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Am J Physiol Regul Integr Comp Physiol 310: R1169–R1176, 2016.
First published March 30, 2016; doi:10.1152/ajpregu.00391.2015.

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Submitted 8 September 2015; accepted in final form 27 March 2016

Azzout-Marniche D, Chalvon-Demersay T, Pimentel G, Chaumontet C, Nadkarni NA, Piedcoq J, Fromentin G, Tomé D, Gaudichon C, Even PC. Obesity-prone high-fat-fed rats reduce caloric intake and adiposity and gain more fat-free mass when allowed to self-select protein from carbohydrate:fat intake. *Am J Physiol Regul Integr Comp Physiol* 310: R1169–R1176, 2016. First published March 30, 2016; doi:10.1152/ajpregu.00391.2015.—We tested the hypothesis that, for rats fed a high-fat diet (HFD), a prioritization of maintaining protein intake may increase energy consumption and hence result in obesity, particularly for individuals prone to obesity ("fat sensitive," FS, vs. "fat resistant," FR). Male Wistar rats ($n = 80$) first received 3 wk of HFD (protein 15%, fat 42%, carbohydrate 42%), under which they were characterized as being FS ($n = 18$) or FR ($n = 20$) based on body weight gain. They then continued on the same HFD but in which protein (100%) was available separately from the carbohydrate:fat (50:50%) mixture. Under this second regimen, all rats maintained their previous protein intake, whereas intake of fat and carbohydrate was reduced by 50%. This increased protein intake to 26% and decreased fat intake to 37%. Adiposity gain was prevented in both FR and FS rats, and gain in fat-free mass was increased only in FS rats. At the end of the study, the rats were killed 2 h after ingestion of a protein meal, and their tissues and organs were collected for analysis of body composition and measurement of mRNA levels in the liver, adipose tissue, arcuate nucleus, and nucleus accumbens. FS rats had a higher expression of genes encoding enzymes involved in lipogenesis in the liver and white adipose tissue. These results show that FS rats strongly reduced food intake and adiposity gain through macronutrient selection, despite maintenance of a relatively high-fat intake and overexpression of genes favoring lipogenesis.

high-fat diet; obesity prone; protein intake; protein leverage; dietary self-selection

DIETARY FAT IS OFTEN CONSIDERED RESPONSIBLE for the high prevalence of adiposity (5) because it is an energy-dense nutrient, lends greater flavor and palatability to food, has a reduced thermogenic effect (34), and has a poorly controlled intake (9). Genetic and epigenetic factors also influence who in a given population will develop adiposity. It is also well established that humans (4, 19, 32), as well as rodents (1, 20), are not uniformly sensitive to adiposity gain under high-fat diets (HFDs). Increasing dietary protein levels has been reported to induce various metabolic and behavioral effects converging to reduce body weight (BW) and body fat gain (18, 28), even in conditions of high-fat feeding (24). Proteins therefore seem to be superior to carbohydrates in promoting satiety, diet-induced thermogenesis, and fat loss (2, 3, 6, 15, 33). It is, however, not known whether individuals sensitive to adiposity gain under HFD ("fat sensitive", FS, as opposed to "fat resistant", FR) react similarly under high-protein diets.

It is also recognized that, when placed on a dietary self-selection (DSS) regimen, rats (and most species) are generally able to select an adequate diet to meet nutritional and physiological needs for growth and maintenance. In experiments in which DSS animals were offered the choice between two mixed diets with different protein contents, BW gain and food intake were similar to rats fed single, standard mixed diets (10, 14, 17, 23, 35). In contrast, when the choice offered to DSS animals was between a pure protein and a carbohydrate:fat mixture of the usual standard (low-fat) maintenance diet (which ensures stability of the dietary carbohydrate:fat ratio, a factor known to affect energy balance) (21, 22, 27), BW gain and food intake were significantly reduced, and the dietary protein:energy ratio increased up to 40% (21). This suggests that, when fed the usual maintenance diet, these rats ate more to increase protein intake.

The goal of this study was to test whether such a response is observed in rats fed a HFD and how it may differ between FR and FS individuals. Outbred Wistar rats initially fed a HFD, characterized as prone or resistant to the diet according to adiposity gain, were given a free choice between two food jars. One contained the protein of the HFD (P100), only, and the other its carbohydrate:fat mixture (C50:L50: 50% energy from carbohydrate and 50% energy from lipids). Rats were characterized for DSS and meal pattern, caloric and protein intake, BW, body composition, and adiposity. Gene expression profiling was used to measure metabolic adaptations in liver and adipose tissue, as well as neuropeptides and receptors in brain areas implicated in feeding control [arcuate nucleus (ARC)] and reward [nucleus accumbens (NAcc)].

MATERIALS AND METHODS

Experimental Design

The study was approved by the French National Animal Care Committee (number 11/027) and conformed with the European legislation on the use of laboratory animals. The experimental plan is outlined in Fig. 1. Five groups, each consisting of 16 male, 7-wk-old Wistar rats initially weighing 224.6 ± 1.9 g (Harlan-France), were used. A group was delivered every other week and was given 1 wk of adaptation to the housing conditions (22°C ± 1, humidity 60%, 12-h:12-h light/dark cycle, lights on at 08:00). During the experimental period, rats were weighed three times per week. First, the rats were

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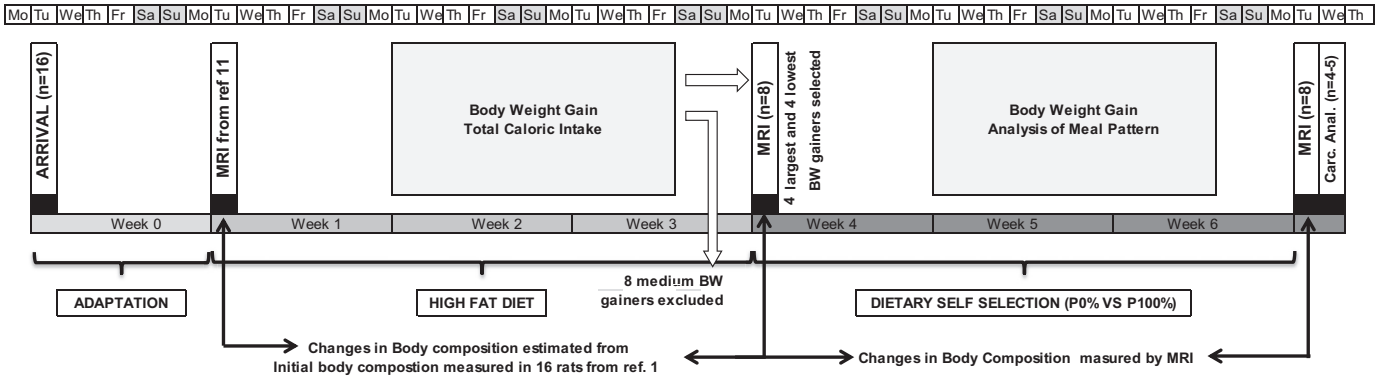

Fig. 1. Experimental plan applied to each of the 5 groups of 16 rats involved in the study.

put on a HFD (Table 1) for 3 wk. Subsequently, from each group of 16 rats, the four that gained the most weight were selected as FS and the four that gained the least weight were selected as FR. The eight intermediate rats were removed from the study. The eight selected rats had body composition measured by MRI, then were assigned for another 3 wk to a DSS regime between the P100 and the C50:L50 diets (Table 1). At the end of the DSS, there was another round of MRI to measure body composition. Then at least two FS and two FR rats from each group of eight (26 rats in total) were randomly chosen for a more detailed body composition analysis. This was performed by dissection and weighing of the main organs and tissues. Collection of brain, liver, and adipose tissue samples were used for analysis of gene expression by q-PCR. In these rats, the dissection was performed 2 h after ingestion of a standardized P100 meal following overnight fasting to normalize the feeding status of the individuals and to study the brain and peripheral organ status in relation to protein intake.

Analysis of DSS and Spontaneous Activity

The eight rats selected as FR or FS were maintained for 4 days (4 rats during the second week and 4 rats during the third week of DSS) in a cage equipped with two weighed food cups (sensitivity 0.01 g) for meal pattern recording (see Refs. 8 and 21 for details). Each cage was also positioned on an activity platform equipped with force transducers that produced an electrical signal (V) proportional to the intensity of the work generated by rat movement. The activity level was obtained by adjusting the force transducer signal amplitude to 1 kg BW (signal in V/BW in kg). Data were acquired at 100 Hz, and mean values were binned every 2 s (see Ref. 7 for details).

Table 1. *Composition of HFD, P100, and C50:L50 diets*

	HFD	P100	C50:L50
Weight content, g/kg			
Milk proteins	170.0	902.7	0.0
Starch	436.6	0.0	537.9
Sucrose	71.1	0.0	87.6
Soy oil	225.0	0.0	277.2
Minerals	35.0	35.0	35.0
Vitamins	10.0	10.0	10.0
Cellulose	50.0	50.0	50.0
Choline	2.3	2.3	2.3
Energy content, %			
Protein	14.4	100.0	0.0
Carbohydrate	42.9	0.0	50.1
Fat	42.8	0.0	49.9
Energy density, kJ/g	19.82	15.11	20.91
Food quotient	0.847	0.825	0.850

HFD, high-fat diet; P100, pure protein diet; C50:L50, carbohydrate:fat diet.

Analysis of Body Composition and Final Discrimination Between FR and FS Rats

Analysis of body composition was done by MRI. Images were acquired on a 7T Bruker Pharmascan system (running Paravision 4) using a Bruker 50-mm inner diameter tunable quadrature radio frequency resonator. Anesthesia was administered with isoflurane in oxygen-supplemented air. Breathing rate and rectal temperature (maintained at 36–38°C using warm air) were monitored. A Turbo-RARE-3D sequence was used to acquire fat-sensitive T_2-weighted images (TR/TE = 750/42 ms, FOV 75 × 50 × 50 mm, matrix = 128 × 96 × 96, 4–5 overlapping images acquired to cover the whole rat). On average, including calibration and positioning, each rat was unconscious for ~40 min. 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 fat mass on the assumption of a 0.9-g/cm^3 density, and fat-free mass (FFM) was determined by subtracting this from the rat weight on the day of the scan.

Because of the large number of rats used in the study (n = 80) and resource constraints, we did not perform MRI before the HFD, instead estimating initial body composition from MRI results in 16 male Wistar rats of the same age and weight involved in a recent study (1) (Table 2). As described above, it was body weight gain during the HFD that was used to preselect four FR and four FS rats from the 16 in each delivery to be analyzed by MRI at the end of the HFD. This is because we had previously shown that weight gain was a reasonable proxy to use for adiposity gain during the HFD (1). In these selected rats, body composition was measured again by MRI at the end of the DSS period so that changes in FFM and fat mass in these rats during the DSS period could be measured directly. For the final analysis at the end of the study, all 40 selected rats were pooled and reallocated as FR or FS based on adiposity gain computed from MRI measurements for better precision. Few rats switched groups, confirming that body weight gain was a good proxy for adiposity gain.

Dissection and weighing of the main organs and tissues at the end of the study provided a more detailed description of body composition. It revealed large discrepancies in the measurement of fat mass between MRI and dissection in the two largest FS rats, which weighed 511 g and 540 g and were indeed too large to be properly placed in the MRI tunnel. These rats were excluded from data analysis, thus reducing the number of FS rats from 20 to 18.

Blood Samples

Blood samples (100 μl) were taken from the tail vein of fed rats in the morning, between 10:00 and 12:00 (light period), during the second week of each HFD and DSS regime to assay blood glucose, plasma lactate, triglycerides (TG), high-density lipoproteins (HDL), total cholesterol (CHOL), glycerol (GLY), ketone bodies (KB), and

Table 2. *Body weight and body composition at different stages of study*

	FR		FS		
	Mean	SE	Mean	SE	*P* Value
Weight at onset of HFD	261.1	2.24	260.2	3.22	NS
	Body composition by MRI at onset of HFD estimated from that measured in 16 WT and age-matched rats (1)				
BW, g	261.0 ± 2.8				—
Total fat, g	15.5 ± 1.0				—
FFM, g	245.5 ± 2.2				—
Adiposity, %	5.9 ± 0.4				—
	Body composition by MRI at end of HFD				
BW, g	343.3	8.25	364.4	5.52	0.042
Total fat, g	57.7	2.13	87.4	2.17	0.000
FFM, g	285.6	6.96	277.0	5.85	NS
Adiposity, %	16.8	0.45	24.1	0.67	10^{-8}
	Body composition by MRI at end of DSS				
BW, g	395.0	10.18	424.9	7.72	0.026
Total fat, g	72.6	4.33	95.2	5.47	0.003
FFM, g	322.4	8.52	329.7	6.69	NS
Adiposity, %	18.3	0.92	22.3	1.11	0.009
	Body composition by dissection at end of DSS				
BW, g	396.7	11.4	430.1	11.0	0.046
Subcutaneous fat	15.8	1.18	21.2	2.02	0.032
Abdominal fat	19.4	1.43	28.2	2.24	0.004
Mesenteric fat	5.65	0.51	8.39	0.76	0.007
Total fat	40.9	2.67	57.8	3.92	0.002
Carcass	173.9	7.47	184.3	5.12	NS
FFM	355.8	9.79	372.3	9.23	NS
Adiposity, %	10.26	0.52	13.38	0.78	0.003

Applicable values are means ± SE. BW, body weight; FFM, fat-free mass; FR, fat-resistant rats; FS, fat-sensitive rats; WT, wild-type; DSS, dietary self-selection.

free fatty acids (FFA). Blood glucose was immediately assayed using an automatic analyzer (Life-Scan, One Touch Vita). The remaining blood was centrifuged (3,000 *g*, 15 min, 4°C), and plasma was stored at −80°C until assay using an Olympus AU 400 automatic chemical analyzer.

Gene Expression Analysis

RNA was extracted from brain (ARC nucleus and NAcc), epididymal adipose, and liver tissue using TRIzol reagent (Invitrogen). Concentration was assessed using a nanodrop spectrophotometer at 260 nm, and RNA integrity was confirmed by agarose gel electrophoresis. Retrotranscription was performed on 0.4 μg of RNA using the High-Capacity cDNA Archive Kit 116 Protocol (Applied Biosystems).

Gene expression was measured by real-time PCR on an ABI 7300 (Applied Biosystems) using Power SYBR GREEN PCR MIX (Applied Biosystems). The primer sequences of genes were designed with Primer Express software, and the sequence of each primer is described in Table 8.

In liver, we studied mRNA encoding proteins involved in glycolysis [glucokinase (GK), liver-pyruvate kinase (L-PK)], lipid metabolism [acetyl-coA carboxylase (ACC), fatty acid synthase (FAS)], and lipid transport and oxidation [peroxisomal acyl-coenzyme A oxidase 1 (ACOX1), carnitine palmitoyltransferase 1a-liver isoform (CPT1a), cluster of differentiation 36 (CD36), peroxisome proliferator-activated receptor-α (PPAR-α), and peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α)].

Neuropeptide Y (NPY), Agouti-related peptide (AgRP), proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing factor (CRF), serotonin receptor 1B (5HT1B), serotonin receptor 2C (5HT2C), and serotonin receptor 6 (5HT6) were assayed in the ARC, and dopamine receptor 1 (DR1), DR2, DR3, κ-opioid receptor (KOR), μ-opioid receptor (MOR), δ-opioid receptor (DOR), 5HT1B, 5HT2C, 5HT3A, and 5HT6 were assayed in the NAcc at the end of the DSS period exactly 2 h after ingestion of a 3-g (45.8 kJ) P100 meal.

Real-time PCR was performed using 5 ng of cDNA in addition to 15 μl of the reagent mix containing RNase-free water, PCR mix, and forward and reverse primers. Negative controls were used to detect potential contamination (control without RT or RNA). The threshold (CT) was set with the constant value for all genes and samples to quantify gene expression, and the mRNA concentration was calculated as follows: $2^{-\Delta CT}$, where $\Delta CT = CT\ Gene - CT\ 18S$. Data are means ± SE expressed as a percentage of the values of the FR rats.

Statistical Analysis

Data are presented as means ± SE. Statistical analyses were performed using R version 3.1.3. Between-group comparisons were performed by Student's *t*-test or, when the comparisons extended to the HFD and DSS periods, by two-factor ANOVA with analysis of the interaction between groups and diet (HFD vs. DSS). Pairwise comparisons were performed with post hoc Bonferroni tests for multiple-comparison correction.

RESULTS

BW and Body Composition

At the onset of HFD, FR and FS rats had the same BW (261.15 ± 2.24 and 260.2 ± 3.22 g, respectively) and could be considered young, but mature, adult rats that needed no extra dietary protein to sustain growth. FS rats gained significantly more weight during the HFD (Fig. 3*A*). However, as is usually observed in rats fed a HFD, a progressive decrease in BW gain was observed with time in both groups (Fig. 2, *A* and *B*). During the third week, FS rats did not gain significantly more weight than FR ones (Fig. 2*B*).

At the end of the HFD period, BW, total body fat, and adiposity were significantly larger in FS rats, but FFM was not different (Table 2). The computation of gain in fat mass and FFM during HFD, derived from a body composition estimate at the onset of the HFD using data acquired elsewhere, may have introduced some uncertainties in the exact changes at individual levels. This may have prevented observation of significant differences at the level of FFM gain (Table 3). However, given the very large differences between FR and FS rats, it cannot be challenged that fat mass (Fig. 3*B*) and adiposity (Fig. 3*C*) increased very significantly in FS rats.

When switched to DSS, FR and FS rats had similar rates of BW gain (Figs. 2 and 3*A*). The gains in body fat and adiposity were extremely reduced in both FR and FS rats, the reductions being significantly stronger in FS rats (Fig. 3, *B* and *D*). However, although differences were reduced, FS rats continued to have more fat and higher adiposity levels at the end of the 3 wk of DSS (Table 2). The gains in FFM were not significantly changed by DSS but tended to be higher in FS rats ($P < 0.1$). There was also a significant diet × group interaction, indicating that DSS had a more positive effect on the evolution of FFM in FS rats (Fig. 3*C*).

Analyses of body composition by dissection and weighing of the main organs and tissues in 26 rats representative of the FR and FS groups showed that FS rats had more subcutaneous,

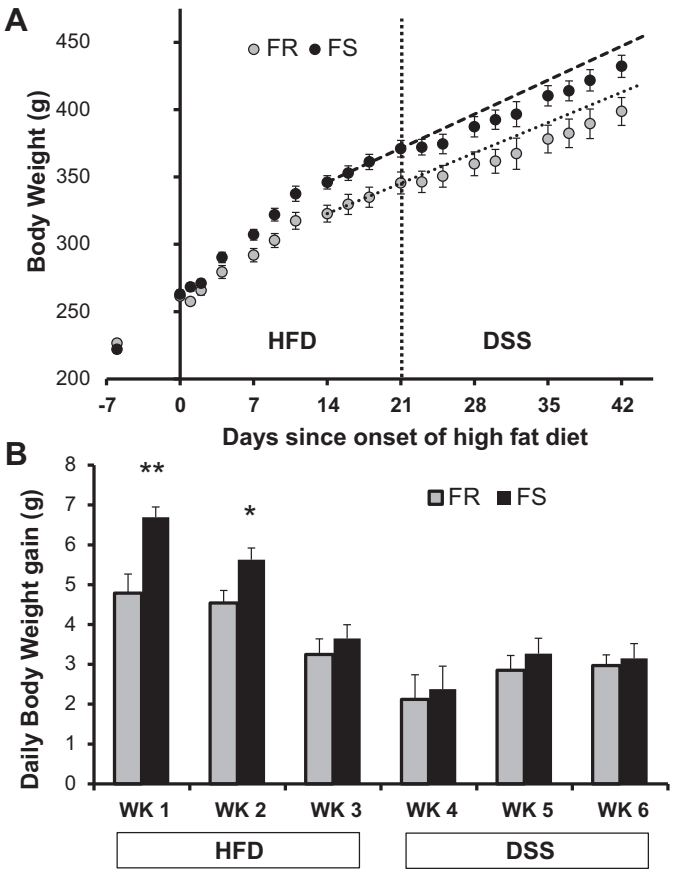

Fig. 2. *A*: evolution of body weight (BW) during the study. Both groups had the same weight at arrival and at the onset of the high-fat diet (HFD) period. The difference in BW between groups became significant at *day 7* and remained so until the end of the study. Dashed lines are extrapolated regression lines of BW gains during the last week of HFD. They show that dietary self-selection (DSS) induced an initial reduction in BW gain before return to a normal growth rate. *B*: weekly evolution of BW in fat-resistant (FR) and fat-sensitive (FS) rats during the study. Daily BW gain was larger in FS rats during the first 2 wk of HFD but not during the third week. Note that the larger weight gain of FS rats during HFD was due to a larger gain in fat mass (see Table 2). $*P < 0.05$, $**P < 0.01$.

abdominal, and mesenteric fat than FR ones and confirmed that FFM was not different between groups (Table 2).

Caloric Intake

Caloric intake, measured during *weeks 2* and *3* of the HFD, was similar in FR and FS rats regardless of how it was expressed, absolute (Table 3) or adjusted to BW or FFM (data not shown). When allowed to select between the P100 and the C50:L50 diets, FR and FS rats reacted similarly; they reduced total caloric intake by 40% and carbohydrate:fat intake by 50%, although they increased protein intake by 13%. As a result, the proportion of protein in the diet increased from 14% to ~27% ($P < 10^{-16}$), and the proportion of fat decreased from 42% to 37% ($P < 10^{-16}$).

DSS and Intensity of Spontaneous Activity During DSS

Total and night caloric intakes were not different between FR and FS rats, but day caloric intake was significantly larger in FS rats because they ingested significantly more protein during the day (Table 4). In contrast, the pattern of ingestion of the C50:L50 diet was the same for FR and FS rats. Most of the meals (>75%) were taken from a single food cup. When mixed meals occurred, the ingestion sequence C50:L50 first, then P100, was slightly favored in both FR and FS rats ($P < 0.1$). C50:L50 meals were approximately two times larger than P100 meals. The level of spontaneous activity was similar in FR and FS rats. Time spent resting was also not different between groups.

Blood and Plasma Parameters

No difference was observed between FR and FS rats during the HFD or the DSS period (Table 5).

Gene Expression in Brain, Liver, and Adipose Tissue

Gene expression in the brain. NPY, AgRP orexigenic neuropeptides, POMC, CART, CRF anorexigenic neuropeptides, 5HT1B, 5HT2C, and 5HT6 serotine receptors were assayed in the ARC because of their central role in the control of food intake (Table 6). DR1, DR2, and DR3 (dopamine receptors), KOR, MOR, and DOR (opioid receptors), and 5HT1B, 5HT2C, 5HT3A, and 5HT6 (serotonin receptors) were assayed in the NAcc because of their central role in reward. No differences were observed between FR and FS rats.

Gene expression in the liver and adipose tissue. In the liver, GK gene expression, encoding the rate-limiting enzyme of glycolysis, was 44% higher in FS rats (Table 7). This suggests that glycolysis may have been increased in FS rats. However, no significant differences were observed for PK. For hepatic lipid metabolism, mRNA-encoding FAS, which generates palmitic acid by catalyzing the condensations between malonyl-CoA and acetyl-CoA, was also higher in FS rats. However, the mRNA-encoding ACC, which is involved in the synthesis of malonyl-CoA from acetyl-CoA, was not significantly different. Moreover, the expression of ACOX, responsible for peroxisomal long-chain fatty acid oxidation, was 20% higher in FS rats, whereas no changes were observed for mRNA encoding proteins involved in fatty acid mitochondrial oxidation (CPT1, PPAR-α, and PGC-1α) or in fatty acid transport

Table 3. *Components of caloric intake (kJ) during HFD and DSS*

	HFD		DSS				
	FR	FS	FR	FS			
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Diet Effect	Group Effect	Diet × Group Effect
Total	453.12 (14.03)	475.53 (7.65)	274.99 (8.53)	284.10 (8.42)	<0.0001	NS	NS
Fat + HCHO	389.68 (12.07)	408.95 (6.58)	201.70 (8.40)	209.23 (8.00)	<0.0001	NS	NS
Protein	63.44 (1.96)	66.57 (1.07)	73.29 (5.78)	74.87 (3.87)	0.004	NS	NS

HCHO, high carbohydrate.

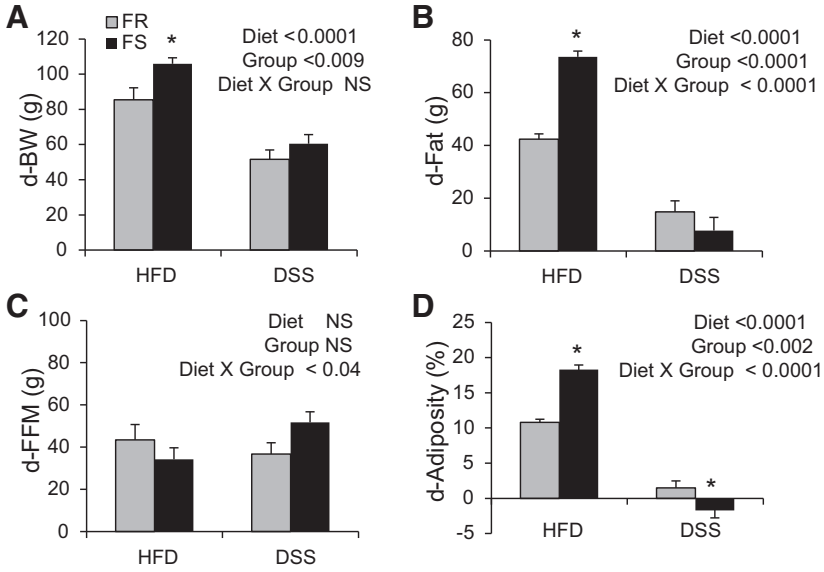

Fig. 3. Comparison of BW, fat mass, fat-free mass (FFM), and adiposity gains during the HFD and DSS periods. Changes in fat mass and FFM during HFD were estimated from body composition values at the onset of HFD measured in a previous study (see Table 2). *A*: BW gain. *B*: fat mass gain. *C*: FFM gain. *D*: adiposity gain. *$P < 0.05$. d, difference.

(CD36). Taken together, these results suggest that both lipogenesis and peroxisomal fatty-acid oxidation were increased in FS rats.

In white adipose tissue, expressions of FAS and ACC were significantly higher in FS rats, indicative of a higher potential for lipogenesis.

DISCUSSION

This study analyzed protein intake and meal patterns in a DSS design in which rats previously classified as FR or FS were allowed to select between pure protein (P100) and a fat-carbohydrate mixture in which carbohydrates and lipids each accounted for 50% of energy (C50:L50). This specific design (the use of only 2 diets: protein vs. a protein-free carbohydrate:fat mixture) was chosen to avoid interference between changes in protein intake and changes in the carbohydrate:lipid ratio selected in parallel (21, 22, 28). The results showed that both FR and FS rats strikingly reduced caloric intake but maintained and even increased protein intake, that body fat and adiposity gains were significantly reduced with a more pronounced effect in FS than in FR rats, and that FFM gain was preserved and even slightly increased in FS rats. In addition, mRNA expression measured at the end of the study, 2 h after ingestion of a calibrated P100 meal, indicated that expression of genes coding for hepatic and adipose lipogenesis and hepatic peroxisomal oxidation was larger in FS rats than in FR rats.

Table 4. *Meal pattern components during DSS*

Meal Pattern During DSS	FR Mean (SE)	FS Mean (SE)	*P* Value
	Day/night intakes, kJ		
Total day	79.6 (6.02)	102.2 (7.93)	0.03
Total night	195.4 (6.91)	181.9 (10.8)	NS
C50:L50 day	63.0 (5.28)	74.6 (6.41)	NS
C50:L50 night	138.7 (5.73)	134.7 (8.33)	NS
Protein day	16.6 (2.45)	27.7 (3.51)	0.01
Protein night	56.7 (4.69)	47.2 (4.60)	NS
	% Ingested during day		
Total	28.7 (1.83)	36.3 (3.03)	0.04
C50:L50	30.7 (1.88)	36.3 (3.35)	NS
Protein	22.2 (2.64)	37.4 (4.76)	0.01
	Meal size, kJ		
C50:L50	21.2 (1.49)	22.7 (1.17)	NS
Protein	10.3 (0.77)	10.6 (0.51)	NS
	Meal number		
Total meal number	14.1 (0.66)	13.7 (0.83)	NS
Protein meals	4.05 (0.34)	4.06 (0.37)	NS
C50:L50 meals	6.55 (0.44)	6.17 (0.72)	NS
Mixed meals	3.45 (0.37)	3.39 (0.46)	NS
	Spontaneous physical activity		
Intensity, V/kg	10.3 (0.38)	9.75 (0.30)	NS
Time resting, %	24.9 (1.27)	28.0 (1.05)	NS

Plasma Parameters and Gene Expression

In the present study, plasma parameters measured during the HFD and their changes induced by macronutrient selection during DSS were not different between FR and FS rats. This result indicates that the short 3-wk exposure period to HFD was sufficient to allow for the selection between FR and FS rats, but too short to allow for the emergence of metabolic dysfunctions and changes in plasma parameters.

Central control of food intake is integrated in two main regions. In the ARC (homeostasic control), NPY/AgRP and POMC/CART/CRF control the balance between orexigenic and anorexigenic signaling. The NAcc is a key center of the reward system that coordinates GABA, opioid dopaminergic, and serotoninergic pathways. In this study, gene expression in the NAcc measured 2 h after ingestion of a P100 test meal was not different between FR and FS rats (see Table 8 for a list of genes used in this study). This is consistent with the observation that FS rats did not exhibit more preference for protein than did FR rats. Moreover, no difference was observed at the level of the orexigenic and anorexigenic peptides in the ARC. This result contrasts with the effect of a high-fat meal reported in a previous study (1) (same HFD as in this study), inducing a paradoxical trend for a larger expression of mRNA for AgRP, POMC, and CART in the hypothalamus of FS rats compared

Table 5. *Blood glucose and plasma metabolites*

	HFD		DSS				
	FR	FS	FR	FS			
mmol/	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Diet Effect	Group Effect	Diet × Group Effect
Glucose (n = 18–20)	6.47 (0.16)	6.67 (0.17)	126.15 (2.09)	127.03 (2.39)	NS	NS	NS
Lactate (n = 18–20)	2.792 (0.241)	2.653 (0.195)	2.555 (0.151)	2.631 (0.146)	NS	NS	NS
TG (n = 8–12)	1.115 (0.110)	1.315 (0.131)	1.360 (0.110)	1.421 (0.157)	NS	NS	NS
HDL (n = 8–12)	0.485 (0.036)	0.632 (0.044)	0.493 (0.051)	0.532 (0.039)	NS	NS	NS
Cholesterol (n = 8–12)	2.054 (0.130)	2.306 (0.107)	2.054 (0.151)	2.313 (0.144)	NS	NS	NS
Glycerol (n = 8–12)	0.239 (0.022)	0.261 (0.023)	0.263 (0.028)	0.231 (0.018)	NS	NS	NS
Ketone bodies (n = 8–12)	0.055 (0.009)	0.038 (0.006)	0.051 (0.011)	0.044 (0.010)	NS	NS	NS
FFA (n = 8–12)	0.600 (0.053)	0.695 (0.057)	0.742 (0.075)	0.683 (0.049)	NS	NS	NS

Blood was taken from the tail vein of fed rats in the morning between 10:00 and 12:00 (light period) during the second week of HFD feeding and the second week of DSS. TG, triglyceride; HDL, high-density lipoprotein; FFA, free fatty acid.

with FR rats. This suggests that DSS allowed FS rats to normalize their central response to feeding or that the defective response previously observed was specific to the ingestion of carbohydrate and/or fat.

In contrast to the lack of differences at the central level, a higher expression of genes encoding enzymes involved in lipogenesis, such as FAS in the liver and ACC and FAS in white adipose tissue, was observed in FS rats. This result concurs with the higher sensitivity of FS rats to the HFDs. Moreover, mRNA encoding GK, but not L-PK, was increased in FS rats. Although activity of these enzymes is also regulated through posttranscriptional processes, these results indicate that, after 3 wk of the DSS period, ingestion of a protein meal raised glycolysis and lipogenesis to a higher level in FS rats compared with FR rats. This did not result in higher fat deposition, possibly thanks to the reduced caloric and carbohydrate intake during DSS. In addition, FS rats also exhibited higher levels of mRNA encoding for the synthesis of ACOX1, probably leading to a higher rate of hepatic long-chain fatty oxidation in peroxisomes, which may have favored a lower fat storage in these rats during DSS.

Table 6. *mRNA expression (arbitrary units) in arcuate nucleus and nucleus accumbens (expression relative to 18S)*

	FR (n = 9)	FS (n = 9)	
	Mean (SE)	Mean (SE)	
		Arcuate nucleus	
AgRP	32.35 (3.10)	29.75 (4.06)	NS
POMC	22.50 (3.67)	22.60 (3.38)	NS
CART	15.00 (2.26)	17.05 (3.25)	NS
CRF	50.50 (20.52)	36.88 (11.03)	NS
5HT1B	39.67 (5.17)	42.34 (4.71)	NS
5HT2C	14.74 (2.04)	24.35 (8.08)	NS
5HT6	11.36 (1.32)	9.63 (1.38)	NS
NPY	7.63 (1.45)	7.67 (1.45)	NS
		Nucleaus accumbens	
DR1	2.26 (0.31)	2.40 (0.21)	NS
DR2	2.89 (0.45)	3.94 (0.38)	NS
DR3	11.86 (1.94)	13.50 (1.55)	NS
KOR	11.34 (1.17)	12.71 (0.97)	NS
MOR	2.25 (0.21)	2.33 (0.16)	NS
DOR	1.88 (0.14)	2.13 (0.13)	NS
5HT1B	9.87 (1.02)	10.70 (0.66)	NS
5HT2C	8.41 (0.54)	9.52 (0.49)	NS
5HT3A	1.87 (0.22)	2.16 (0.16)	NS
5HT6	3.73 (0.49)	4.47 (0.35)	NS

BW, Body Composition, and DSS

Food intake involves a concomitant control of energy and protein sufficiency. Even if it is presently accepted that caloric intake has priority over protein intake, it has been proposed that in some situations the priority can be shifted to protein (25, 30) [see also the protein leverage hypothesis (11, 31)]. As obese rats are suspected to use food less efficiently to stimulate protein deposition (12, 30), the regulation of protein balance could make them more sensitive to overconsumption of energy under a HFD (13, 29).

Interestingly, in FS rats, the higher gain in adiposity during the HFD and stronger effect of DSS in reducing adiposity gain occurred, while caloric intake remained similar in FR and FS rats throughout the study. This indicates that phenotypic differences between FR and FS rats were due to differences in the metabolic adaptations to the diets. However, it is difficult to consider that the small decrease in the fat content or the increase in the protein content of the diet during DSS were the

Table 7. *mRNA expression (arbitrary units) in liver and adipose tissue (expression relative to 18S)*

	FR (n = 15)	FS (n = 13)	
	Mean (SE)	Mean (SE)	
		Liver	
ACC	66.8 (6.0)	73.2 (10.3)	NS
FAS	26.5 (2.5)	44.9 (7.8)	0.03
GK	8.02 (1.23)	15.41 (2.54)	0.01
L-PK	335.5 (42.3)	514.4 (125.5)	NS
ACOX	2950 (162)	3516 (174)	0.03
CPT1A	176.6 (14.7)	201.7 (23.4)	NS
PPARg	3.10 (0.26)	3.70 (0.40)	NS
PGC1a	1.79 (0.183)	1.76 (0.35)	NS
CD36	213.9 (27.6)	280.4 (32.8)	NS
PPARa	397.6 (36.0)	466.3 (63.7)	NS
BAAT	1562 (116)	1763 (128)	NS
		Adipose tissue	
ACC	540 (59.6)	969.4 (138.7)	0.01
FAS	827 (81.4)	2344 (281.9)	0.00
GLYAT	0.146 (0.022)	0.102 (0.016)	NS
CD36	8851 (1249)	9429 (993)	NS
ATGL	11.6 (1.60)	15.1 (3.31)	NS

Table 8. *Primer sequences*

		Up	Down
ACC	Acetyl-coA carboxylase	5′-CAACGCCTTCACACCACCTT-3′	5′-AGCCCATTACTTCATCAAAGATCCT-3′
ACOX 1	Peroxisomal acyl-coenzyme A oxidase 1	5′-AAG-AAA-TCC-CCA-CTG-AAC-AAA-ACA-3′	5′-CCC-AGG-GAA-ACT-TCA-AAG-CTT-3′
AGRP	Agouti-related protein precursor	5′-TGGTGCCCTTGACCAAAGTT-3′	5′-AATTTCTGCCCCCACAGATG-3′
CART	Cocaine and amphetamine regulated	5′-CCGAGCCCTGGACATCTACTC-3′	5′-AAATACTGACCAGCTCCTTCTCATG-3′
CD36	Cluster of differentiation 36	5′-CAG-CCT-CCT-TTC-CAC-CTT-TTG-3′	5′-AAG-GCG-TTG-GCT-GGA-AGA-A-3′
CPT1	Carnitine palmitoyltransferase 1a-liver isoform	5′-TCT-CTG-GAT-GCG-GTA-GAA-AAG-G-3′	5′-CTC-TAT-ATC-CCT-GTT-CCG-ATT-CGT-3′
CRF	Corticotropin-releasing factor (CRh ou CRF)	5′-CCG-CAG-CCG-TTG-AAT-T-3′	5′-TTC-TTC-ACC-CAT-GCG-GAT-CA-3′
DR1	Dopamine receptor 1	5′-GCC-CCG-AGG-CTC-CAT-CT-3′	5′-ACG-GCA-TGA-GGG-ATC-AGG-TA-3′
DR2	Dopamine receptor 2	5′-CCA-TCA-GCA-TTG-ACA-GGT-ACA-CA-3′	5′-CAG-TAA-CTC-GGC-GCT-TGG-A-3′
DR3	Dopamine receptor 3	5′-TGT-GGC-CGA-CCT-GCT-AGT-G-3′	5′-CTC-CAC-CTG-TCA-CCT-CCA-AGT-AC-3′
DOR	Opioid receptor, δ (Oprd1)	5′-CGT-GCT-CGT-CAT-GTT-TGG-AA-3′	5′-AAG-GCC-AGA-TTG-AAG-ATG-TAG-ATG-T-3′
FAS	Fatty acid synthase	5′-TGC-TCC-CAG-CTG-CAG-3′	5′-GCC-CGG-TAG-CTC-TGG-GTG-TA-3′
GK	Glucokinase	5′-TTG-AGA-CCC-GTT-TCG-TGT-CA-3′	5′-AGG-GTC-GAA-GCC-CCA-GAG-T-3′
5HT1B	5-Hydroxytryptamine (serotonin) receptor 1B, G protein-coupled (Htr1b)	5′-CGC-CAA-CCT-CTC-CCA-CAA-3′	5′-GCG-ATG-GAG-TCC-TGG-TAA-ATG-3′
5HT2C	5-Hydroxytryptamine (serotonin) receptor 2C (Htr2c)	5′-GCA-GCC-GAG-TCC-GTT-TCT-C-3′	5′-TTG-GCC-TAT-GCT-TGC-AGG-TA-3′
5HT6	Serotonin receptor (5HT6)	5′-AAC-ATA-GCT-CAG-GCC-GTG-TGT-3′	5′-CAG-CCA-TGT-GAG-GAC-ATC-GA-3′
5HT3A	5-Hydroxytryptamine (serotonin) receptor 3a (Htr3a)	5′-CCC-CAC-GTC-CAC-AAA-CTC-AT-3′	5′-CCC-CAC-CGA-CAG-CAT-CTG-3′
KOR	Opioid receptor, κ	5′-GCA-TTT-GGC-TAC-TGG-CAT-CA-3′	5′-GAC-ATC-CAC-ATC-TTC-CCT-GAC-TT-3′
L-PK	Liver-pyruvate kinase	5′-TGA-TGA-TTG-GAC-GCT-GCA-A-3′	5′-GAG-TTG-GTC-GAG-CCT-TAG-TGA-TC-3′
MC4R	Melanocortin 4 receptor	5′-GGG-AAA-GCC-ACA-AAA-AAC-GA-3′	5′-GGC-GCT-ACT-GAA-AGC-TCA-CTC-T-3′
MOR	Opioid receptor, μ	5′-CAC-GGC-TAA-TAC-AGT-GGA-TCG-A-3′	5′-GGG-CAA-TGG-AGC-AGT-TTC-TG-3′
NPY	Neuropeptide Y	5′-CTC-TGC-GAC-ACT-ACA-TCA-ATC-TCA-3′	5′-GTG-TCT-CAG-GGC-TGG-ATC-TCT-T-3′
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-α	5′-TGC-GGG-ATG-ATG-GAG-ACA-3′	5′-GCG-AAA-GCG-TCA-CAG-GTG-TA-3′
POMC	Proopiomelanocortin precursor	5′-AGG-CCT-TTC-CCC-TAG-AGT-TCA-A-3	5′-GTC-GGC-CTT-CTC-GGT-ATC-C-3′
PPAR-α	Peroxisome proliferator-activated receptor α	5′-TGG-CAA-TGC-ACT-GAA-CAT-CGA-G-3′	5′-CCG-AAT-AGT-TCG-CCG-AAA-GAA-G-3′

only factors that allowed for the decrease in caloric intake. Indeed, decreasing fat in the diet from 42% to 37% is not sufficient to strongly affect food intake. In rats, it has been repeatedly observed that, when the three macronutrients are combined in a single diet, the protein:energy ratio must increase above 40% to induce a significant decrease in caloric intake (3, 27, 28). Therefore, the possibility for rats to separately adjust protein vs. energy intake during DSS was probably critical in reducing adiposity gain. This is consistent with observations from a previous study that compared rats allowed to select between a protein-free and a 55% casein diet and those allowed to select between 15% and 55% casein diets. Results showed that the former ate more protein, decreased caloric intake, and gained less weight (26).

Meal Pattern and DSS

Meal pattern analysis showed that rats ate from both the C50:L50 and P100 food jars (i.e., mixed protein with carbohydrate and fat) in only 25% of cases. This is consistent with a previous report that observed that rats that spontaneously selected a high level of fat intake (52%) ingested most of their meals from a single food jar (24). This behavior strongly contrasts with the observation that, for rats selecting between a P100 and a high-carbohydrate carbohydrate:fat mixture (carbohydrate 88%, lipids 22%), ~70% of the meals were mixed meals (21). A high proportion of mixed meals (56%) was also observed in conditions where rats had a choice between the three macronutrients and ingested only 15–20% of lipids (14, 16). These observations indicate that, when ingesting a HFD, rats prefer to ingest protein separately, whereas, when ingesting a low-fat diet, they ingest proteins mixed with carbohydrates. FS rats also ingested more protein and more energy than FR ones during the light period. This is in line with previous observations (21), which demonstrated that rats that selected between protein and a low-fat carbohydrate:fat mixture ingested very small amounts of food during the light period and almost exclusively protein meals. These different feeding strategies probably contribute to improvement of oxidation rather than storage of the ingested nutrients and reduce energy requirements and limit fat deposition in the long term.

Perspectives and Significance

Taken together, these results and those of previous studies (21, 26) show that the possibility for rats to finely adjust protein intake according to the available carbohydrate:fat mixture allows them to develop feeding strategies that enable a reduction of energy intake and fat mass gain. In-depth analysis of the consequences of these adjustments on the metabolic fate

of the ingested nutrients, in particular on the cycles of lipolysis/lipogenesis, is required to identify the underlying mechanisms and to envisage possible adaptation to human nutrition.

ACKNOWLEDGMENTS

The authors thank Angélique Simonin for excellent technical assistance.

GRANTS

The study was funded with the resources of the laboratory.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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