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Dalila D. Azzout-Marniche, Catherine C. Chaumontet, Nachiket A Nadkarni, Julien Piedcoq, Gilles Fromentin, et al.. Food intake and energy expenditure are increased in high-fat-sensitive but not in high-carbohydrate-sensitive obesity-prone rats.. *AJP - Regulatory, Integrative and Comparative Physiology*, 2014, 307 (3), pp.R299-309. 10.1152/ajpregu.00065.2014 . hal-01173413

HAL Id: hal-01173413

<https://hal.science/hal-01173413>

Submitted on 28 May 2020

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Food intake and energy expenditure are increased in high-fat-sensitive but not in high-carbohydrate-sensitive obesity-prone rats

Dalila Azzout-Marniche,^{1,2} Catherine Chaumontet,^{1,2} Nachiket A. Nadkarni,^{1,2} Julien Piedcoq,^{1,2} Gilles Fromentin,^{1,2} Daniel Tomé,^{1,2} and Patrick C. Even^{1,2}

¹Institut National de la Recherche Agronomique, Centre de Recherche en Nutrition Humaine d'Ile-de-France (CRNH-IdF), UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France; and ²AgroParisTech, CRNH-IdF, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France

Submitted 11 February 2014; accepted in final form 28 May 2014

Azzout-Marniche D, Chaumontet C, Nadkarni NA, Piedcoq J, Fromentin G, Tomé D, Even PC. Food intake and energy expenditure are increased in high-fat-sensitive but not in high-carbohydrate-sensitive obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 307: R299–R309, 2014. First published June 4, 2014; doi:10.1152/ajpregu.00065.2014.—Obesity-prone (OP) rodents are used as models of human obesity predisposition. The goal of the present study was to identify preexisting defects in energy expenditure components in OP rats. Two studies were performed. In the first one, male Wistar rats ($n = 48$) were fed a high-carbohydrate diet (HCD) for 3 wk and then a high-fat diet (HFD) for the next 3 wk. This study showed that adiposity gain under HCD was 2.9-fold larger in carbohydrate-sensitive (CS) than in carbohydrate-resistant (CR) rats, confirming the concept of “carbohydrate-sensitive” rats. Energy expenditure (EE), respiratory quotient (RQ), caloric intake (CI), and locomotor activity measured during HFD identified no differences in EE and RQ between fat-resistant (FR) and fat-sensitive (FS) rats, and indicated that obesity developed in FS rats only as the result of a larger CI not fully compensated by a parallel increase in EE. A specific pattern of spontaneous activity, characterized by reduced activity burst intensity, was identified in FS rats but not in CS ones. This mirrors a previous observation that under HCD, CS but not FS rats, exhibited bursts of activity of reduced intensity. In a second study, rats were fed a HFD for 3 wk, and the components of energy expenditure were examined by indirect calorimetry in 10 FR and 10 FS rats. This study confirmed that a low basal EE, reduced thermic effect of feeding, defective postprandial energy partitioning, or a defective substrate utilization by the working muscle are not involved in the FS phenotype.

obesity-prone; energy balance; locomotor activity; indirect calorimetry; mRNA; blood parameters

GENETIC AND EPIGENETIC FACTORS make some individuals more sensitive than others to diet-induced obesity (20, 45). Outbred polygenetic rodents exhibit this variability in individual responses to diets (30, 50).

In rats, sensitivity to obesity has mainly been studied in response to a high-fat diet (HFD), and numerous differences between obesity-prone (OP) and obesity-resistant (OR) rats have been reported. After adaptation to a HFD and development of obesity, OP rats present intrinsic differences in nor-epinephrine metabolism (25), altered arcuate neuropeptide Y (NPY) expression (26), impairment in growth hormone secretion (24), leptin resistance (29, 31), defective counter-regulatory responses to insulin-induced hypoglycemia (46), lower interscapular brown adipose tissue mitochondrial uncoupling

protein-1 content and proton conductance (18), and reduced dopamine levels in the nucleus accumbens (37). It is, however, difficult in these studies to conclude whether the observed differences are the cause or the consequence of the HFD-induced obesity. Studies conducted early after high-fat feeding are less numerous, but high plasma triglycerides (TG) (11, 23), elevated leptin, insulin, TG (11), tissue-specific changes in lipoprotein lipase (11, 36), and greater expression of the opioid enkephalin in various areas of the hypothalamus (7) early in response to high-fat (HF) feeding have been described as possible defects that may contribute to the sensitivity to HF feeding.

Large differences in adiposity gain can also be observed under a high-carbohydrate diet (HCD) in mice (51) and in rats (15, 34). Obesity under HC feeding develops more visceraally, and in our hands, only half of the rats prone to obesity under HC feeding are also prone to obesity under HF feeding and vice versa. Therefore, the mechanisms responsible for the predisposition to obesity under HC feeding are not necessarily the same as those that promote body fat gain under HF feeding. Accordingly, from now on, we divide OP rats into fat-sensitive (FS, as opposed to fat-resistant, FR) and carbohydrate-sensitive (CS, as opposed to carbohydrate-resistant, CR) rats. In search of metabolic and/or behavioral factors able to predict for fat or carbohydrate sensitivity, we previously reported that in young lean rats fed a standard HCD, rats that would later demonstrate a sensitivity to a HFD had smaller meal size and larger meal number while rats that would later demonstrate a sensitivity to HCD had lower intensity of motor activity and higher ingestion speed (15). These parameters are, however, difficult to use as predictive factors to separate individuals because of significant overlap in individuals' data. In a following study, we performed a detailed analysis of the components of energy expenditure in FR/FS and CR/CS rats that revealed that FS rats did not exhibit any defect in any components of energy expenditure before being fed a HFD, but that CS rats had higher postmeal glucose oxidation and lower postmeal lipid oxidation than CR rats (34).

In previous studies (15, 34), CS and FS rats were mainly studied while fed a HCD or during transition from a HCD to a HFD. The goal of the present study was to further evaluate body weight, body composition, components of energy expenditure, and feeding and activity patterns in CS and FS rats, more particularly after adaptation to HF feeding. In a first experiment, 48 rats were classified as CR or CS and FR or FS, according to the evolution of their adiposity measured by MRI during 3 wk of HC feeding and then 3 wk of HF feeding. During the third week of HF feeding, meal pattern, spontane-

Address for reprint requests and other correspondence: P. C. Even, AgroParisTech, CRNH-IdF, UMR914 Nutrition Physiology and Ingestive Behavior, F-75005 Paris, France (e-mail: patrick.even@agroparistech.fr).

ous activity and total energy expenditure (TEE) were measured by indirect calorimetry coupled with recording of food intake and spontaneous activity. At the end of the study, the rats were killed; blood was taken for analysis of plasma circulating substrates; and tissues were sampled for quantification of mRNA expression for various genes in the liver, adipose tissue, and hypothalamus. In a second study, the components of energy expenditure were studied in detail under HF feeding in 10 FS and 10 FR rats during a cycle of fasting and controlled refeeding, according to a procedure previously described (14, 34).

MATERIALS AND METHODS

The protocol conformed to the European legislation on the use of laboratory animals and was approved by the French Ethical Committee no. 11-027.

Experiment 1

Design. In a first experiment, male Wistar rats ($n = 48$) (Harlan), weighing ~ 225 g (range 193–252 g) and 7 wk old, were delivered as six groups of eight, with groups arriving sequentially over a 9-mo period. Rats recovered in the laboratory for 1 wk on a synthetic high-carbohydrate diet (HCD; Table 1) prior to any procedures. A 12:12-h light-dark cycle (lights on at 0800) was maintained throughout the experiment.

The design of the experiment was similar to that of Nadkarni et al. (34). The eight rats in each batch were scanned by MRI to measure their body fat content a week after arrival. Using these data, in general, for each batch, the two leanest and two fattest rats were kept, and the other four discarded from the study. The idea was to exploit the preexisting (small) differences in starting adiposity that develop after weaning to favor preselection of CR and CS rats [the maintenance diet of rats (chow) being a high-carbohydrate-low fat diet] and thus limit the number of rats excluded from the study. After the first MRI measurement, the rats were continued for another 3 wk on the synthetic HCD and reanalyzed for body adiposity at the end of this period. At this point, CR and CS rats were segregated according to their increase in body adiposity during the HCD period. The rats were then fed for 3 wk on the HFD (Table 1) prior to another round of MRI. At this point, FR and FS rats were segregated on the basis of their gain in body adiposity during HFD. During the third week of HFD, the rats

were housed for five consecutive days in metabolic cages with free access to the HFD for measurements of TEE, feeding behavior, and spontaneous motor activity. At the end of the study, the rats were anesthetized with halothane and killed by decapitation; then blood was collected. At this stage of the study a significant number of rats were too large to enter the MRI tunnel, or even if they could enter, their size caused image artifacts. Therefore, final body composition was measured by dissection and weighing of the main organs and tissues (16).

Calorimetry. MONITORING OF TEE, FEEDING BEHAVIOR AND SPONTANEOUS ACTIVITY UNDER HFD. Because of technical constraints, measurements of respiratory exchange, feeding pattern, and motor activity were performed in only 4 of the 6 groups of rats included in the study ($n = 16$). The goal was to obtain for each rat measures of meal pattern, spontaneous movement, TEE, and respiratory quotient (RQ) during HFD. All four rats in a group were housed at 1800 in individual metabolic cages equipped with a weighed food cup (sensitivity better than 0.05 g) and with an activity platform placed below the cage (sensitivity better than 1 g) (see Ref. 14 for details). For gas analysis, the cages were multiplexed, meaning all connected to the same gas analyzers. Thus $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for each cage during 2 min every 10 min (2 min for each cage plus 2 min on an empty cage-room air, to correct values for room $O_2\%$ and $CO_2\%$). Measurements were performed over 4 days. *Day 1* in the metabolic cage was used for habituation. $\dot{V}O_2$, $\dot{V}CO_2$, caloric intake (CI), and spontaneous activity were measured on *days 2, 3, and 4*. Data were analyzed only when the feeding pattern could be confidently analyzed, in particular, when no food spillage was observed in the cage and/or suspected from the trace. This required exploiting only 2 out of the 3 days of recording in 10 out of the 16 rats. Metabolic rate was computed from $\dot{V}O_2$ and $\dot{V}CO_2$, according to the Weir formula (13, 17). TEE, CI, and activity values reported are the results of the average values of 2 or 3 days of recording.

ADJUSTMENT OF TEE, CI, AND SPONTANEOUS ACTIVITY TO BODY SIZE AND COMPOSITION. As calorimetry sessions took place between the second round of MRI and dissection, the fat-free mass (FFM) and fat mass (FM) of the rats during the calorimetry sessions were estimated by linear extrapolation. The same technique was used in Nadkarni et al. (34) between the first two rounds of MRI. Here, as there, the technique was considered valid since the FFM+FM weights predicted by the extrapolation matched very well the actual measured body weight (BW) during calorimetry. The TEE and CI data were adjusted to per kilogram FFM and by a more recent attempt to take into account the metabolic activity of FM using $(FFM + 0.2 \times FM)$ as the metabolically active body mass (2, 14). Spontaneous activity signal recorded from force transducers was normalized to per kilogram BW to take into account the fact that, for a given intensity of activity, the signal from the force transducers was proportional to the weight of the animal.

CALCULATION OF ENERGY BALANCE AND FOOD EFFICIENCY. Energy balance (EB) was computed from changes in body composition [assuming that 4.8 kJ/g are fixed in lean tissues and 34 kJ/g in adipose tissue (43)] for the whole 21 days of HFD feeding (EBMRI) and from differences between CI and TEE measured during the 5 days of food intake and calorimetry measurements (EBCalo). Food efficiency during calorimetry measurements was computed as the ratio of energy deposited to caloric intake (kJ/kJ).

Blood and tissue sampling. At the end of *experiment 1*, blood was collected from the trunk of the animal for measurements of TG, glycerol, ketone bodies, high-density lipoprotein (HDL), and cholesterol. Pieces of liver and epididymal fat were collected for assaying TG content and mRNA expression of genes, encoding key enzymes involved in different metabolic pathways, such as lipogenesis and glycerolipid biosynthesis (acetyl-CoA carboxylase, fatty acid synthase, glycerol-3-phosphate acyltransferase 1 mitochondrial), glycolysis (glucokinase), lipolysis and β -oxidation

Table 1. *Macronutrient composition of the HCD and HFD*

	HCD	HFD
Weight content, g/kg		
Milk proteins	140.0	170.0
Starch	622.4	436.6
Sucrose	100.3	71.1
Soy Oil	40.0	225.0
Minerals	35.0	35.0
Vitamins	10.0	10.0
Cellulose	50.0	50.0
Choline	2.3	2.3
Energy content, %		
Protein	14.7	14.4
Carbohydrate	75.9	42.9
Fat	9.4	42.8
Energy density, kJ/g	15.95	19.82
Food quotient	0.946	0.847

Macronutrients were prepared by the *Atelier de Preparation des Aliments*, INRA, Jouy en Josas, France. Energy density is computed assuming a metabolizable energy of 16.7 kJ/g for carbohydrates and proteins and 37.7 kJ/g for fat. Food quotient is computed assuming a quotient of oxidation of 1.0 for carbohydrates, 0.825 for proteins, and 0.70 for lipids. HCD, high-carbohydrate diet; HFD, high-fat diet.

(monoglycerol lipase and carnitine acyltransferase 1, a liver isoform), lipoprotein trafficking (apolipoprotein A and E), and glycerol metabolism (glycerokinase) in the liver, acetyl CoA carboxylase, fatty acid synthase (FAS), leptin, and lipoprotein lipase in adipose tissue. The hypothalamus was dissected, put in TRIzol (Invitrogen) and snap frozen for measuring mRNA expression of neuropeptide Y, proopiomelanocortin, melanocortin 4 receptor, cocaine- and amphetamine-regulated transcript, Agouti-related peptide, insulin receptor, leptin receptor (long cytoplasmic form), fat mass, and obesity-associated genes.

Experiment 2

Design. Forty male Wistar rats (Harlan), weighing ~225 g (range 193–252 g) and 7 wk old, were delivered as five groups of eight, with groups arriving sequentially over a 6-mo period. Rats recovered in the laboratory for 1 wk on the synthetic high-carbohydrate diet prior to being maintained on the HFD for 3 wk. A 12:12-h light-dark cycle (lights on at 0800) was maintained throughout.

Calorimetry. During the third week of HF feeding, since *experiment 1* showed a strong correlation between BW gain and adiposity gain during HFD (see Fig. 3), rather than using MRI body composition measurements of adiposity, selection for further study was simply based on body weight gain; the two rats that gained the most and the two that gained the least weight in each group of eight were housed, in turn, for 24 h in a calorimetry device for a detailed analysis of the components of energy metabolism, as described previously (14, 34). In brief, the rats were housed in the cage at 1800 with water but were food-deprived overnight. Temperature in the cage was maintained at 26°C to reduce the cost of thermoregulation (14). The next day at 1000, a test meal of 60 kJ (3 g) of the HF diet was given to the animal, and data collection continued until 1700. As previously described (13, 14), the calorimetry system allowed separation of resting and activity-related $\dot{V}O_2$ and $\dot{V}CO_2$, thus precise itemizing of the various components of total energy expenditure. Briefly, the simultaneous recording of $\dot{V}O_2$, $\dot{V}CO_2$, and the intensity of spontaneous activity at a high rate (1 point every 2 s) feeds a model of changes expected in $\dot{V}O_2$ and $\dot{V}CO_2$ values as a function of changes in the intensity of motor activity [cage volume and air flow are used to model the delay and damping of the response, and it is assumed that resting metabolic rate (RMR) and the cost of activity remain constant]. Model predictions are then compared with the actual measurements using a numerical filtering process (Kalman) that is tuned to maintain the model predictions close to the actual measurements. This leads to the computation over time of RMR and cost of activity values that fit the predictions of the model and the values actually recorded by the calorimetry device. This allows separation of resting and activity-related $\dot{V}O_2$ and $\dot{V}CO_2$, thus precise itemizing of the various components of TEE. $\dot{V}O_2$ and $\dot{V}CO_2$ (ml/min) were converted to metabolic rate (Watts) using an adaptation of the Weir equation [(16.3 $\dot{V}O_2$ + 4.57 $\dot{V}CO_2$)/60]. RQ was computed as the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$. The thermic effect of feeding (TEF) was calculated from the increase in RMR induced by the test meal (and

was, thus, free from any noise due to variations in spontaneous activity).

Body adiposity was measured by MRI the Monday that followed the measurements to control for the difference in adiposity between rats and to adjust energy expenditure to the metabolic body size (FFM + 0.2 × FM) (14). This same procedure applied in rats fed a HCD has been previously published (34).

Body composition by MRI analysis. Images were acquired on a 7T Bruker Pharmascan system (running Paravision 4) using a Bruker 50 mm ID tunable quadrature RF resonator (For details, see Ref. 34.) Images were registered, and then fat pads were segmented semiautomatically (by fuzzy c-means) in MIPAV 4.3.0. Adipose volume was converted to grams of FM on the assumption of a density of 0.9 g/cm³, and FFM was determined by subtracting this from the weight of the rat on the day of the scan.

RNA extraction and RT-PCR. Small pieces of liver and adipose tissue were taken and frozen in liquid nitrogen. To avoid RNA degradation, the hypothalamus was immediately extracted from the fresh brain by making an incision medial to the piriform lobes caudal to the optic chiasma and anterior to the cerebral crus to a depth of 2–3 mm and was put directly in TRIzol reagent and frozen in liquid nitrogen. This extraction was centered on the arcuate nucleus, which is the main site of expression of proopiomelanocortin (POMC) and NPY but also included part of the dorsal motor nucleus and lateral hypothalamus nuclei. Total RNA was extracted from liver, adipose tissue, and hypothalamus tissues with TRIzol reagent (Invitrogen, Breda, Netherlands). Concentrations of RNA samples were measured on a NanoDrop ND-1000 UV-Vis spectrophotometer. RNA integrity was checked by ethidium bromide staining. 0.4 µg of total RNA in a final volume of 10 µl was reverse transcribed using a high-capacity cDNA archive kit protocol (Applied Biosystems). Real-time PCR was performed to measure RNA expression using a ABI 7300 (Applied Biosystems using Power SYBR Green PCR Master Mix), as previously described (44). Reactions were performed as follows: denaturation for 10 min at 95°C, 40 cycles at 95°C for 15 s, followed by 1 min at 60°C (amplification). Negative controls (reactions without reverse transcriptase or RNA) were used to monitor for contamination. The efficiency was estimated using a series of five-fold dilutions of the sample and checked for each run. A melting curve was performed to check for the absence of contamination. The primer sequences of target genes are given Table 2. Gene expression was calculated as $2^{-\Delta CT}$. 18S RNA was used as the housekeeping gene in liver and adipose tissue, and RPL-13A RNA in hypothalamus.

Statistical analysis of postprocessed data. Results are reported as means ± SE. Between-group comparisons were done by Student's *t*-tests in Excel. *P* ≤ 0.05 was considered as significant. To select between sensitive and resistant rats in *experiment 1*, we used the evolution of adiposity (defined as FM/BW) during the HCD (CR/CS) and HFD (FR/FS) periods. For each period, the mean and the

Table 2. Primer sequences of target genes

Gene	Upregulated	Downregulated
FAS	5'-TGCTCCAGCTGCAG-3'	5'-GCCCGGTAGCTCTGGGTGTA-3'
ACC	5'-CAACGCCTTCACACCACCTT-3'	5'-AGCCATTACTTTCATCAAGATCCT-3'
Lept	5'-CAAGTCCAGGATGACCAAAA-3'	5'-ATACGACTGCGTGTGTGAAAT-3'
LPL	5'-GGACTGAGGATGGCAAGCA-3'	5'-GGCAGGTTGAAGGAATGTT-3'
NPY	5'-TTTTCTAGTTTCCCCCACA-3'	5'-CCTGGTGGTGGCATGCAT-3'
POMC	5'-AGGCCCTTCCCTAGAGTTCAA-3'	5'-GTCGGCCTTCTCGGTATCC-3'
MC4R	5'-GGGAAAGCCACAAAAACGA-3'	5'-GGCGCTACTGAAAGTCACTCT-3'
CART	5'-CCGAGCCCTGGACATCTACTC-3'	5'-AAATACTGACCAGCTCCTTCTCATG-3'
AGRP	5'-TGGTGCCCTTGACCAAGTT-3'	5'-AATTCTGCCCCACAGATG-3'
IR	5'-CCCAATGGCAACATCACACA-3'	5'-CAGCTCGCTGTCTCTGCTT-3'
Ob-R	5'-ACGATGCTTACCACGTACT-3'	5'-GGAAGCCCTGACGAAAAA-3'
FTO	5'-GAGCGGAAGCTAAGAACTGA-3'	5'-GCCACTGCTGATAGAACTCATCA-3'

Student confidence interval (SCI) of adiposity gain at $\alpha = 0.05$ were computed as $SD \times 1.96 / \text{SQRT}(n)$ (with SD for standard deviation and SQRT for square root). Resistant rats were defined as those whose adiposity gain was lower than the mean minus SCI, while sensitive rats were defined as those whose adiposity gain was higher than the mean plus SCI. During HCD, mean adiposity gain of all of the 24 rats was 3.74% with a 95% confidence interval of 0.76%, and during HFD, mean adiposity gain was 6.34% with a 95% confidence interval of 0.86%. Following this classification, and thanks to the initial preselection based on the initial adiposity of the rats, of the 24 rats included for the whole study, 10 rats that gained less than 2.99% adiposity were classified as CR, 8 that gained more than 4.50% were classified as CS, and only six were discarded as intermediate. Similarly, under a HFD, 10 rats that gained less than 5.48% were classified as FR, 7 that gained more than 7.20% were classified as FS, and 7 were discarded as intermediate.

For the sake of comparison between rats that varied largely in body size and composition, energy expenditure and caloric intake were adjusted to fat free mass (FFM) and active metabolic mass (AMM) computed as $(\text{FFM} + 0.2 \times \text{FM})$ (14).

RESULTS

Evolution of BW and Body Composition During HCD and HFD (Experiment 1)

Individual responses of the rats to the HCD and HFD showed that CS rats were not necessarily FS and vice versa (Fig. 1A), with four out of the eight CS rats also being FS, and 6 out of the 10 CR being FR. However, a significant correlation between adiposity gain during HCD and HFD was observed (Fig. 1B). The 0.29 value of the R^2 coefficient indicates that sensitivity to one of the diets predicts only one-third of the sensitivity to the other diet.

During HCD, adiposity gain was $1.98\% \pm 0.19$ in CR rats and $5.81\% \pm 0.47$ in CS ones, meaning CS rats gained 2.9 times more adiposity than CR rats (Fig. 2A). This occurred without significant differences in BW gain, as the difference between CR and CS rats lay exclusively in FM gain, while the gain in FFM was much the same. In FS rats, adiposity, but also BW and FFM, all increased significantly more than in FR ones (Fig. 2B). During a HFD, adiposity gain was $4.52\% \pm 0.22$ in FR rats and $9.11\% \pm 0.53$ in FS ones (see Table 3). FS rats gained 2 times more fat, 1.6 times more weight and 1.2 times more FFM than FR rats (Fig. 2D). CS rats also gained more adiposity than CR ones during HFD but only 1.44 times more and the gains in BW and FFM were not significantly different (Fig. 2C).

Considering all rats together, the ratio of visceral to subcutaneous fat deposition was significantly larger under HCD (2.73 ± 0.23) than under HFD (1.39 ± 0.13) ($P < 0.001$) (Fig. 2), but the potential adverse effect of such deposition in CS rats was moderated by the fact that under HCD they increased fat storage subcutaneously (~ 3 times) more than viscally (~ 2 times) so that the ratio of visceral to subcutaneous fat deposition was only 2.05 ± 0.23 vs. 3.17 ± 0.35 in CR rats ($P = 0.02$). However, considered in the long-term, such a specific visceral deposition of fat with a high-carbohydrate diet should be considered to be a potential aggravating factor compared with the more subcutaneous fat deposition observed under high-fat feeding.

Taken together, these results indicate that the accumulation of fat during HCD develops more viscally than subcutaneously, and although adiposity gain was lower in absolute terms in CS rats, the difference in adiposity gain was relatively larger between CR and CS rats than between FR and FS rats. Because

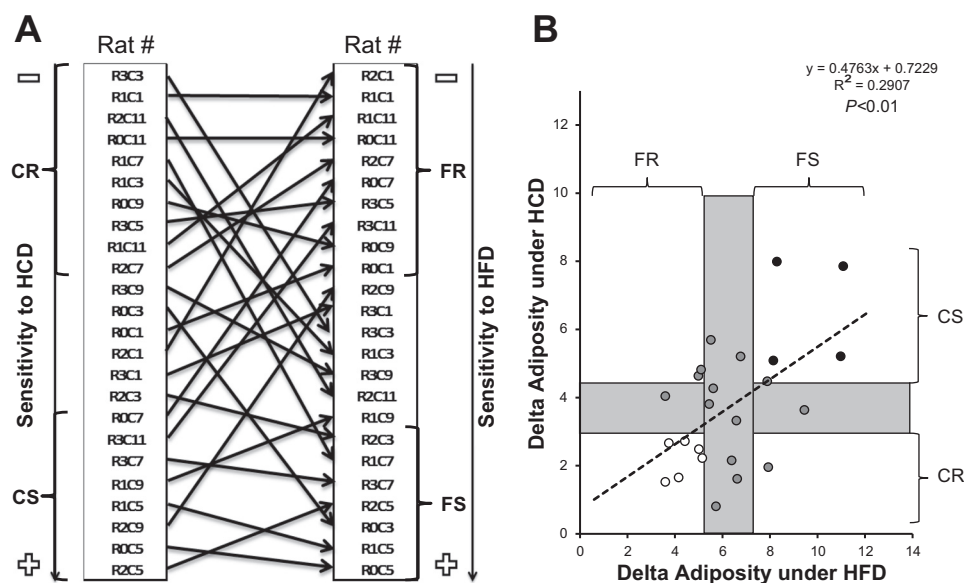


Fig. 1. A: respective sensitivity levels of individuals to high-carbohydrate diet (HCD) and high-fat diet (HFD). Top to bottom: classification from the lesser to the higher sensitivity to the HCD (left) and HFD (right). Arrows describe the evolution of each individual in the classification. Descending arrows mean less sensitive to HCD and more to HFD, ascending arrows more sensitive to HCD and less to HFD, and horizontal arrows sensitive or resistant to both. B: correlation between adiposity gains under HFD and HCD. Horizontal shaded area limits the intermediate gain of adiposity between HCD sensitive (above) and HCD resistant ones (below). Similarly, vertically shaded area limits the intermediate gain of adiposity between HFD-resistant rats (left) and HFD-sensitive rats (right). Open symbols denote rats resistant to both diets, while black symbols denote rats sensitive to both diets, and gray symbols indicate rats resistant-intermediate to one diet and sensitive-intermediate to the other. Despite significance, the correlation shows that resistance or sensitivity to one diet is poorly predictive of the resistance or sensitivity to the other ($R^2 = 0.29$).

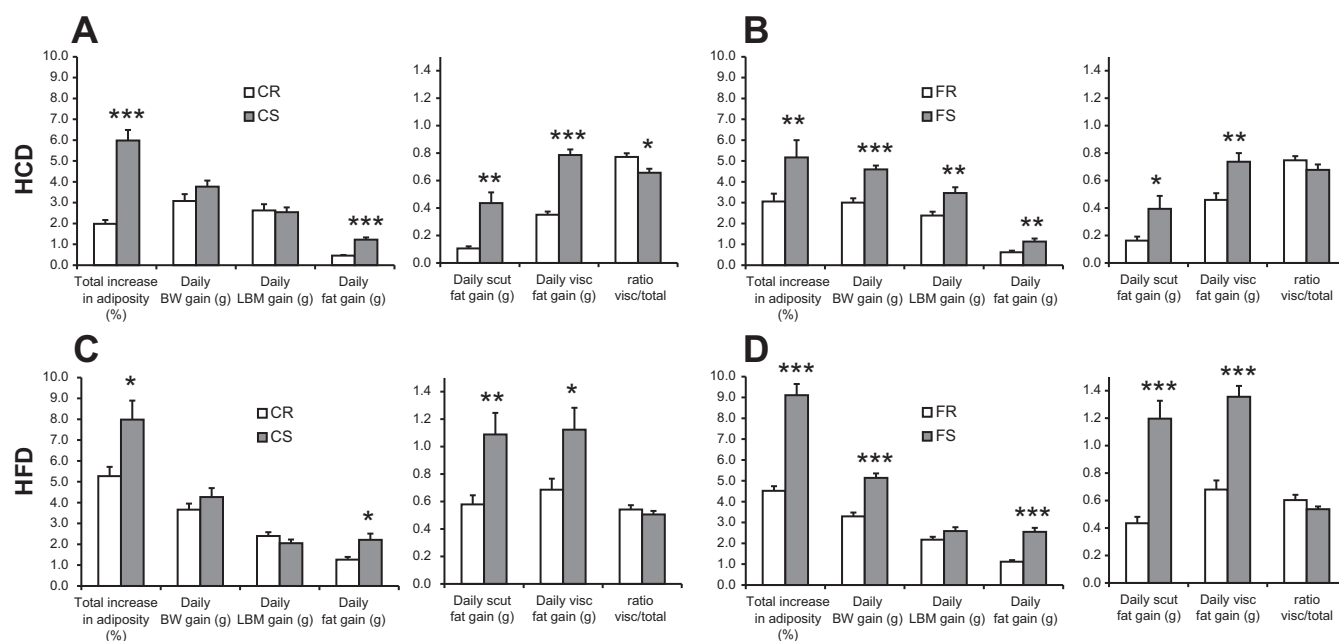


Fig. 2. Evolution of body composition in carbohydrate-resistant-carbohydrate-sensitive (CR-CS) (A and C) and fat-resistant-fat-sensitive (FR-FS) (B and D) rats. Note that CS rats are characterized by increased adiposity without significant differences in fat-free mass (FFM) and body weight (BW) gain, whereas in FS rats, adiposity, FFM, and BW are all significantly increased. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of the high correlation between adiposity gain and BW gain under HFD (Fig. 3), BW can be taken as a proxy of adiposity gain when rats are fed a HFD. This characteristic was used in *experiment 2* to remove the necessity of MRI. In contrast, under HC feeding, BW gain only poorly reveals the differences in adiposity gain between individuals (Fig. 3), and dual photon beam absorptiometry, MRI, or an equivalent noninvasive method, is required to separate CR from CS rats.

Table 3. mRNA expression (arbitrary units) in adipose tissue (expression relative to 18S) and in the hypothalamus (expressed relative to RPL 13A)

	FR (n = 10)	FS (n = 7)	P
<i>Adipose Tissue (Expression Relative to 18S)</i>			
ACC	1.56 ± 0.171	2.01 ± 0.653	NS
FAS	43.2 ± 7.17	51.15 ± 10.7	NS
Lept	5.55 ± 0.624	7.81 ± 0.950	0.06
LPL	0.320 ± 0.0334	0.299 ± 0.048	NS
<i>Hypothalamus (Expressed Relative to RPL 13A)</i>			
NPY	3.013 ± 0.408	3.523 ± 0.258	NS
AGRP	4.810 ± 0.690	6.662 ± 0.587	0.08
POMC	33.026 ± 5.641	50.540 ± 4.354	0.04
CART	1.724 ± 0.119	2.006 ± 0.039	0.09
MC4-R	3.530 ± 0.235	3.783 ± 0.242	NS
INSULIN-R	10.650 ± 0.536	11.293 ± 0.603	NS
FTO	13.673 ± 1.450	15.195 ± 1.606	NS
ObR	8.720 ± 0.699	7.879 ± 0.902	NS
CRF	17.002 ± 0.038	16.938 ± 0.078	NS

ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; Lept, leptin; LPL, lipoprotein lipase; NPY, neuropeptide Y; AGRP, Agouti-related peptide; IR, insulin receptor; POMC, proopiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; MC4R, melanocortin-4 receptor; INSULIN-R, insulin receptor; FTO, fat mass and obesity-associated gene; ObR, leptin receptor, long cytoplasmic form; CRF, corticotropin-releasing factor; FR, fat-resistant; FS, fat-sensitive; NS, nonsignificant.

CI, TEE, and EB under HFD (Experiment 1)

CR and CS rats did not exhibit any difference in CI or TEE regardless of the procedure used to adjust the data for differences in body size and composition, nor differences in the structure of feeding [meal size, meal number, speed of ingestion, day/night distribution (data not shown)] nor in the pattern of spontaneous activity (Fig. 4, *top*). In FS rats, caloric intake was 25% larger ($P = 0.02$) than in FR rats, but adjustment to FFM or to AMM reduced this difference to 15% ($P = 0.03$) and 13%, respectively ($P = 0.06$) (Fig. 4, *bottom*). TEE was 20% larger in FS rats, but adjustment to FFM or AMM reduced the difference to 10% (NS) and 8% (NS), respectively (Fig. 4, *bottom*). Taken

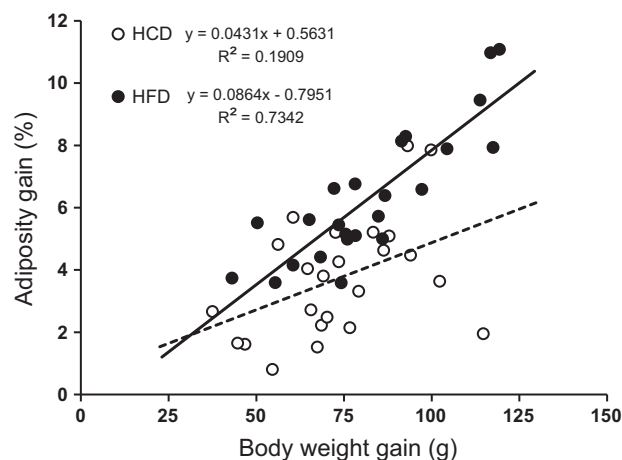
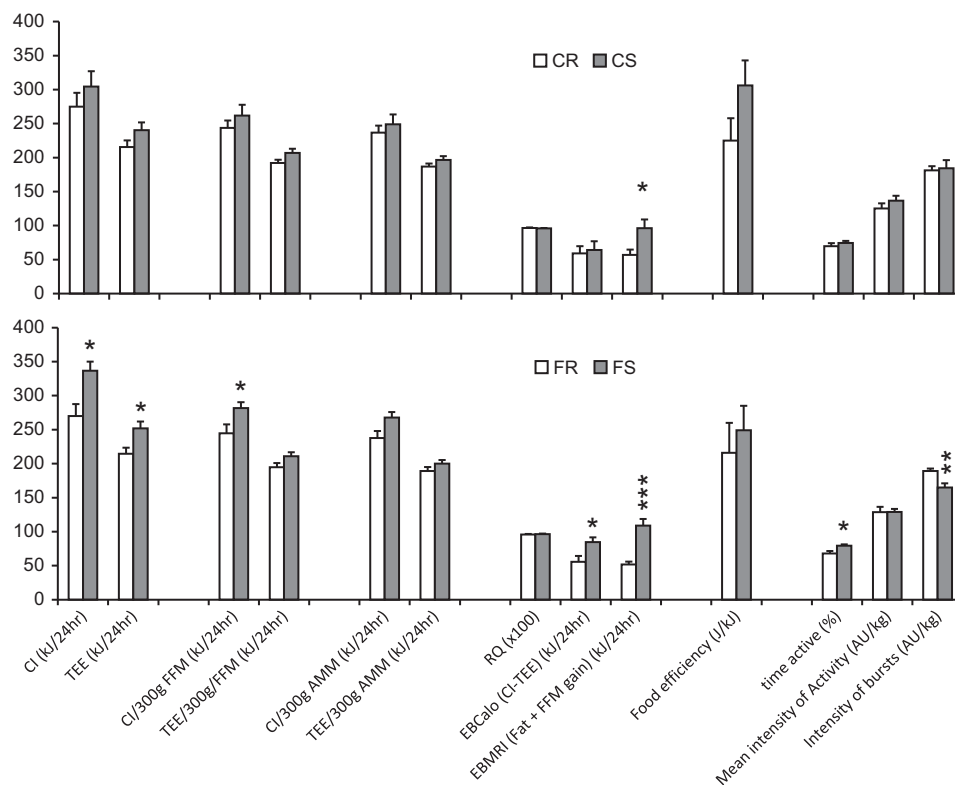


Fig. 3. Correlation between BW gain and adiposity gain under HCD and HFD. Adiposity gain is highly correlated to BW gain under HFD, which permits us to use BW gain as a proxy for the sensitivity to HFD. In contrast, under a HCD, adiposity gain is poorly correlated to BW gain, and analysis of body composition is required to discriminate between sensitive and resistant rats.

Fig. 4. Components of energy balance in CR ($n = 7$) and CS ($n = 6$) (top) and FR ($n = 7$) and FS ($n = 5$) rats (bottom) after adaptation to the HFD. During the 3 wk on a HFD, CS rats did not differ from CR rats on any components of energy expenditure, but energy balance (EB) during the 21 days of a HCD, estimated from changes in body composition, was larger in CS rats. FS rats, in contrast, exhibited a higher caloric intake (CI) and energy expenditure (EE) on a per rat basis but not after adjustment to active metabolic mass (AMM = FFM + $0.2 \times$ FM), where FFM is fat-free mass and FM is fat mass. EB was also significantly higher during the 21 days of HFD, as well as during the 5 days during which components of EE were measured. Mean intensity of activity was the same as in FR rats, but was dispatched differently, FS rats spending more time active but exhibiting significantly lower intensity of activity bouts. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (AU, arbitrary units).



together, these observations show that FS rats tend to eat significantly more but have a level of energy expenditure not significantly higher than that of FR rats, or, said another way, in FS rats, the increase in EE (8–10%) under HF feeding did not fully compensate for the increase in CI (13–15%), which explains the more positive energy balance of these rats.

EB computed from EBCalo and EBMRI (see MATERIALS AND METHODS) produced the same values in CR and FR rats, but EB computed from EBMRI resulted in larger values than EBCalo in CS and, in particular, in FS rats (Fig. 4). Detailed analysis of body weight gain during the HFD period showed that FS rats indeed gained slightly less BW during the 4 days in the metabolic cages than during the overall 21 d of HFD (5.48 vs. 4.06 g/d, $P = 0.13$), while FR ones continued to gain weight identically (3.70 g/day vs. 3.63 g/day, $P = 0.92$). It can, thus, be inferred that FS rats (and to a lesser extent CS rats) ate less while in the metabolic cages than in their home cages while their respective FR and CR partners maintained a constant intake. Nevertheless, these results show that, under HFD, FS rats gained more fat and weight because they increased CI more than TEE. The difference being small, food efficiency did not appear significantly increased in FS rats and no differences in RQ were observed with FR ones (Fig. 4, bottom).

Considering spontaneous activity, FS rats spent significantly more time active (Fig. 4, bottom). However, mean daily work due to activity was similar to that in FR ones because when active, FS rats developed bursts of activity of significantly lower intensity than FR rats (Fig. 4, bottom). In addition, significant inverse relationships were found between the intensity of the burst of activity and body fat gain

($r^2 = 0.39$, $n = 12$; $P < 0.02$), adiposity gain ($r^2 = 0.39$, $n = 12$; $P < 0.02$) and energy balance computed from changes in body composition measured by MRI (EBMRI) ($r^2 = 0.40$, $n = 12$; $P < 0.02$).

Detailed Analysis of the Components of Energy Expenditure in FR/FS Rats under HFD (Experiment 2)

On the basis of the results of *experiment 1* (Fig. 3), in *experiment 2*, FR and FS rats were segregated according to BW gain. Accordingly, FS rats gained 5.88 ± 0.36 g/day and FR ones 3.22 ± 0.36 g/day ($P < 0.0001$) during HFD, and their mean weights at the end of the HFD period were 395.1 ± 10.3 g vs. 326.4 ± 8.7 , respectively ($P < 0.001$). Analysis of body composition by MRI performed at the end of the HFD period also indicated that FS rats had significantly more body fat (52.66 ± 3.55 vs. 37.39 ± 3.32 g, $P < 0.01$).

The time-course changes in RMR and RQ measured on the FR and FS rats from 10 h before to 6 h after ingestion of the 60-kJ HFD test meal are shown in Fig. 5. RMR was significantly higher in FS rats (Fig. 5A), but only as a result of their larger body size as an adjustment to FFM (Fig. 5B) and to AMM (Fig. 5C) completely erased the difference. Relative to meal time (0 h), premeal (–10 to 0 h) and postmeal (0 to 6 h), RQs were also similar in the two groups (Fig. 5D), indicating that the ratio of lipid to glucose oxidation was similar in FR and FS rats in the postabsorptive, as well as in the fed state. The other components of energy expenditure (Fig. 6) also showed no differences between FR and FS rats. Taken together with the data of *experiment 1*, these results suggest that the FS

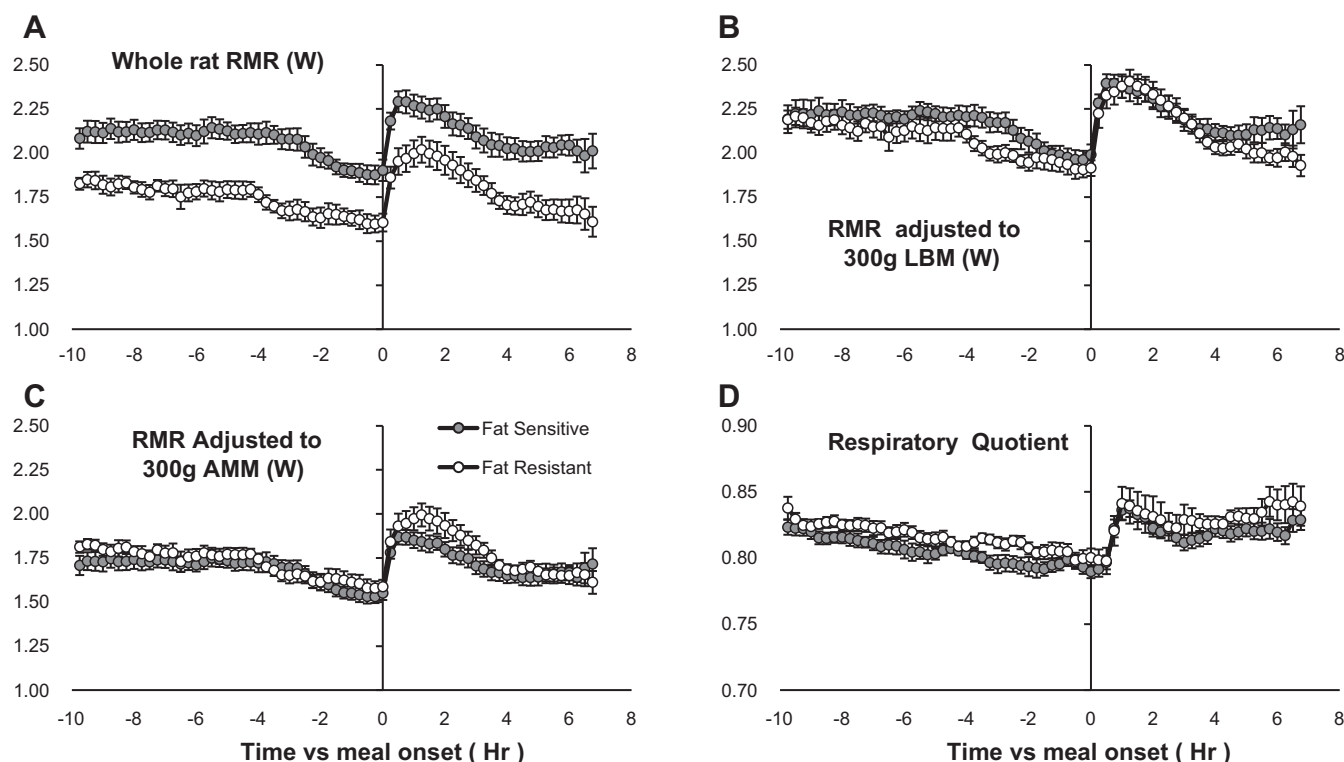


Fig. 5. Evolution (means \pm SE) of resting metabolic rate (RMR) (A: whole rat, B: adjusted to FFM, C: adjusted to AMM) and respiratory quotient (RQ) in the fasted state (-10 to 0 h) and in response to ingestion of the 60-kJ test meal of the HFD (0 to 6 h) in FR ($n = 10$) and FS ($n = 7$) rats. A: whole rat RMR was larger in FS rats, but adjustment of RMR to LBM or AMM (B and C) shows that this was only the result of the larger body size of FS rats. In A and D, it is also apparent that postingestive RQ, thermic effect of feeding (TEF), and meal-induced changes in RQ are similar in FR and FS rats (see also Fig. 6) [Note that TEF must not be estimated from B and C because adjustment to body size overestimates meal-induced changes in RMR in smaller rats (here FR ones)].

rats did not suffer any defect in the various components of energy expenditure.

Blood Parameters and mRNA Expression in the Liver, Adipose Tissue, and the Hypothalamus in FR and FS Rats after HFD (Experiment 1)

Plasma TG was higher in FS than in FR rats (2.61 ± 0.12 vs. 2.06 ± 0.12 , $P < 0.01$). No differences were observed for ketone bodies, free fatty acids, total cholesterol, or HDL

cholesterol (results not shown). TG in the liver ($4.99\% \pm 0.52$ in FS vs. $4.13\% \pm 0.33$ in FR) and epididymal adipose tissue ($92.1\% \pm 2.3$ in FS vs. 89.8 ± 2.7 in FR) were not different in FR and FS rats. These results indicate that after 3 wk of HFD, the FS rats did not yet suffer complications due to obesity.

No differences in gene expression of enzymes involved in glucose and lipid metabolism were observed between groups in the liver (data not shown). In adipose tissue, only

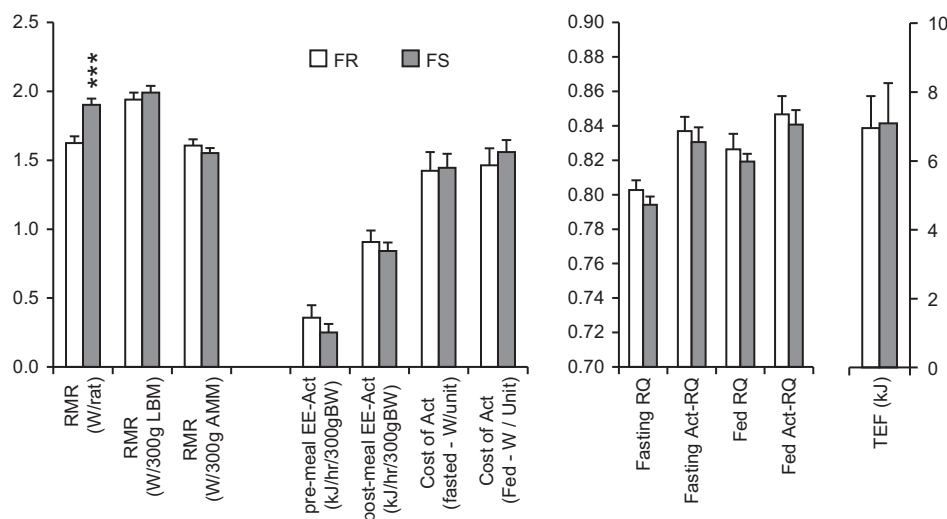


Fig. 6. Components of energy expenditure in FR ($n = 10$) and FS ($n = 7$) rats during a cycle of fasting and refeeding under HFD showed that only whole rat RMR was significantly larger in FS rats but exclusively because of the larger LBM and MMA of FS rats. Indeed, if adjusted to body size, none of the components of energy expenditure differed between FR and FS rats, indicating that in this model, the predisposition to obesity under HFD did not result from any metabolic defect at the level of RMR, TEF, substrate oxidation, EE with activity, and cost of activity. *** $P < 0.001$.

leptin mRNA tended to be greater in FS rats ($P = 0.056$) (Table 3), which was in accordance with their higher adiposity. In the hypothalamus, POMC was higher and Agouti-related peptide (AGRP) and cocaine- and amphetamine-regulated transcript (CART) tended to be higher as well (Table 3).

DISCUSSION

The present study confirms that significant differences in body adiposity can develop during HFD but also during HCD feeding, and reveals that sensitivity or resistance to adiposity gain under HCD only partly covers the population of rats sensitive or resistant to HFD. These results together with analysis of body composition, components of energy expenditure, spontaneous activity, and metabolic markers indicate that the sensitivities to a HFD or a HCD have different origins and that FS rats do not exhibit any defect in any component of energy expenditure.

Although adiposity gain is lower in absolute terms in CS rats under HCD than in FS rats under HFD, the difference in adiposity gain is larger between CR and CS rats under HCD than between FR and FS rats under HFD. This confirms previous observations that individuals can be classified not only by their HFD sensitivity (classical obesity-prone) but also by their HCD sensitivity (15, 34). This is observed in conditions in which the HCD was based on the AIN93 recommendation, in which most of the carbohydrate is provided as starch (38) in contrast to those diets designed to induce overfeeding and/or insulin resistance through the introduction of a high proportion of sucrose, as in the few previous studies that reported differences in sensitivity to HCD (10). Indeed, we observed a significant correlation between adiposity gain under HCD and HFD, but as testified by the rather low R^2 value, the level of adiposity gain under HFD explains only 30% of the adiposity gain from the HFD, suggesting that only some of the mechanisms underlying sensitivity to HCD are involved in the sensitivity to HFD and vice versa. An important observation was that in CS rats on a HCD, dietary fat accumulates significantly more viscerally than in FS rats on a HFD, which in the long term may be an aggravating factor for CS rats, considering that visceral fat is more deleterious than the subcutaneous sort (6, 9). However, we also observed that both CS and FS rats increased subcutaneous fat deposition more than visceral fat deposition and that in absolute terms, CS rats finally deposited ~ 2 times less visceral fat than FS rats. Thus, long-term studies are required to estimate the evolution of pathologies in CS rats. It is poorly probable that these differences were the result of the fact that the rats were first submitted to HCD because, in practice, in most studies before being submitted to a HFD, rats are fed on chow, i.e., on a HCD. Also, BW and BW gain were higher during HFD than during HCD; therefore, the larger visceral fat deposition under HCD cannot be the result of a higher growth rate during the HCD. Differences in adiposity gain were also recently observed in C57BL/6 mice fed a chow diet (meaning a HCD close in composition to the one used here) associated with differential expression of proteins involved in energy metabolism, glycolysis, and fat synthesis in visceral adipose tissue (51). This suggests that 1) preferential visceral fat accumulation may be a common trait of high-carbohydrate diets, 2) sensitivity to HCD is probably a common feature of various species, and 3) metabolic pathways orienting fat depo-

sition viscerally rather than subcutaneously are involved. The fact that liver TG was highest in CS rats suggests that lipid synthesis in the liver may be the source of this specific distribution. In clinical practice, this may be a parameter to consider before dietary interventions, because in subjects with a high visceral to subcutaneous fat ratio, low-fat diets may not necessarily be advisable.

Comparing TEE and CI and considering the consistent results provided after adjustment to FFM only or AMM ($\text{FFM} + 0.2 \times \text{FM}$) (2, 14) indicates that after adaptation to HFD, there were no differences in TEE or CI between CR and CS rats, confirming their overall low sensitivity to HFD, whereas FS rats ate more and had a larger TEE than FR ones. On the other hand, these data also show that in FS rats TEE is not decreased, but increased less than CI, which explains their more positive EB. Therefore, FS rats do not gain more fat because of a reduced TEE during HFD or, since RQ was also not different, because of a reduced rate of lipid oxidation, but because they ate more. This result is confirmed by the detailed analysis of the components of energy expenditure, which did not reveal any defects. FS rats, indeed, exhibited increased RMR, but this increase completely disappeared after adjustment for differences in body composition. RQ, reflecting the relative rates of glucose and lipid oxidation, was the same in the fasting state, as well as in response to ingestion of the test meal, indicating that the capacity of the FS rats to adjust glucose and lipid oxidation to overnight food restriction, as well as to respond to ingestion of a high-fat test-meal, was the same as in FR rats. This was also true for fuel use by the working muscles, as testified by the same activity (Act)-RQ and the same cost of activity in FR and FS rats. The present data, together with previous studies (15, 34), thus, strongly suggest that there is no defective regulation of energy metabolism in FS rats. This is not true for CS rats, for which we previously reported exaggerated changes in glucose and lipid oxidation in response to ingestion of a high-carbohydrate test meal (34).

On the energy intake side of the energy balance equation, many arguments are in favor of a defective control of food intake in FS rats. HFDs are generally less satiating than HCDs (33, 49), and FS rats are suspected to be less sensitive to the satiating effect of fat (12) or to be more responsive to the palatability of HFDs, which seems to be the case here. In addition, the fact that FS rats, but not FR ones, reduced their caloric intake when housed in the calorimetry cages suggests that they had less motivation for feeding than the FR ones. Such lower motivation to eat was also reported in *ob/ob* mice following operant training (39) and by Shin et al. in 2011 (42), who also reported that high-fat diet-induced obesity paradoxically decreases motivation for food reward, as measured by incentive runway and progressive ratio lever press performance. They concluded, and the present results are in line with this, that while obese rats readily indulge in easily available palatable fatty food, they are not ready to make an extra effort to obtain it.

Only a few studies have investigated the expression of hypothalamic neuropeptides in FS or FR animals, with inconsistent results. Resistance to the central anorectic effect of leptin and insulin seems to be present, even before the onset of obesity (27–29). In this study, we observed no difference in the mRNA expression of insulin and leptin receptors, but this does not preclude the fact that entry of leptin into the brain may be

impaired, in particular, following the observed increase in plasma TG (see below). POMC and AGRP expression in the arcuate nucleus is lower in diet-induced obese (DIO) mice than in diet-resistant (DR) mice after 22 wk of HFD (21), but no difference was observed in arcuate NPY or POMC mRNA between DIO and DR rats after 4 wk on a high-energy diet (46). Here, we show that hypothalamic AGRP mRNA expression tended to be larger in FS rats ($P = 0.06$), but that of POMC, which exerts an anorectic effect, was also increased ($P < 0.04$). The polymorphism of outbred FS (and probably CS) rats probably explains the difficulty of describing a clear brain profile. On the other hand, although many studies have demonstrated that the level of mRNA expression can be used as an indicator of functional significance of the neurotransmitter system, confirmation is still needed at the protein and functional levels.

FS rats have been characterized as “high plasma TG responders” (11, 23). In our hands, FS rats on a HFD (but not on a HCD, data not shown) had increased plasma TG, which agrees with the results of many previous studies and the hypothesis that increased TG levels in response to high-fat feeding is a factor predicting and perhaps predisposing rats for sensitivity to a HFD. One suggested mechanism is that TG impairs leptin signaling in the brain (4), which would stimulate feeding. Reduced whole body (8, 41) and liver FFA oxidation (22) in response to a high-fat diet have also been quoted as predisposing factors. In this study, however, plasma FFA and ketone bodies are not affected and whole body RQ measured under free feeding conditions was the same in FR and FS rats, which indicates that after 2–3 wk of adaptation and despite a still higher rate of weight gain, there was no difference in the ratio of glucose to lipid oxidation at the whole body level. This result is in line with a previous report from our laboratory that after introduction of a HFD, adjustment of whole body fat oxidation to the higher fat content of the diet (as measured from the speed of adjustment of whole body RQ to the food quotient) tended to be slower in FS rats but was adjusted within 4–5 days (15). The present results also agree with a report by Novak et al. (35), who observed that in response to high-fat feeding, RQ decreased to the same extent in OP and OR rats (35). In addition, we also reported that the RQ and TEF responses to a HFD test meal were not different between the FR and FS rats of this study during the 3 wk they were maintained on the HCD (34), and we show here that after 3 wk of HFD, there is still no difference in the metabolic responses to ingestion of a HF test meal between FR and FS rats. Thus, our results suggest that a defective lipid oxidation may participate in the rapid rate of weight gain of FS rats during the first few days of adaptation to HFD (dynamic phase), but not to the sustained increase in BW gain observed over the long term. It is, however, disturbing that RQ was not higher in FS rats because if FS rats stored more fat than FR ones, they should have oxidized a lower proportion of the dietary fat, and the RQ should necessarily be higher (32). One possibility to explain this apparent discrepancy is that in FS rats, more energy may be derived from polysaccharide catabolism by the microbiota, thus delivering short-chain fatty acids into the circulation (47). Indeed, studies of germ-free and conventionalized mice revealed that the microbiota promote absorption of monosaccharides from the gut lumen, with a resulting induction of de novo hepatic lipogenesis, and that *ob/ob* mice are able to extract

more energy from ingested food substances than their lean littermates (3, 47).

A decreased level of physical activity in obesity-prone mice fed a HFD has previously been reported (5, 35), but this does not definitely preclude less energy being expended with activity because activity is usually measured with beam breaks, which does not take into account the fact that the cost of moving is higher in heavier OP rats or mice. For example, Novak et al. (35) reported a decreased overall activity measured by beam breaks in diet-induced obese rats (~28 vs. 34 estimated from their Fig. 1 in Ref. 35), but the obese rats were much heavier than the lean ones (352 g vs. 250 g). Thus, the energy expended with activity as computed by activity \times weight (kg) was, in fact, higher in OP than in OR rats (18.9 vs. 15.95). What Novak et al. (35) also observed was rather small differences in horizontal activity but striking differences in vertical activity. Considering this point with the fact that we observed that the intensity of the bursts of activity but not overall activity was different between FR and FS rats, it seems that the pattern of activity and the types of activity in which the FR and FS rats engage rather than overall activity may be different and contribute to the sensitivity or resistance to diet-induced obesity. An interesting point is that in this study, we observed an inverse correlation between intensity of the bursts of activity and adiposity gain, fat gain, and energy balance, and in a study that we published in 2011, we observed a strong inverse correlation between the sensitivity of CR and CS rats to HCD and the intensity of the bursts of activity under HCD ($r^2 = 0.76$) and furthermore, that this correlation disappeared within days of the rats being switched to the HFD (15). That bursts of activity are of smaller intensity in CS rats only under HCD and in FS rats only under HFD suggests that spontaneous activity can be a component involved in the predisposition to body fat gain, whatever the fat content of the diet. Differences in muscle fiber types have been reported between FS and FR rats (1) and lean and obese subjects (48). Whether such differences can affect the stereotypic behavior of the rats and/or the control of energy metabolism in working muscles and affect energy balance remains to be elucidated. Wade et al. (48), for example, reported that fatter men with a low proportion of slow muscle fibers combusted less fat during cycle ergometry. In our hands, however, we did not observe such a defect in fat oxidation in obese Zucker rats running on a treadmill (40) despite the fact that it was reported that obese Zucker rats have a greater proportion of fast-twitch fibers (19), and in the present, as well as in our previous study (34), no differences in Act-RQ and in the energetic cost of activity were observed, suggesting that the reduced intensity of activity bursts is not accompanied by clear defects in the metabolism of the working muscles. However, we still did not perform a detailed analysis of the short-term changes in energy metabolism induced by the bursts of activity. Such specific studies must be designed to further analyze if differences in the intensity of bursts can affect substrate oxidation in muscles and energy balance.

In conclusion, the present data together with that which we previously published suggest that the propensity to gain weight on a HFD in FS rats is not necessarily sustained by a low metabolic rate, a low energy expended with activity, a low cost of activity or a poor capacity to oxidize fat, but only by an excess CI (~15%) not fully balanced by a compensatory

increase in EE (~10%). High blood TG levels appear to be a constant in FS rats fed a HFD and can possibly stimulate feeding by impairing leptin signaling to the brain. A common point of CS and FS rats is an altered pattern of spontaneous activity, characterized by a reduced intensity of the bursts of activity when the rats are fed the diet to which they are sensitive.

Perspectives and Significance

Significant increases in adiposity can develop under a HCD in rats that can otherwise be sensitive or resistant to HFD-induced obesity, and the data we obtained hitherto suggest that different metabolic flaws may operate. At a phenotypic level, CS rats on a HCD, contrary to FS rats under HFD, do not enlarge their LBM, and fix more fat viscerally, and thus are more difficult to identify. Since carbohydrate sensitivity has also been reported in mice, it is plausible that this characteristic may be present in humans. Obviously, the nutritional treatment to apply to carbohydrate- and fat-sensitive individuals must be different. In our hands, FS rats seem to be particularly sensitive to the reward signals of palatable high-fat food, suggesting that future studies should focus on the reward signaling in these animals. In contrast, CS rats exhibit a defective substrate partitioning in response to ingestion of a high-carbohydrate meal, and future studies should focus on glucose responsiveness, insulin sensitivity, and liver metabolism to clarify the underlying mechanisms.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.A.-M., C.C., and P.C.E. conception and design of research; D.A.-M., C.C., J.P., and P.C.E. performed experiments; D.A.-M., C.C., N.A.N., J.P., and P.C.E. analyzed data; D.A.-M., N.A.N., D.T., and P.C.E. interpreted results of experiments; D.A.-M., C.C., N.A.N., G.F., D.T., and P.C.E. edited and revised manuscript; D.A.-M., C.C., N.A.N., J.P., G.F., D.T., and P.C.E. approved final version of manuscript; D.T. and P.C.E. drafted manuscript; P.C.E. prepared figures.

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