Circulating Hormones

The findings here and in published reports (9, 20, 22, 39) clearly demonstrate a suppressive effect of dietary fat (30% or more) on insulin levels. This is evident here in animals given a choice of macronutrient diets, as well as a single diet rich in fat. Fatty acids, which rise with an increase in fat intake (35), are found to inhibit pancreatic secretion of insulin (13, 46). It is of interest that insulin administration, in turn, has a preferential suppressive effect on the ingestion of fat (8).

It has also been demonstrated that a high-fat diet causes an increase in circulating glucose in both rats (3, 22) and mice (28). The findings described above provide strong support for a positive relationship between dietary fat and circulating glucose, an association detected in multiple groups and in both the single-diet and choice-diet paradigms. This rise in glucose very likely reflects the development of insulin resistance, a known consequence of a fat-rich diet (6, 20, 35, 39). Dietary fat, associated with an increase in fatty acid oxidation (14, 42, 44), can impede the binding of insulin to its receptor, decrease glucose transporters, and blunt insulin-induced decrease in hepatic glucose output and glucose utilization (20, 22, 23, 41). This rise in glucose, along with an increase in the insulin-to-glucose ratio, reflecting insulin resistance, is evident after only a few weeks on the diets and is seen in a situation in which rats voluntarily choose a fat-rich diet.

The present findings show that CORT levels are higher in rats consuming a high-carbohydrate diet compared with the control diet. This may reflect a counterregulatory action to prevent a reduction in glucose in response to the rise in insulin. In contrast, the high-fat diet with 60% fat had only a small effect on CORT compared with the control diet with 30% fat. It has been reported that a rise in dietary fat from 4 to 20% can stimulate the hypothalamic-pituitary-adrenal axis, possibly reflecting a chronic stress response (40). This pattern was not observed in the present study, in either the single-diet or choice-diet paradigms, when fat increased from 10 to 30%. A number of differences in the test procedures may explain these different results, including the nature of the diets used, the amount of fat in the diet, the length of time on the diets, and the fact...
that food intake and body weight gain are not altered by a rise in fat to 20%.

**Dietary Fat, Blood Glucose, and Body Fat**

Of particular importance is the close association between dietary fat, glucose levels, and body fat. A positive correlation among these three variables, although evident across the total population of subjects, is strongest under two conditions. These are conditions in which dietary fat rises above 30% and in animals that are naturally prone to obesity. Whereas dietary fat is sufficient to produce higher glucose levels in the total population, the expression of this trait is strongest in animals with a natural tendency to deposit body fat. The greater efficiency of these obesity-prone rats is reflected in their greater weight gain in a state of hyperinsulinemia and increased insulin-to-glucose ratio, presumably indicating a state of reduced insulin sensitivity (17, 26). It is also associated with the behavioral trait of 15% greater daily caloric intake, reflecting a specific increase in fat consumption.

**Dietary Carbohydrate, Hormones, and Body Fat**

Carbohydrate alone is related inversely to body weight gain and body fat (1, 38). Across the total population, ingestion of this nutrient is negatively correlated with body fat. Moreover, animals that consume a high-carbohydrate diet invariably have a lower body weight than fat-prefering rats.

There is one condition in the present study, however, in which carbohydrate contributes to weight gain, body fat, and circulating glucose. This is in fat-prefering, obesity-prone rats that are in a hyperinsulinemic state. In these subjects, carbohydrate accounts for 10–25% of their total diet, and the ingestion of this nutrient is positively correlated with circulating insulin, a relationship not seen in obesity-resistant rats. Furthermore, both carbohydrate and insulin in these rats are positively related to circulating glucose, which in turn is a consistent correlate of body fat. This contribution of carbohydrate to body fat, in animals on macronutrient diets, may be similar to results obtained in a different paradigm in which a sugar solution added to a balanced diet results in the development of obesity (1, 21, 36).

Measurements of the adrenal steroid CORT show this hormone to be at high circulating levels in animals that choose or are given a high-carbohydrate diet. Moreover, similar to carbohydrate ingestion, it is inversely related to body weight and body fat in the total population. Under no conditions and in no subgroups on the macronutrient diets is CORT found to be higher in obesity-prone subjects, in contrast to the hypercortisolism sometimes observed in profoundly obese animals (6, 11, 15, 25) and humans (16). Thus, in rats within a normal range of adiposity, high levels of CORT are associated with a high-carbohydrate diet and lower body weight.

The normal function of CORT, similar to the effect of carbohydrate ingestion, is to maintain glucose homeostasis. In addition to stimulating carbohydrate and fat ingestion (11, 25), CORT enhances glucose production and reduces glucose utilization in certain tissues (27). Although CORT levels are high under conditions of high-carbohydrate ingestion, in the high-carbohydrate eaters on macronutrient diets they are also found to be positively related to the consumption of protein, which may help in providing substrates for further gluconeogenesis (27). The importance of CORT, as it relates to insulin in body weight regulation, has been clearly outlined in a review by Dallman et al. (11). This relationship may help to explain why, in some instances, the ratio of insulin to CORT is more consistently related to body weight than is insulin alone. Similar to carbohydrate ingestion, CORT alone normally shows an inverse relation to body fat; however, this hormone becomes positively related under conditions of high stress or chronic CORT administration at high doses (5, 31) or with extreme obesity in genetic strains (6, 18).

**Obesity-Prone vs. Obesity-Resistant Rats**

In the rats on macronutrient diets, obesity-prone and obesity-resistant subjects can be clearly distinguished when consuming a high-fat diet, more than on a low-fat diet and with lower body fat. Their distinct traits include increased fat ingestion and higher circulating levels of glucose and insulin. Of particular interest is the finding that these traits are positively related to body fat in the obesity-prone but not obesity-resistant rats, even though the latter are consuming the same high-fat diet. One additional component to this profile is carbohydrate ingestion, which is normally inversely related to adiposity, but in obesity-prone rats is positively related to their body fat.

Other laboratories have investigated obesity-prone rats, although generally under conditions providing a single diet with differential carbohydrate and fat content. On a high-fat diet, obesity-prone animals, in addition to greater weight gain and adiposity, exhibit increased energy efficiency and lipogenic capacity in adipose tissue, as well as decreased lipolytic enzymes in muscle (17). Levin and Routh (26) have also observed higher glucose-stimulated norepinephrine levels in the blood of obesity-prone Sprague-Dawley rats. The significance of these traits, and whether they are a cause or an effect of the high-fat or high-caloric diet, need to be explored in future studies.

**Perspective**

The present findings reveal clear relationships between the ingestion of specific nutrients and changes in circulating hormones. They identify circulating glucose as a close marker of both dietary fat and body fat, possibly reflecting a state of insulin resistance. With the multiple measures recorded, the results provide some perspective on how behavior can contribute to obesity, even within a few weeks on macronutrient diets. The susceptibility of animals prone to obesity is
The ultimate goal of this research is to understand the mechanisms controlling eating behavior and body weight. The integrative approach taken in this investigation, involving both behavioral and physiological measures, will hopefully provide a helpful foundation for future studies of such mechanisms. The brain, and in particular the hypothalamus, very likely has a central role in the control of the behavioral, endocrine, and physiological patterns described here. Evidence obtained in other studies (6, 25, 26) indicates that specific neurochemicals in the central nervous system, including galanin and neuropeptide Y, may have differential functions in the control of nutrient ingestion and metabolism. This control is reflected not only by the impact of the neurochemicals on ingestion and metabolic patterns but also by the effect of nutrient intake on the activity of endogenous neurochemicals. When this control is disturbed, such as in obesity-prone animals on a high-fat diet, the brain peptides may also be altered. The task at hand is to identify the critical factors that are causally related to an animal’s overeating and propensity toward obesity on fat-rich diets.

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