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Low-carbohydrate diets affect energy balance and fuel homeostasis differentially in lean and obese rats

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Morens, C., V. Sirot, A. J. W. Scheurink, and G. van Dijk. Low-carbohydrate diets affect energy balance and fuel homeostasis differentially in lean and obese rats. *Am J Physiol Regul Integr Comp Physiol* 291: R1622–R1629, 2006. First published August 10, 2006; doi:10.1152/ajpregu.00128.2006.—In parallel with increased prevalence of overweight people in affluent societies are individuals trying to lose weight, often using low-carbohydrate diets. Nevertheless, long-term metabolic consequences of those diets, usually high in (saturated) fat, remain unclear. Therefore, we investigated long-term effects of high-fat diets with different carbohydrate/protein ratios on energy balance and fuel homeostasis in obese (*fa/fa*) Zucker and lean Wistar rats. Animals were fed high-carbohydrate (HC), high-fat (HsF), or low-carbohydrate, high-fat, high-protein (LC-HsF-HP) diets for 60 days. Both lines fed the LC-HsF-HP diet displayed reduced energy intake compared with those fed the HsF diet (Zucker, -3.7%) or the HC diet (Wistar rats, -12.4%). This was not associated with lower weight gain relative to HC fed rats, because of increased food efficiencies in each line fed HsF and particularly LC-HsF-HP food. Zucker rats were less glucose tolerant than Wistar rats. Lowest glucose tolerances were found in HsF and particularly in LC-HsF-HP-fed animals irrespective of line, but this paralleled reduced plasma adiponectin levels, elevated plasma resistin levels, higher retroperitoneal fat masses, and reduced insulin sensitivity (indexed by insulin-induced hypoglycemia) only in Wistar rats. In Zucker rats, however, improved insulin responses during glucose tolerance testing and tendency toward increased insulin sensitivities were observed with HsF or LC-HsF-HP feeding relative to HC feeding. Thus, despite adverse consequences of LC-HsF diets on blood glucose homeostasis, principal differences exist in the underlying hormonal regulatory mechanisms, which could have benefits for B-cell functioning and insulin action in the obese state but not in the lean state.

fa/fa Zucker rats; glucose tolerance; insulin sensitivity

OBESITY HAS REACHED EPIDEMIC proportions in most affluent societies, and this is associated with a dramatic rise in metabolic/endocrine (e.g., Type 2 diabetes) and cardiovascular diseases (14). Ironically, these trends are paralleled by a large number of individuals trying to lose weight (24) and by the large sums of money spent on weight loss products and services (6). Although guidelines for successful weight loss include a reduction of caloric intake combined with increased physical exercise (20, 37), considerable public interest has been aimed at the use of low-carbohydrate diets to attain successful weight loss (27). For example, the “Atkins diet” (at least 45 million copies of books sold over 40 years) is currently embraced by over 20 million people worldwide (2).

In accordance with public enthusiasm are several recently published studies reporting, in obese people, an improvement of sustained weight loss after 6 mo on a low-carbohydrate (“ketogenic”) diet compared with the results obtained with a more conventional low-fat, low-calorie diet (8, 17, 27, 38). These differences in weight loss, however, disappeared after 12 mo, and associated metabolic correlates (e.g., insulin resistance and glucose tolerance) only showed marginal improvements.

One potential problem associated with chronic ingestion of a low-carbohydrate diet is that it usually contains lots of fat to compensate for the reduction of calories due to lack of carbohydrates. This way, low-carbohydrate diets can contain up to 60% of fat and, not infrequently, mostly in the form of saturated fat (16). For years, scientists and health officials have warned against the adverse health consequences of saturated fat, as it can lead to dislipidemia, insulin resistance, and glucose intolerance. It may, therefore, be hypothesized that these effects also occur with the consumption of a low-carbohydrate (ketogenic) diet with a high fat content. Thus, to assess the health consequences of increased dietary fat, irrespective of carbohydrate content, the present study was designed to investigate the effects of chronic consumption of high-fat diets with different carbohydrate/protein ratios on energy balance and fuel homeostasis in normal Wistar and in obese *fa/fa* Zucker rats. The latter animals were included in our study as a rodent model of obesity and insulin resistance. The *fa/fa* Zucker rat has a single mutation in the gene encoding the leptin receptor (13), which renders this rat less sensitive to leptin (1, 23). Although homologous mutations are extremely rare in the human population (7), and thus probably not underlying the current epidemic of obesity and metabolic syndrome in affluent societies, there is support for the notion that leptin resistance is a correlate of human obesity, as well as of the development and progression of the metabolic syndrome (39). Therefore, studying the effects of chronic consumption of high-fat diets with different carbohydrate/protein ratios in leptin-resistant obese *fa/fa* Zucker rats may be highly relevant for judgment of the potentially beneficial or adverse health consequences in leptin-resistant individuals.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats ($n = 36$) were obtained from the breeding colony maintained by the Department of Animal Physiology at the University of Groningen. Male Zucker *fa/fa* (HsdOla: ZUCKER *Lep^{fa}*, $n = 40$) rats were purchased from Harlan Nederland (Horst, The Netherlands) at the age of 6 wk. All rats were allowed 10

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days to habituate to their housing conditions (i.e., individual housing in rooms with controlled humidity and temperature, 12:12-h light-dark cycle, with lights on from 0300 to 1500). Then, three cohorts, matched for mean body weight within the *fafa* Zucker group (247 ± 5 g) and within the Wistar group (264 ± 5 g), were switched to one of the different experimental diets. These pelleted isocaloric diets were 1) a high-carbohydrate diet (HC, standard laboratory chow), 2) a high-(saturated) fat diet (HsF) and 3) a low-carbohydrate, high-(saturated) fat, high-protein (LC-HsF-HP) diet. The composition of the diets is presented in Table 1. The HsF and LC-HsF-HP diets were prepared by adding casein (Merck by Boom, Meppel, The Netherlands), corn oil (Albert Heijn, Zaandam, The Netherlands), beef tallow (Ossewit, NV Vandemoortele Roosendaal, Roosendaal, The Netherlands), cellulose (Bufa, Uitgeest, The Netherlands), guar gum (Merck by Boom), and vitamins and salt mixtures (prepared with components purchased from Sigma Aldrich, Zwijndrecht, The Netherlands) to the standard laboratory chow diet (RMH-B, Hope Farms, Woerden, The Netherlands) up to the levels given in Table 1.

Food intake and body weight were assessed daily. All methods and experiments were approved and checked by the Animal Care Committee of the University of Groningen.

Surgery. Between day 35 and day 40 of the experiment, under N₂O-isoflurane anesthesia, rats were implanted with two indwelling silicon catheters, inserted in the right and left jugular vein to allow blood sampling and infusions, respectively. Surgery was performed as described previously (26). Both cannulas were externalized on top of the skull and secured with dental cement. Animals were injected with Finadyne (1 g/kg body wt, Schering-Plough, Maarssen, The Netherlands) for analgesia following surgery.

Intravenous glucose tolerance test. On one day between day 45 and day 50, food was removed at lights on, and animals were subjected to an intravenous glucose tolerance test (IVGTT), which was performed during the last hours of the light phase. Specifically, the rats were connected to sampling and infusion tubing, which extended out of the rat cages for remote blood sampling and intravenous infusion, respectively. After connection to the tubing, animals were left undisturbed for 45 min before starting the experiment. Two baseline blood samples (100 μ l) were taken 10 and 5 min before the start of an infusion of a 10% glucose solution (in sterile saline) at a rate of 0.1 ml/min for 20 min. Blood samples (100 μ l) 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. After each sample was collected, an equivalent volume of sterile saline was given back to the rats to avoid major disturbances in hemodynamics.

Insulin sensitivity test. Seven days after the IVGTT, animals were food-deprived overnight and were subjected to an insulin sensitivity test (IST) during the last hours before the dark phase. Again, rats were

connected to the blood sampling tubing 45 min before the first blood sample. After a baseline blood sample (100 μ l) was collected, rats were injected intraperitoneally with a solution of insulin (Velosulin, Novo Nordisk Farma, Bagsvaerd, Denmark) at a dose of 5 U/kg and 0.5 U/kg for the *fafa* Zucker and the Wistar rats, respectively. Blood (100 μ l) was then sampled after 15, 30, 45, and 60 min for glucose and insulin measurements.

Blood and tissue collection and analysis. On day 60, rats were killed by decapitation under CO₂ anesthesia. 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. Plasma glucose levels were assessed by the ferricyanide method of Hoffman (11). Plasma levels of insulin, leptin, resistin, and adiponectin were measured by commercial radioimmunoassay kits (RI-13 K, GL-32 K, and MADP-60 HK, respectively; Linco Research, St. Charles, MO). Plasma concentration of free fatty acids, triglycerides, and ketones were assessed using a commercial kit (Boehringer Mannheim, Mannheim, Germany). Carcass analysis was performed by assessing wet weights of liver, retroperitoneal fat mass (both sides), gastrointestinal tract, kidneys (both), and eviscerated carcasses.

Calculations and statistical analysis. Results are expressed as means $\pm$ SE. Areas under the curve (AUC) of blood glucose and plasma insulin responses were calculated using the trapezoidal method relative to baseline and over the full period of sampling following the start of intravenous glucose infusion (i.e., IVGTT). Food efficiencies (FE; mg body wt/kcal) were calculated as the ratio between body weight (g) gained over the first month of dietary treatment and the total amount of food (kcal) ingested during that period of time. All statistical analyses were done using SPSS 11.0 (Chicago, IL) and were performed within each rat line. 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, daily energy intake, and food efficiencies. Concerning the IVGTT and IST experiments, the diet effect (HsF, LC-HsF-HP, and HC) was evaluated using an ANOVA with repeated measures, with one within-subject factor (time) and one between-group factor (diet = HsF, LC-HsF-HP, or HC). Tukey's test was used for post hoc analysis. The level of significance was set at $P < 0.05$ for all tests.

RESULTS

Body weight and energy intake and efficiency. Figure 1 presents the average daily energy intake (A), body weight gain (B), and the FEs (C) calculated over the first month of the experiment, that is, before rats underwent surgeries. As expected, clear differences were observed between *fafa* Zucker

Table 1. Composition of the diets

	Standard Chow diet, HC		High-(Saturated) Fat Diet		High-(Saturated) Fat, High-Protein Diet	
	g/kg	%energy	g/kg	%energy	g/kg	%energy
Protein	228	23	200	20.4	340	34.7
Crude (lab chow)	228		69		18	
Added casein			131		322	
Fat (saturated fat)	55 (0.7)	14	260 (94.3)	60.2	260 (99.6)	60.2
Lab chow fat	55		17		4	
Added corn oil			74		77	
Added beef tallow			169		179	
Carbohydrates	625	63	190	19.4	50	5.1
Lab chow						
Polysaccharides	600		182		48	
Simple sugars	25		8		2	
Metabolizable energy		3.8 kcal/g		3.9 kcal/g		3.9 kcal/g

HC, high carbohydrate.

Fig. 1. A: average daily energy intake (kcal) calculated between day 0 and day 21 (i.e., before the surgeries were performed) in *fa/fa* Zucker and Wistar rats fed a high-carbohydrate (HC), high-fat (HsF), or low-carbohydrate, high-fat, high-protein (LC-HsF-HP) diet. B: body weight gain (g) calculated over the same period of time in *fa/fa* Zucker and Wistar rats fed a HC, HsF, or LC-HsF-HP diet. C: food efficiencies (mg body wt/kcal) of *fa/fa* Zucker and Wistar rats fed a HC, HsF, or LC-HsF-HP diet. Values are presented as means $\pm$ SE; * $P < 0.05$, ** $P < 0.01$.

and Wistar rats irrespective of diet, with energy intake, body weight gain, and FEs all being elevated in the *fa/fa* Zucker rats relative to the Wistar rats. Within each rat line, diet composition caused differential effects on above-mentioned parameters. For example, although diet composition failed to affect body weight gain of the Wistar rats, body weight gain in the *fa/fa* Zucker rats fed the HC was markedly lower ($P < 0.01$) than in the *fa/fa* Zucker rats fed the HsF diet or the LC-HsF-HP diet.

Despite the similar body weight gain in Wistar rats fed the different diets, the average daily energy intake was markedly reduced in the LC-HsF-HP fed rats compared with HC fed rats ($P < 0.01$). The 24-h-energy intake was also significantly lower for the rats fed the HsF diet compared with those fed the HC diet ($P < 0.05$). In the *fa/fa* Zucker rats, however, average daily energy intake was significantly elevated in the HsF group ($P < 0.01$) relative to the HC group. Increasing the percentage of proteins at the expense of carbohydrates (LC-HsF-HP diet) induced a significant reduction in energy intake ($P < 0.01$), with a 24-h energy intake equivalent to that of the *fa/fa* Zucker rats fed the HC diet.

FEs were significantly elevated in both *fa/fa* Zucker and Wistar rats fed the HsF and LC-HsF-HP diets compared with those fed the HC diet. As opposed to energy intake and body weight gain being affected differentially by diet composition in Wistar and *fa/fa* Zucker rats, the direction of change of FEs by

diet composition appeared comparable in Wistar and *fa/fa* Zucker rats.

IVGTTs. Between day 45 and day 50, animals were subjected to an IVGTT (Fig. 2, A–D) to assess their glucose tolerance and associated insulin responses. The ANOVA with repeated measures revealed a significant time $\cdot$ diet effect on plasma glucose levels in Wistar rats ($P = 0.0001$). Plasma glucose levels were significantly lower in the HC-fed rats at $t = 20$ and $t = 26$ min ($P < 0.05$) after the start of the infusion compared with those measured in the rats fed the HsF diet, as well as the LC-HsF-HP diet ($P < 0.05$ at 20 min only). No interaction effect was found in *fa/fa* Zucker rats. With respect to the AUCs of the glucose responses, however, there was a significant diet effect in both Wistar ($P = 0.030$) and *fa/fa* Zucker ($P = 0.005$) rats, with the lowest AUCs calculated for the rats fed the HC diet compared with the HsF, and particularly compared with the LC-HsF-HP diets (see Fig. 2 for details).

In both *fa/fa* Zucker and Wistar rats, there was a significant time $\cdot$ diet interaction on plasma insulin profiles during the IVGTT ($P = 0.066$ and $P = 0.041$, respectively). The pattern of the plasma insulin response of the *fa/fa* Zucker rats fed the HC diet was markedly different compared with those observed in the other two groups. The basal plasma insulin level was higher (39.9 ± 5.1 ng/ml, $P < 0.05$) in the *fa/fa* Zucker rats fed the HC diet than in those fed the HsF or LC-HsF-HP diets

Fig. 2. Intravenous glucose tolerance test. A: plasma glucose (mM) in *fa/fa* Zucker rats. B: plasma insulin (ng/ml) in *fa/fa* Zucker rats. C: plasma glucose (mM) in Wistar rats. D: plasma insulin (ng/ml) in Wistar rats. Rats were fed either an HC, HsF, or LC-HsF-HP diet. Values with the same letter are significantly different, $P < 0.05$. The dotted lines indicate when the infusion was performed. The bars represent the areas under the curve calculated for each parameter (between 0 and 50 min). Values are presented as means $\pm$ SE; * $P < 0.05$.

(23.6 ± 2.9 and 23.2 ± 2.9 ng/ml, respectively). Moreover, the plasma insulin levels in the *fa/fa* Zucker rat fed the HC diet remained higher during the 10 first min of the glucose infusion, and no further glucose-induced increase was observed. However, after the end of the infusion, plasma insulin decreased. No significant differences were observed among groups in Wistar or in *fa/fa* Zucker rats regarding the AUCs of the insulin responses.

ISTs. The intraperitoneal insulin injection induced an increase of plasma insulin together with a decrease of plasma glucose as presented in Fig. 3, A–D. In the *fa/fa* Zucker rats, the intraperitoneal injection of insulin resulted in similar increases in plasma insulin levels, which plateaued after $t = 30$ min. No significant diet effect was found on plasma glucose levels. In the Wistar rats, the intraperitoneal injection of insulin led to increments in the plasma insulin level, and these were associated with reductions in plasma glucose levels as well. The decline from baseline in plasma glucose levels was significantly less pronounced in the HsF (-5.1%) and the LC-HsF-HP-fed (-11.3%) rats compared with HC fed (-26.6%) rats 15 min after the intraperitoneal insulin injection ($P < 0.05$, HsF and LC-HsF-HP vs. HC). Interestingly, the Wistar rats could be assigned, in each dietary group, to subcohorts, either responding with an increase of plasma insulin (IST+) or not (IST–) to the intraperitoneal insulin injection. Although most animals in the HC ($n = 7$ out of 11) and HsF diet groups ($n = 8$ out of 11) reacted with IST+, only 3 out of 11 rats (27%) did in the LC-HsF-HP group.

Plasma fuels and hormones, and body composition. After decapitation of the rats, blood was collected to measure fuels and hormones, and the results of these analyses are shown in Table 2. In both *fa/fa* Zucker and Wistar rats, plasma insulin levels were significantly affected by diet composition

(ANOVA, diet effect, $P < 0.0001$ and $P = 0.045$, in the *fa/fa* Zucker and Wistar rats, respectively). The *fa/fa* Zucker rats fed the HC diet had markedly elevated basal insulin levels (43.3 ± 2.9 ng/ml) compared with those fed the HsF or LC-HsF-HP diets (30.7 ± 2.5 and 24.7 ± 2.0 ng/ml, respectively). Concerning the Wistar rats, basal insulin was significantly reduced in the LC-HsF-HP group compared with the HsF group (1.7 ± 0.1 vs. 2.2 ± 0.2 ng/ml, $P < 0.05$). Within both rat lines, leptin levels remained unaffected by diet composition. Adiponectin tended to be elevated in *fa/fa* Zucker rats fed the HC diet compared with the LC-HsF-HP and especially the HsF group ($P > 0.05$). The ANOVA revealed a significant diet effect on adiponectin and resistin levels in Wistar rats fed the HC diet compared with those fed the LC-HsF-HP diet, with significantly decreased adiponectin and significantly increased resistin plasma levels. No such differences were detected between the HsF and LC-HsF-HP groups. Plasma glucose and FFA levels remained unaffected by the composition of the diets, in both the *fa/fa* Zucker and the Wistar rats. Plasma levels of triglycerides were significantly affected only in the *fa/fa* Zucker rats, with elevated levels in the HsF and LC-HsF-HP groups relative to the HC fed rats. In contrast, plasma ketone levels were increased only in the Wistar rats fed the HsF or the LC-HsF-HP diets compared with those fed the HC diet, and this difference was significant for the LC-HsF-HP group only (196.6 ± 11.6 vs. 145.9 ± 10.2 $\mu\text{g/ml}$ in the HC group, $P < 0.05$). Such an effect was not observed in the *fa/fa* Zucker rats.

At the end of dietary treatments, the observed effects of diet on body weight in the *fa/fa* Zucker rats had disappeared, and again, no significant effects were observed in Wistar rats. Within each rat line, there was no significant effect of the diet on organ and tissue weight. This was probably due to the relatively large individual differences in body weight among

Fig. 3. Insulin sensitivity test. A: plasma glucose (mM) in *fa/fa* Zucker rats. B: plasma insulin (ng/ml) in *fa/fa* Zucker rats. C: plasma glucose (mM) in Wistar rats. D: plasma insulin (ng/ml) in Wistar rats. Rats were fed either a HC, a HsF, or a LC-HsF-HP diet. Each arrow indicates the time of the intraperitoneal injection.

animals within each dietary group. When normalized for body weight, however, the ANOVA revealed a significant diet effect on retroperitoneal fat mass and liver weight in the Wistar rats, but not in the *fa/fa* Zucker rats, as shown in Table 3. Post hoc analysis revealed reduced liver weight in the LC-HsF-HP-fed Wistar rats compared with the HC fed ones. Retroperitoneal fat mass was heavier in the HsF and LC-HsF-HP-fed Wistar rats than in those fed the HC diet.

Correlation analysis. For the Wistar rats and within each dietary group, the unresponsiveness to the IST seemed to be paralleled by relatively higher resistin and lower adiponectin levels; those differences were significant only in the LC-HsF-HP-fed rats (for plasma resistin, IST⁻: 2.9 ± 0.5 vs. IST⁺: 0.9 ± 0.1 ng/ml, $P < 0.05$; for plasma adiponectin IST⁻: 1.2 ± 0.1 vs. IST⁺: 1.8 ± 0.3 ng/ml, $P < 0.05$).

DISCUSSION

There is an increasing number of reports suggesting that low-carbohydrate diets may be beneficial in the treatment of obesity in humans (4). However, the long-term metabolic consequences still remain a matter of debate. Adverse health consequences might be expected to occur with prolonged ingestion of low-carbohydrate diets, since these usually contain large amounts of (saturated) fat. The present study focused on the metabolic consequences of HsF diets with a high or a low carbohydrate/protein ratio in obese *fa/fa* Zucker rats and lean Wistar rats, and these effects were compared with results obtained in rats fed a HC control diet.

Unlike what has been reported in humans, lean and genetically obese rats chronically fed a LC-HsF-HP did not have a

Table 2. Plasma concentration of fuels and hormones measured after 60 days of dietary treatment

Diet	Zucker <i>fa/fa</i> Rats			Diet Effect <i>P</i> value	Wistar Rats			Diet effects <i>P</i> value
	HC	HsF	LC-HsF-HP		HC	HsF	LC-HsF-HP	
Glucose, mmol/l	7.7 ± 0.2	7.7 ± 0.1	7.6 ± 0.2	ns	7.7 ± 0.2	7.5 ± 0.1	8.1 ± 0.2	ns
Free fatty acids, mmol/l	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	ns	0.8 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	ns
Triglycerides, mmol/l	$1.1 \pm 0.1^{a,b}$	1.3 ± 0.1^a	1.2 ± 0.1^b	0.007	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	ns
Ketones, μ g/ml	95.6 ± 11.2	96.1 ± 18.6	95.7 ± 8.5	ns	145.9 ± 10.2^c	171.6 ± 11.6	196.6 ± 11.6^c	0.019
Insulin, ng/ml	$43.3 \pm 2.9^{a,b}$	30.7 ± 2.5^a	24.7 ± 2.0^b	<0.0001	2.0 ± 0.1	2.2 ± 0.2^c	1.7 ± 0.1^c	0.045
Leptin, ng/ml	34.6 ± 7.4	25.3 ± 8.1	29.8 ± 3.7	ns	3.9 ± 0.4	4.2 ± 0.5	3.8 ± 0.3	ns
Adiponectin, μ g/ml	4.9 ± 0.3	4.0 ± 0.2	4.4 ± 0.4	ns	1.8 ± 0.1^c	1.5 ± 0.1	1.4 ± 0.1^c	0.034
Resistin, ng/ml	2.0 ± 0.3	1.6 ± 0.3	2.1 ± 0.3	ns	1.4 ± 0.3^c	1.6 ± 0.1	2.4 ± 0.4^c	0.033

Values are expressed as means $\pm$ SE. Statistical comparisons are for treatment within genotype. Values with the same letter within a row are significantly different ($P < 0.05$); ns, not significant.

Table 3. *Body composition after 60 days of dietary treatment*

Diet	Zucker <i>fa/fa</i> Rats				Wistar Rats			
	HC	HsF	LC-HsF-HP	Diet effect <i>P</i> value	HC	HsF	LC-HsF-HP	Diet effect <i>P</i> value
Liver	3.77±0.25	3.19±0.13	3.61±0.15	ns	3.11±0.07 ^a	2.88±0.10	2.72±0.07 ^a	0.012
Kidneys	0.73±0.16	0.56±0.02	0.66±0.06	ns	2.7±0.1	2.7±0.2	2.9±0.1	ns
Retroperitoneal fat	4.32±0.20	4.36±0.32	4.28±0.17	ns	1.23±0.09 ^{a,b}	1.64±0.10 ^a	1.64±0.11 ^b	0.016
Gastrointestinal tract (full)	9.84±0.35	9.53±0.47	9.07±0.36	ns	7.51±0.71	7.72±0.88	6.65±0.53	ns
Eviscerated carcass	78.2±0.9	76.0±1.0	76.9±0.3	ns	79.3±0.7	79.4±0.4	80.1±0.2	ns

Values are presented as percentage of body weight and expressed as means ± SE. Statistical comparisons are for treatment within genotype. Values with the same letter within a row are significantly different ($P < 0.05$).

lower body weight gain than rats either on a HsF diet with a high-carbohydrate/protein ratio or on a HC lab chow control diet. In the obese *fa/fa* Zucker rats, but not in the Wistar rats, body weight gain was even amplified on the HsF, as well as on the LC-HsF-HP diets compared with what was observed with the HC diet. These effects, however, were transient and disappeared at the end of the dietary treatment, and this effect may have been paralleled by the similar weights of retroperitoneal fat pads (i.e., a correlate of body adiposity) in the *fa/fa* Zucker rats at the end of the different dietary treatments. Analogous transient effects of diets with different macronutrient content on body weight in *fa/fa* Zucker rats have previously been reported by Vasselli et al. (34), and these authors suggested that this was the result of a ceiling effect or “breakpoint” imposed by maximal storage capacity of adipose tissue in these animals. In the Wistar rats, however, relative weights of retroperitoneal fat pads in the HsF and LC-HsF-HP fed animals were higher compared with those observed in Wistar rats fed the HC diet, indicating increased body adiposity despite similar body weights.

Even though body weight was not reduced by the HsF and LC-HsF-HP diets, there were reductions in energy consumption in the HsF-fed, and particularly in the LC-HsF-HP-fed Wistar rats (−12.4%) compared with the HC-fed ones. Anorexigenic effects of a diet with a high-fat content may be attributed to its “ketogenic” character, which would obviously be even more accentuated when carbohydrates are replaced by protein in such a diet. This scenario is of course relevant to our study in which plasma ketone levels of HsF-fed Wistar rats and particularly of LC-HsF-HP-fed Wistar rats were increased relative to the level found in HC fed rats. In contrast to the Wistar rats, plasma ketone levels were not different among diet groups of obese *fa/fa* Zucker rats. This is in line with other studies underscoring congruous inhibited fat oxidation and reduced ketogenesis in *fa/fa* Zucker rats (e.g., 31). Another mechanism that may have contributed to reduced energy intake, at least in the LC-HsF-HP group is that the elevated dietary protein content has specific satiating effect (for a review, see Ref. 29), and these effects may persist when the diet is also high in fat. This “protein” effect of the LC-HsF-HP diet may not be limited to Wistar rats, as *fa/fa* Zucker rats also had reduced consumption of the LC-HsF-HP diet compared with those fed the HsF diet. An effect of elevated dietary protein content to cause a reduction of food intake in *fa/fa* Zucker rats is in line with reports of Peret et al. (22) and Vasselli and Maggio (33).

As mentioned above, relative reductions in intake of the LC-HsF-HP (Wistar and *fa/fa* Zucker rats) and of the HsF

(Wistar rats only) diets were not associated with a lower body weight gain. One implication of this phenomenon is that the consumption of diets with a high fat content (or conversely with a low carbohydrate content) renders animals more efficient with their ingested calories (5, 10, 32). These results are in agreement with our previous findings showing that high-fat feeding, irrespective of protein/carbohydrate content, increases food efficiency in Wistar rats, as well as in rats rendered obese by a chronic pharmacological impairment of the central melanocortin system (18). Our current data show that leptin signaling is not required for this effect.

In general, *fa/fa* Zucker rats were less glucose tolerant and extremely hyperinsulinemic compared with Wistar rats, which underscores the nature of the obesity and insulin resistance model that these leptin receptor-deficient animals represent. Within both rat lines, glucose tolerances were reduced in rats fed the HsF and particularly the LC-HsF-HP diet compared with those fed the HC diet. Regarding the Wistar rats, the observed glucose intolerances during the IVGTT were associated with similar, or slightly increased insulin responses, which is indicative of a reduced whole body insulin-induced glucose uptake (5, 12, 28, 35). And, indeed, insulin sensitivity—indexed by the magnitude of insulin-induced hypoglycemia during the IST—was most attenuated in Wistar rats fed the LC-HsF-HP diet compared with those fed the HC diet. At first sight, these data may be interpreted to indicate that diet-induced changes in insulin sensitivity contribute to alteration in glucose homeostasis in the Wistar rats. Consistent with this idea would be the observation that plasma levels of adiponectin and resistin—hormones that respectively increase (9) and reduce (15, 36) glucose tolerance and insulin sensitivity—were consistently altered by the LC-HsF-HP diet in the Wistar rats. Furthermore, analysis of the individual data collected during the IST revealed that 8 out of 11 of the LC-HsF-HP fed Wistar rats did not respond at all to the intraperitoneal injection of insulin, either with a decrease in plasma glucose or with an elevated plasma insulin level. Closer inspection revealed that the “insulin-nonresponders” (IST−) had significantly increased plasma resistin (+222%) and decreased adiponectin (−33%) levels compared with those that did respond to the injection (IST+). In addition, the IST− rats also presented an increased—though not significant ($P = 0.077$)—food efficiency (IST−: 0.048 ± 0.002 and IST+: 0.042 ± 0.001 g/kcal). At this point, we do not know whether the reduced insulin levels found in plasma were the result of increased clearance or blunted passage from the abdominal cavity to the bloodstream. The first possibility does not seem very likely, as no differences in baseline (i.e., endogenous) levels of insulin

were observed between IST+ and IST− rats. To consider the second hypothesis, it might be imagined that exogenous insulin uptake by the abdominal capillary beds is highly variable among animals fed a ketogenic diet, and that “adipocytokines,” such as resistin play a role in this mechanism. Although we have no direct evidence for such an effect, this idea may be corroborated by the recent findings of Mu et al. (19) showing that resistin has potent stimulatory effects on capillary angiogenesis, which potentially could impair insulin transport across capillary walls. Such an effect on capillary beds may be caused by resistin originating from adipose tissue or by resistin secreted from macrophages (21). We are currently investigating these potential mechanisms, as they could be relevant for diabetic patients on insulin therapy.

As opposed to the Wistar rats, no large differences in plasma insulin levels after insulin injection were observed between *fa/fa* Zucker rats in the different dietary groups, nor were there large differences in plasma levels of resistin and adiponectin within and between dietary groups. An issue that deserves attention in this respect is the fact that, while different dietary groups of *fa/fa* Zucker had comparable insulin levels at baseline before the IST, *fa/fa* Zucker rats fed the HC diet were markedly hyperinsulinemic at baseline before the IVGTT relative to the other dietary groups. In the case of the IVGTT, this elevated basal plasma insulin level was followed by a highly disturbed—and in some animals even absent—insulin response during the IVGTT. These effects could be the result of post-ingestive consequences of the HC diet in the *fa/fa* Zucker rats, since the period of food deprivation prior to the IVGTT (8–9 h during the light phase) was shorter than prior to the IST (overnight and light phase). Although this was not detrimental for glucose homeostasis in *fa/fa* Zucker rats fed the HC diet in the present study, it may be anticipated that obese subjects of a species with a lower maximal or more “labile” B-cell capacity than *fa/fa* Zucker rats [i.e., such as humans (25)] could be at risk of developing B-cell failure to compensate the insulin resistance frequently associated with obesity. In that respect, consuming a diet with a relatively low carbohydrate content might be less detrimental. Finally, future experiments will be necessary to indicate the effects of the quality of added dietary protein and fatty acids, which have been used in the formulation of the low-carbohydrate diet in the present study. This is necessary since protein and fatty acid sources used to prepare the low-carbohydrate diet (i.e., casein and mostly saturated fat, respectively, in the present experiment) differed from those that were already supplied in the HC diet. It is possible that these different sources of protein and fatty acids in the HsF and LC-HsF-HP diet also have specific effects on energy balance and glucose homeostasis (3, 30).

In conclusion, our work shows that consuming a LC-HsF-HP diet causes impaired glucose tolerance in lean Wistar as well as obese *fa/fa* Zucker rats, an effect that is probably due to the high level of saturated dietary fat. Although this indicates similar adverse consequences of such a diet on blood glucose homeostasis in lean and obese rats, there appear to be principal differences in the underlying hormonal regulatory mechanisms, which could have benefits for B-cell functioning and insulin action in the obese, but not in the lean state. Because no reductions in body weight and retroperitoneal fat pad weights were observed in lean and obese rats when fed the LC-HsF-HP diet (despite reductions in energy intake, which

appeared to be compensated by increases in food efficiency), these findings may be of particular importance for individuals for whom the diet is not very beneficial in their attempt to lose weight. Future studies should perhaps be focused on body weight effects and health consequences of low-carbohydrate diets with a higher protein content at the expense of dietary fat.

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