

Diet-induced obesity in *ad libitum*-fed mice: food texture overrides the effect of macronutrient composition

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Abstract

Diet-induced obesity in mice can be achieved through the use of diets with different macronutrient compositions and textures. We aimed at determining the contribution of macronutrient composition to obesity development and associated pathophysiological changes in mice. C57BL/6N mice were offered a control, a high-fat or a Western-style diet, either as pellet (H for hard) or with identical composition in powder form (S for soft), resulting in C-S, C-H, HF-H, HF-S, W-H and W-S groups, respectively. Body fat distribution, expression levels of selected target genes in adipose tissues, clinical chemistry and hormone concentration in the blood, as well as liver TAG content were measured. The most striking finding was that all mice fed the different powder diets developed obesity with similar weight gain, whereas among the mice fed the pellet diets, only those given the HF and W diets became obese. This allowed us to separate diet-specific effects from obesity-mediated effects. Irrespective of the food texture, the W diet induced a more severe hepatosteatosis and higher activities of serum transaminases compared with the two other diets. Adipose tissue gene expression analysis revealed that leptin and adiponectin levels were not affected by the dietary composition *per se*, whereas uncoupling protein 1 and 11 β -hydroxysteroid dehydrogenase type 1 levels were decreased by both dietary composition and changes in body weight. In conclusion, diets differing in macronutrient composition elicit specific pathophysiological changes, independently of changes in body weight. A diet high in both fat and sugars seems to be more deleterious for the liver than a HF diet.

Key words: Diet texture: Obesity: High-fat diets: Western diet: Intrahepatic TAG

Obesity has reached epidemic levels in Western countries. It reduces life expectancy and is considered as a key factor in the development of the metabolic syndrome⁽¹⁾. Obesity results from a sustained imbalance between energy intake and energy expenditure by physical activity and is characterised by the storage of excessive TAG in adipose tissue and in additional ectopic depots, such as in the liver and muscle⁽²⁾. In understanding the genetic and environmental basis of obesity, animal models have proven to be useful by allowing manipulations technically or ethically not feasible in human subjects⁽³⁾. Although monogenic⁽⁴⁾ and pharmacologically induced⁽⁵⁾ models of obesity have provided insights into critical pathways, the polygenic nature of obesity calls

for more realistic approaches to generate rodent-based obesity models.

In this context, high-fat diets have been applied to induce obesity in rodents since the early 1950s⁽⁶⁾ and have been shown to cause pathophysiological changes similar to those found in human obesity⁽⁷⁾. These changes are strain-dependent: some strains, such as the AKR/J mouse, are obesity-prone while others, such as the SWR/J mouse strain, are obesity-resistant^(8,9). Dietary fat influences body adiposity quantitatively^(8,10) but also qualitatively, depending on the fatty acid composition of the diet^(10,11). However, it is not yet clear whether fat *per se* has an obesogenic effect. Some studies have reported that animals fed an isoenergetic high-fat

Abbreviations: 11- β -hsd-1, 11 β -hydroxysteroid dehydrogenase type 1; BAT, brown adipose tissue; C-H, control diet in pellet (H for hard) form; C-S, control diet in powder (S for soft) form; EAT, epididymal adipose tissue; HF-H, high-fat diet in pellet form; HF-S, high-fat diet in powder form; IHTG, intrahepatic TAG; MAT, mesenteric adipose tissue; WAT, white adipose tissue; W-H, Western-style diet in pellet form; W-S, Western-style diet in powder form.

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diet had a greater body-weight gain than animals fed a control or low-fat diet⁽¹²⁾, but others have failed to show any major differences⁽¹³⁾. In human subjects, total energy intake, and not dietary fat content alone, has been shown to determine body fat accumulation^(14,15). Therefore, it seems sensible to induce obesity in rodent models not only by increasing the amount of dietary fat but also by means of hyperphagia. Cafeteria diets have been introduced for this purpose: animals are offered a choice of several palatable food items of variable composition, appearance and texture in addition to a (most often) non-purified diet. These diets have been shown to induce obesity based on hyperphagia in both rats and mice^(16,17). Furthermore, diets used in feeding trials can substantially vary with respect to their hardness and diets presenting a hard texture have been shown to cause reduced body-weight gains in rodents^(18–21).

We decided to compare the effects of three different purified diets on body-weight development and physiological parameters in C57BL/6N mice when identical diets were either provided in powder form or as pellets. Mice were given a control (C) diet (4.2% fat, w/w), a high-fat (HF) diet (34% fat, w/w) or a Western-style (W) diet (17% fat, w/w). The latter consisted of three differently flavoured diets, with exactly the same energy density and macronutrient composition, offered simultaneously to the mice. Based on the analysis of body fat compartments, gene expression in visceral and brown adipose tissue (BAT), plasma clinical chemistry and hormone/cytokine concentration, health status was assessed against an obese phenotype background.

Materials and methods

Animals and experimental protocols

Conventional 8-week-old male C57BL/6N mice were obtained from Charles River Laboratories and individually maintained in a controlled environment (12 h light–dark cycle, 22°C), and had free access to water and food. A first cohort of mice was fed a non-purified diet (catalogue no. V1534; Ssniff GmbH) for 2 weeks and thereafter divided into three groups (*n* 12) with a similar mean body weight. Mice were then fed for 12 weeks group-specific pellet diets (H for hard) (catalogue no. E15000-04, E15741-34 and S0372-E0222, -E0242 and -E0262, respectively; Ssniff GmbH). For the W group, mice had free access to three differently flavoured diets (peanut, banana and chocolate, respectively) given simultaneously and characterised by the same macronutrient composition and an increased content of both fat and sugars. The diet composition is provided in Table 1. Throughout the feeding trial, body weight, food and water consumption were recorded once per week. To correct food intake for loss of food, metal grids were placed below the food containers, allowing the collection of spillage. A second cohort of mice underwent the same dietary treatment but this time identical diets were given as powder (S for soft) in small cups (non-purified diet: catalogue no. V1530; C: E15000-00; HF: E15741-30; W: S0372-E0220, -E0240 and -E0260). As all mice in this second cohort developed the same body weights,

but lower than those observed in the first cohort, we decided to extend the length of the feeding trial to 18 weeks so that their mean body weight matched that of the mice from the first cohort fed the HF diet. Moreover, to determine whether the difference in age played a significant role in the effects observed, a third cohort of mice underwent the same dietary treatment but this time only the C and HF diets, in powder and pellet forms, were given to the mice for 12 weeks.

Food intake test

After 8 weeks of feeding, mice from the third cohort were subjected to a food intake test: the amount of food consumed 30, 60 and 120 min after the diets were provided again following overnight deprivation was measured.

Sample collections

At the end of the feeding trial, mice in a non-fasting state were anaesthetised using isoflurane and blood was collected from the retro-orbital sinus. Mice were then killed by cervical dislocation. Liver, epididymal (EAT), retroperitoneal and perirenal, mesenteric (MAT), inguinal adipose tissue and interscapular BAT samples were collected, weighed with a precision balance and snap-frozen in liquid N₂. In addition, at the end of the feeding trial, body size of mice from the third cohort was measured as the nasal–anus length. Their caecum was collected, weighed with a precision balance and snap-frozen in liquid N₂. Moreover, during the last week of this feeding trial, faeces produced were collected, dried at 50°C to constant weight and ground. Faecal gross energy content was determined using an isoperibol bomb calorimeter (model number 6300; Parr Instrument GmbH), with benzoic acid used as a standard. All procedures applied throughout the present study were conducted according to the German guidelines for animal care and approved by the state ethics committee under the reference number 209.1/211-2531-41/03.

Chemical analysis

Serum alanine and aspartate aminotransferase activities and glucose concentration were determined using Piccolo[®] Lipid Panel Plus Reagent Discs and a Piccolo Blood Chemistry Analyzer (Hitado Diagnostic Systems).

Serum insulin, leptin and resistin concentrations were determined using a MILLIPLEX MAP Mouse Serum Adipokine Panel (Millipore GmbH) according to the manufacturer's instructions with an inter-assay CV ≤ 12% and an intra-assay CV ≤ 5%.

To determine hepatic TAG content, liver samples were ground in liquid N₂ and dissolved in 0.9% NaCl. TAG were extracted as follows: after centrifugation (Biofuge 15R; Heraeus Laboratory Centrifuges) for 10 min at 10 000 *g*, supernatants were incubated in alcoholic KOH (30 min, 70°C). Magnesium sulphate was added at a final concentration of 0.15 mol/l and, after centrifugation for 10 min at 10 000 *g*, TAG concentration was determined using a commercial enzymatic colorimetric kit following the manufacturer's instructions (Triglycerides liquicolor^{mono}; Human GmbH). Values were

Table 1. Composition of the different diets employed*

	Control	Western style†			High fat
		Flavour 1	Flavour 2	Flavour 3	
GE (MJ/kg)	18.0	21.0	21.0	21.1	25.2
ME (MJ/kg)	15.2	17.9	18.0	18.0	21.4
% Protein	23	17	17	17	19
% Fat	11	36	36	36	60
% Carbohydrates	66	47	47	47	21
Hardness (kP)	2–5				26–34
Casein	240	190	206	200	276.9
Peanut meal, roasted, salted	–	50	–	–	–
Maize starch, modified	498	180	320	50	–
Maltodextrin	–	181	50	64	158
Glucose	100	–	–	–	–
Sucrose	–	120	123	360	80
Cellulose	50	46	50	40	60
Vitamin premix	10	12	12	12	12
Mineral/trace elements	60	60	60	60	61
L-Cystine	–	2.5	2	2	3.5
L-Thr	–	1.5	1.5	1.5	–
Choline chloride	2	2	2	2	2.5
Salt (NaCl)	–	8.9	2.4	2.4	1
Butylhydroxytoluene	–	0.1	0.1	0.1	0.1
Butter fat	–	50	90	–	–
Beef tallow (premier jus)	–	96	–	60	310
Soyabean oil	40	–	2.5	–	30
Coconut fat	–	–	77	–	–
Cocoa butter	–	–	–	98.5	–
Cocoa powder	–	–	–	46	–
Banana flavour	–	–	1	–	–
Chocolate flavour	–	–	–	1	–
Crude protein	208	182	181	184	241
Crude fat	42	171	170	170	340
Crude fibre	50	52	50	54	60
Crude ash	56	65	58	61	61
Starch	488	189	319	62	11
Sugar	108	124	128	362	82
Dextrins	–	178	49	63	156
Na	1.9	5.6	2.9	2.9	2

GE, gross energy; ME, metabolisable energy calculated using Atwater factors.

* Nutrient composition is expressed as g/kg.

† For the Western-style group, mice had free access to the three differently flavoured diets given simultaneously.

normalised to the protein content of the samples, as determined by the Bradford assay⁽²²⁾.

RNA isolation

Total RNA from the EAT, MAT and BAT was isolated using QIAzol[®] lysis reagent (Qiagen GmbH) according to the manufacturer's instructions and further purified using the QIAGEN RNeasy Mini Kit spin columns (Qiagen GmbH). RNA concentration was determined on a NanoDrop ND-1000 UV–Vis spectrophotometer (Peqlab Biotechnologie GmbH) and its quality analysed with an Agilent Bioanalyzer (Agilent Technologies Deutschland GmbH) using Agilent RNA 6000 Nano Chips, according to the manufacturer's instructions.

Real-time quantitative PCR

For each sample, 10 ng of isolated total RNA were used for quantitative PCR using the QuantiTect[®] quantitative,

real-time one-step RT-PCR kit (Qiagen GmbH) following the supplier's protocol. Gene sequences for primers were retrieved from the database Mouse Genome Informatics (<http://www.informatics.jax.org/>). Primers were designed with VectorNTI Advance 10 (Invitrogen) and tested for specificity using BLAST (Basic local alignment search tool) analysis and conventional PCR. The primers used are listed in Table S1 (available online). Quantitative PCR was performed using SYBR Green I dye and a Mastercycler ep realplex apparatus (Eppendorf AG). The following thermal cycling conditions were used: 30 min at 50°C (complementary DNA synthesis), 15 min at 95°C (RT enzyme inactivation) followed by forty cycles at 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The PCR was concluded with a melting curve analysis of the PCR product (1.75°C/min). Quantification cycle (Cq) values were retrieved from realplex 2.0 software (Eppendorf AG) and analysed following the efficiency-corrected method according to Pfaffl⁽²³⁾, using β -actin as the invariant control to normalise the data. Primer efficiency was calculated with LinRegPCR⁽²⁴⁾.

Statistical analysis

For all groups, data are expressed as means with their standard errors. Except for correlation analysis, the first two cohorts of mice were always analysed separately. The third cohort of mice was analysed using two-way ANOVA. Statistical analyses were performed using Prism 4 software (GraphPad Software). Before ANOVA, data were tested for equality of variances and transformed if needed. Tukey's test was used for pairwise comparisons. Differences in liver weight were tested using ANCOVA in SAS (version 9.2; SAS Institute, Inc.) with body weight as a covariate. Differences in weight gain over the feeding period were tested using the MIXED procedure in SAS with time as a repeated factor⁽²⁵⁾. The variables studied were subjected to seven covariance structures: unstructured covariance; compound symmetry; autoregressive order 1; autoregressive moving average order 1; heterogeneous compound symmetry; heterogeneous autoregressive order 1; Toeplitz. The goodness of fit of the models was compared using the Bayesian information criterion. Tukey's test was used as a *post hoc* test. For all tests, the bilateral α risk was $\alpha = 0.05$.

Results

Mice fed the two high-energy pellet diets developed obesity

Feeding mice with the HF (HF-H) and the W (W-H) diets resulted in a significant increase in mean body weight ($P < 0.001$), whereas mice fed the C diet (C-H) remained lean (Fig. 1(A)). Final body weight, cumulative food, energy and water intake, as well as the feed efficiency ratio and food spillage are shown in Table 2. Mice fed the different diets presented statistically different final body weights, with mice given the HF diet being the most obese (C-H: 29.7 (SEM 0.4) g; HF-H: 43.8 (SEM 1.1) g; W-H: 39.7 (SEM 1.3) g; $P < 0.001$). These results are in agreement with the measured energy intake since the higher the energy intake was, the heavier the mice were. Interestingly, the feed efficiency, defined as the amount of energy ingested for a weight gain of 1 g, was approximately 4-fold higher in C-H mice compared with HF-H and W-H mice.

Mice fed the powder diets all developed obesity and ingested similar levels of energy

Over the feeding trial, mice from all three groups showed a marked increase in body weight with no significant difference in weight gain over time ($P = 0.889$) as shown in Fig. 1(B). At the end of the feeding trial, mice did also not present statistically different final body weights (C-S: 45.1 (SEM 1.0) g; HF-S: 45.1 (SEM 1.3) g; W-S: 44.8 (SEM 1.3) g; $P = 0.986$) and body weight was similar to that of mice fed the pellet HF (HF-H) diet. Mice fed the C-S diet had a higher food intake (3.8 (SEM 0.1) g/d) compared with mice fed the W-S (3.1 (SEM 0.1) g/d) and HF-S diets (2.6 (SEM 0.0) g/d) ($P < 0.001$; Table 2). However, when energy intake rates were calculated by taking the different energy densities of the diets into

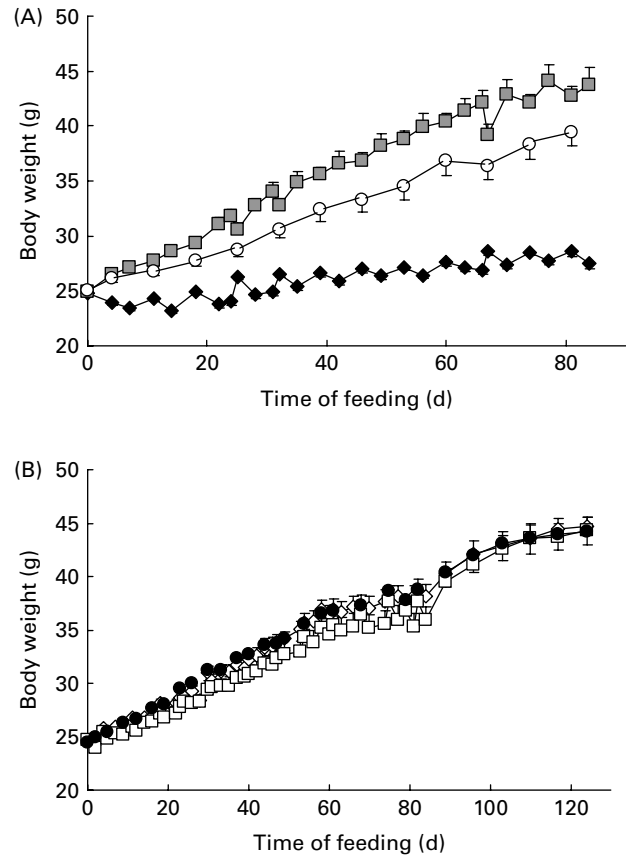


Fig. 1. Body-weight changes in mice receiving the different diets. (A) Body-weight development in mice receiving the different diets provided as pellets (cohorts 1 and 3). Values are means, with their standard errors represented by vertical bars (C-H (◆): n 20; HF-H (■): n 20; W-H (○): n 12). (B) Body-weight development in mice receiving the different powder diets (cohorts 2 and 3). Values are means, with their standard errors represented by vertical bars (C-S (◇): n 20; HF-S (□): n 19; W-S (●): n 12).

account (Table 1), mice in all three dietary groups presented very similar energy intake ($P = 0.111$) and similar feed efficiencies ($P = 0.944$).

Mice fed the pellet control diet displayed increased caecal weight

Mice from the third cohort fed either the C or HF diet in powder and pellet form for 12 weeks showed the same features as described previously, with a lean phenotype when fed the C-H diet and an obese phenotype when fed the C-S, HF-S and HF-H diets (Fig. 1(A) and (B)). In this trial, we also determined whether any changes in overall body length could be observed; however, we did not detect any significant difference ($P = 0.135$; two-way ANOVA), as shown in Table 2. Therefore, body-weight differences originated mainly from different body fat mass. A striking finding, however, was the large increase in caecal weight found only in mice fed the C-H diet. Relative to body weight, caecal weight in these animals was increased 1.8-fold, accounting for 0.6% of body weight, compared with mice fed the C-S, HF-S and



Table 2. Final body weight, cumulative food, energy, water and macronutrient intake in mice receiving the different diets either provided in pellet or powder form*

(Mean values with their standard errors)

	Pellet							Powder						
	Control		High fat		Western style		P	Control		High fat		Western style		P
	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	Mean	SEM	
Weight (g)	29.7 ^a	0.4	43.8 ^b	1.1	39.7 ^c	1.3	<0.001	45.1	1.0	45.1	1.3	44.8	1.3	0.986
Length (cm)	10.0	0.1	10.3	0.1				10.1	0.1	10.1	0.1			
Food (g/d)	3.8 ^a	0.0	3.2 ^b	0.0	3.8 ^a	0.0	<0.001	3.8 ^a	0.1	2.6 ^b	0.0	3.1 ^c	0.1	<0.001
Food spillage (g/d)	0.0 ^a	0.0	0.3 ^b	0.0	0.1 ^c	0.0	<0.001	ND		ND		ND		
Energy (kJ/d)	68.1 ^a	0.5	81.7 ^b	0.7	79.2 ^c	0.7	<0.001	68.4	1.0	65.3	1.0	66.0	1.1	0.111
Feed efficiency (kJ/g)	1693 ^a	312	388 ^b	21	490 ^b	45	<0.001	425	20	421	25	434	36	0.944
Faeces energy (kJ/d)	5.0 ^a	0.2	9.6 ^b	0.4				5.5 ^a	0.1	6.9 ^c	0.4			
Water (ml/d)	4.1 ^a	0.1	2.8 ^b	0.1	3.3 ^c	0.1	<0.001	3.8 ^a	0.1	2.7 ^b	0.1	3.3 ^c	0.1	<0.001
Protein (g/d)	0.7 ^a	0.0	0.5 ^b	0.0	0.5 ^c	0.0	<0.001	0.7 ^a	0.0	0.4 ^b	0.0	0.5 ^c	0.0	<0.001
Fat (g/d)	0.4 ^a	0.0	1.7 ^b	0.0	1.2 ^c	0.0	<0.001	0.4 ^a	0.0	1.3 ^b	0.0	1.0 ^c	0.0	<0.001
Carbohydrates (g/d)	2.1 ^a	0.0	0.6 ^b	0.0	1.5 ^c	0.0	<0.001	2.1 ^a	0.1	0.5 ^b	0.0	1.3 ^c	0.0	<0.001

ND, not detectable.

^{a,b,c} Mean values with unlike superscript letters were significantly different for a given variable ($P < 0.05$).

* Body weight, food and water consumption were recorded once per week.

HF-H diets with identical body weight ($P < 0.001$; two-way ANOVA; Table 3).

Mice fed the control diet as powder or pellets displayed a similar food intake following food deprivation

As shown in Table 4, when mice were presented their respective diet following overnight food deprivation, we did not observe any statistical difference in food intake after 30, 60 or 120 min between mice fed the C-H and C-S diets. Only mice given the HF-H diet presented an increased food intake after 60 and 120 min compared with mice fed the C diets.

Blood chemistry and hormone/cytokine profiles

Mice fed the pellet diets displayed marked differences in blood chemistry and hormone/cytokine profiles. Obese mice from the HF-H and W-H groups displayed significantly increased concentrations of glucose, insulin and resistin when compared with C-H mice (Table 5). On the contrary, mice fed the different powder diets did not exhibit any significant differences in serum glucose, insulin and leptin concentrations; however, serum resistin concentration was significantly increased in mice fed the HF-S diet compared with C-S and W-S mice.

In mice fed the pellet diets, serum alanine aminotransferase activities were increased significantly in the W diet group, while in mice given the HF diet, the increase did not reach significance. For serum aspartate aminotransferase activities, we observed a trend towards an increase in mice fed the energy-rich diets ($P = 0.131$). In mice fed the powder diets, serum alanine aminotransferase and aspartate aminotransferase activities were marginally increased ($P = 0.051$ and 0.096 , respectively) in mice fed the W diet when compared with those receiving the C or HF diet.

All diets – except the control pellet diet – caused increased fat depots, liver weight and intrahepatic TAG concentrations

Organ weight, normalised to body weight, and intrahepatic TAG (IHTG) content are presented in Table 3. Mice fed the W diets presented significantly increased liver weight compared with mice fed the C or HF diet. IHTG content increased 3- to 4-fold in the HF and W groups given the pellet diets ($P < 0.001$). Feeding a W or HF diet in powder form also induced an increase in IHTG compared with the control mice ($P = 0.029$), although mice given the powder C diet already displayed elevated concentrations. When liver weight was plotted against the final body weight in individual mice, in all cases – except for control mice fed the pellet C (C-H) diet that also showed no increase in body weight – a significant correlation was observed (Fig. 2). Moreover, the projected intercepts on the x-axis between 31 and 32 g body weight and the lack of an increase in liver weight in mice that stayed below 32 g body weight suggest that this body weight is the threshold from where on any weight gain, caused by any diet, proportionally increases liver weight.

For the weight of the four depots of white adipose tissue (WAT) collected from mice fed the powder diets, no significant difference was observed, with the exception of the EAT depot in the HF-S group ($P = 0.025$). However, in mice given the HF and W diets provided as pellets, all fat depots increased significantly in relative weight when compared with the C group, which did not display a major weight gain. When compared with the powder diet groups, essentially similar fat depot sizes were observed, with the highest relative mass for the EAT depot, representing 5–6% of total body mass (Table 3). The interscapular BAT was collected as well and similarly revealed a significant expansion with increased body mass. Whereas BAT mass accounted for 0.5–0.6% of final body mass in all mice that became obese, mice fed the powder C and W diets showed a further increase in

Table 3. Organ weight*

(Mean values with their standard errors)

	Pellet						Powder					
	Control			High fat			Control			High fat		
	Mean	SEM		Mean	SEM	P	Mean	SEM		Mean	SEM	P
Liver	5.0 ^a	0.1		5.1 ^a	0.2	0.015	5.1 ^a	0.2		5.2 ^a	0.2	0.001
IHTG†	207 ^a	18		1108 ^b	97	<0.001	854 ^a	125		1038 ^b	124	0.029
Epididymal fat	1.9 ^a	0.1		6.0 ^b	0.1	<0.001	5.6 ^{a,b}	0.2		6.2 ^a	0.2	0.025
Retroperitoneal + perirenal fat	0.8 ^a	0.1		2.6 ^b	0.1	<0.001	2.9	0.1		3.0	0.1	0.873
Mesenteric fat	1.0 ^a	0.1		2.3 ^b	0.1	<0.001	2.3	0.1		2.2	0.2	0.754
Inguinal fat	0.4 ^a	0.0		1.4 ^b	0.1	<0.001	2.0	0.1		2.1	0.1	0.132
Sum of the collected white fat pads	4.2 ^a	0.3		12.3 ^b	0.2	<0.001	12.9 ^{a,b}	0.3		13.6 ^a	0.3	0.050
BAT	0.34 ^a	0.02		0.51 ^b	0.03	<0.001	0.75 ^a	0.02		0.57 ^b	0.03	0.005
Caecum	1.1 ^a	0.1		0.6 ^b	0.1		0.6 ^b	0.0		0.6 ^b	0.0	

IHTG, intrahepatic TAG; BAT, brown adipose tissue.

^{a,b,c} Mean values with unlike superscript letters were significantly different for a given variable ($P<0.05$).

* Organ weight is expressed as a percentage of body weight in each case.

† IHTG is expressed as mg TAG/g protein.

interscapular BAT mass accounting for 0.75% of body mass ($P=0.005$), as shown in Table 3.

Gene expression analysis in adipose tissue

We determined the mRNA expression levels of genes known to be associated with obesity in WAT and BAT (Table 6). In mice receiving the HF and W diets as pellets, an expected large increase in leptin mRNA expression levels was observed in the EAT and MAT samples, compared with mice receiving the pellet C diet and remaining lean. In mice fed the same diets as powder, the differences were not significant, which is in agreement with the observed similar fat depot masses and body weights in these three dietary groups. In all HF and W diet groups, regardless of whether given in pellet or powder form, a significant decline in 11 β -hydroxysteroid dehydrogenase type 1 (11 β -hsd-1) mRNA expression levels was observed in both EAT and MAT depots. This suggests that any expansion of fat depots leads to a reduced mRNA expression level of this gene. In the MAT fat, the resistin mRNA expression level significantly increased in obese mice fed the pellet diets, whereas its expression level in EAT was significantly decreased in mice given the HF diet as pellets. In BAT, we observed a decreased mRNA expression level of the uncoupling protein 1 (*Ucp-1*) in all mice fed the HF and W diets, regardless of whether given in pellet or powder form. The uncoupling protein 3 (*Ucp-3*) mRNA level was reduced in obese mice fed the pellet diets when compared with those on the C diet (C-H) without significant weight gain. The mRNA expression level of the adipose TAG lipase (*Atgl*) was decreased in obese mice fed the pellet diets, whereas in mice given the powder diets, those receiving the W diet displayed an increase in *Atgl* mRNA expression level, despite almost identical body weight and relative BAT mass.

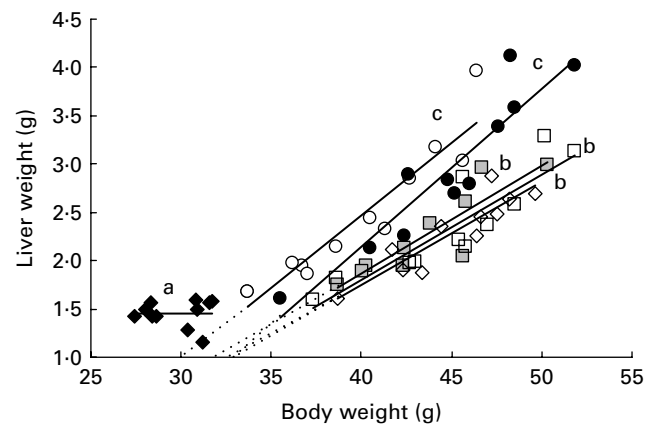


Fig. 2. Liver weight and intrahepatic TAG in mice receiving the different diets. Across all mice and within lines, body weight was significantly correlated with liver weight: r^2 0.67, n 66, $P<0.001$; C-H (◆): r^2 0.00, slope 0.00, n 11, $P=0.955$; HF-H (■): r^2 0.80, slope 0.11, n 10, $P<0.001$; W-H (○): r^2 0.89, slope 0.15, n 12, $P<0.001$; C-S (◇): r^2 0.81, slope 0.11, n 11, $P<0.001$; HF-S (□): r^2 0.80, slope 0.11, n 11, $P<0.001$; W-S (●): r^2 0.86, slope 0.16, n 11, $P<0.001$. ^{a,b,c} For a given group, regression lines with unlike letters indicate a significantly different diet $\times$ texture interaction. There were significant body-weight ($P<0.001$), diet ($P<0.001$) and texture ($P<0.001$) effects.

Table 4. Food intake over short periods of time following overnight food deprivation*
(Mean values with their standard errors)

	Pellet				Powder				<i>P</i>
	Control		High fat		Control		High fat		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
30 min	0.27	0.05	0.46	0.05	0.28	0.04	0.32	0.07	0.084
60 min	0.40 ^a	0.03	0.64 ^b	0.05	0.38 ^a	0.03	0.46 ^{a,b}	0.06	0.004
120 min	0.57 ^{a,b}	0.04	0.70 ^a	0.05	0.47 ^b	0.04	0.58 ^{a,b}	0.05	0.046

^{a,b} Mean values with unlike superscript letters were significantly different for a given variable ($P < 0.05$).

* Food intake is expressed as g of food consumed when diets were presented for different times following overnight deprivation.

Discussion

In the present study, we compared the effects of diets with different macronutrient compositions, provided either as pellets or powder to mice, on the development of obesity and some associated health status markers. In addition to a standard high-starch C diet and a HF diet, we used a W diet with three different flavours. In contrast to the original cafeteria diet introduced by Sclafani & Springer⁽¹⁶⁾, comprising human food items added to a basal diet, we employed defined diets of identical macronutrient composition with 36% of energy coming from fat and 47% of energy from carbohydrates. This enabled us to precisely measure food and energy intake, which can be difficult with the original cafeteria diet due to its complexity⁽²⁶⁾.

The most striking finding of the present study was that all mice, except those given the C pellet diet, gained weight with a similar slope and displayed final body weights of 40–45 g. Interestingly, mice fed the powder diets displayed almost identical daily energy intake rates. The estimated feed efficiency was consequently not different and amounted to approximately 430 kJ/g, independently of the diet. This was not the case for mice fed the pellet diets. Although, here, mice fed the HF diet had a significantly reduced food intake, energy intake in both HF and W diets was significantly higher than that in the C group and feed efficiency was markedly lower ($P < 0.001$), as shown in Table 2. Thus, in powder-fed mice, the hyperphagia frequently associated with the consumption of energy-dense diets in rodents⁽²⁷⁾ as well as in human

subjects⁽²⁸⁾ could not be observed here. All groups presented a similar energy intake and a similar weight gain. Although there is a controversy as to whether flavour variety in diets affects food intake^(29,30), we did not observe major effects except that food intake, when compared with mice fed the HF diet, was higher in mice fed the W diet in both pellet and powder forms.

The different results observed between the pellet and powder variants of the diets are of course striking and point at an effect of food texture on body-weight development. The impact of the hardness of the diet has already been addressed, and it has been shown that mice fed hard pellets had a lower body weight and improved blood glucose concentrations compared with mice fed soft or powder diets^(18,19,21). Rats have been shown to prefer soft pellets rather than the diet they are usually fed⁽³¹⁾. Long-term feeding of soft pellets induced a larger increase in body weight and body fat content and lower postprandial thermogenesis despite similar food intake rates when compared with pellet-fed animals⁽²⁰⁾. Rothwell *et al.*⁽³²⁾ observed that when rats were fed a low-fat or a HF diet given in powder form and greatly differing in energy density, all mice developed the same body weight with similar energy intake rates. In human subjects, diet hardness was found to be a significant determinant of waist circumference, independently of food intake, although no effect was observed on BMI⁽³³⁾. Here, we show for the first time that a pellet-based high-carbohydrate/starch diet fails to trigger obesity, whereas the same diet given in powder form produces an obese

Table 5. Serum clinical chemistry and adipokine concentrations
(Mean values with their standard errors)

	Pellet							Powder						
	Control		High fat		Western style		<i>P</i>	Control		High fat		Western style		<i>P</i>
	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	Mean	SEM	
ALT (units/l)	36 ^a	4	52 ^{a,b}	5	73 ^b	11	0.006	64	5	68	9	122	26	0.051
AST (units/l)	76	8	94	9	103	11	0.131	103	7	93	13	138	19	0.096
Glucose (mmol/l)	11.8 ^a	0.5	14.3 ^b	0.5	15.4 ^b	0.7	<0.001	15.2	1.5	14.8	1.2	15.0	0.7	0.972
Insulin (pmol/l)	19 ^a	3	118 ^b	21	120 ^b	35	<0.001	337	92	410	81	367	118	0.865
Leptin (pmol/l)	33 ^a	7	505 ^b	69	272 ^c	51	<0.001	822	114	560	112	625	161	0.482
Resistin (pmol/l)	47 ^a	5	139 ^b	16	122 ^b	19	<0.001	103 ^a	14	160 ^b	24	116 ^a	18	0.015

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

^{a,b,c} Mean values with unlike superscript letters were significantly different for a given variable ($P < 0.05$).

Table 6. Relative expression of the selected target genes in visceral adipose tissues*
(Mean values with their standard errors)

Target genes	Pellet					Powder				
	Control		High fat		P	Control		High fat		P
	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
Epididymal adipose tissue										
Leptin	1.00	0.17	2.35 ^b	0.11	1.96 ^b	1.00	0.03	1.04	0.07	0.967
Adiponectin	1.00	0.13	0.60 ^b	0.07	0.72 ^{a,b}	1.00	0.09	1.06	0.16	0.376
Resistin	1.00	0.11	0.45 ^b	0.07	0.80 ^{a,b}	1.00	0.19	1.33	0.30	0.143
11- β -hsd-1	1.00	0.08	0.27 ^b	0.03	0.49 ^b	1.00	0.05	0.61 ^b	0.05	0.022
Mesenteric adipose tissue										
Leptin	1.00	0.23	4.06 ^b	0.89	2.90 ^{a,b}	1.00	0.09	1.00	0.15	0.999
Adiponectin	1.00	0.11	1.32	0.12	1.41	1.00	0.10	1.10	0.15	0.472
Resistin	1.00	0.12	2.19 ^b	0.29	2.55 ^b	1.00	0.07	1.38	0.22	0.087
11- β -hsd-1	1.00	0.06	0.42 ^b	0.04	0.61 ^b	1.00	0.07	0.70 ^b	0.07	0.023
Brown adipose tissue										
Ucp-1	1.00	0.13	0.61 ^b	0.08	0.71 ^{a,b}	1.00	0.05	0.61 ^b	0.12	0.003
Ucp-3	1.00	0.18	0.41 ^b	0.06	0.37 ^b	1.00	0.10	0.79	0.15	0.422
Atgl	1.00	0.07	0.72 ^b	0.06	0.76 ^b	1.00	0.04	1.06 ^{a,b}	0.06	0.017

11- β -hsd-1, 11 β -hydroxysteroid dehydrogenase type 1; Ucp, uncoupling protein; Atgl, adipose TAG lipase.
^{a,b} Mean values with unlike superscript letters were significantly different for a given variable (α as superscript for control diet-fed mice, not shown).
^{*} Data are expressed in fold changes compared with control diet-fed mice.

phenotype similar to a HF or W diet. While all mice receiving the high-carbohydrate C diets ingested very similar amounts of food and lost similar quantities of energy through faeces, they displayed quite different body-weight gains. The most striking difference, however, was that feed efficiency was 4-fold higher in the powder diet compared with the pellet variant.

The pellets, as provided here for both the HF and W diets, are softer in texture than those of the C diet with high starch content (Table 1). Thus, those very hard pellets might elicit a higher postprandial thermogenic response^(34,35), and mice consuming them may in addition need to utilise more energy for chewing (although we did not observe any difference in food intake over short periods of time following overnight food deprivation between the groups fed the powder or the pellet C diet, possibly due to adaptation of mice to their respective diet), for efficient handling (i.e. motility) in the stomach and intestine, and for digestion. That energy extraction in the small intestine may be limited from the high-starch pellet diet is suggested by a major increase in caecal mass found in these mice compared with all other animals. When non-digested starch reaches the caecum, microbiota mass increases and increased fermentation delivers SCFA that can be absorbed by the host. Yet, the energy delivered to the host is much less, accounting for about 7.2 kJ/g carbohydrate compared with 17.2 kJ/g when absorbed as glucose⁽³⁶⁾.

Since all mice given the powder diets had the same final body weight and obesity state, as judged by the expansion of fat depots, we could assess the specific effects of diet composition on selected metabolic parameters that characterise an obese state. The organ that was most affected by changes in dietary composition was the liver. Liver weight and IHTG concentrations (in mg/g protein) increased proportionally to total body mass and independently of the diet after a 'threshold' body weight of about 32 g was reached. Increased concentrations of IHTG have been associated with hepatic and peripheral insulin resistance⁽³⁷⁾ and are considered to represent a major determinant of the metabolic syndrome⁽³⁸⁾. The W diet, regardless of whether presented as pellet or powder, increased liver weight more than the HF diet, suggesting that a higher dietary sucrose/dextrin intake may be more deleterious for the liver than a higher fat intake. Recently, Fabbrini *et al.*⁽³⁹⁾ demonstrated in human subjects that the IHTG content, but not visceral adipose tissue mass, was a marker of obesity-related metabolic dysfunctions. We observed in all obese mice – although not feed-deprived – markedly increased plasma glucose and insulin concentrations, suggesting also an impaired glucose tolerance. Plasma TAG did not differ among any group, whereas cholesterol and HDL concentrations were higher in obese mice.

Since adipose tissue plays a central role in energy homeostasis and the development of insulin resistance, notably through adipokines⁽⁴⁰⁾, we measured the concentration of selected adipokines in serum and characterised changes in the mRNA expression levels of selected target genes involved in adipokine secretion or adipose tissue metabolism. Gene expression was studied in two visceral WAT depots and the interscapular BAT. Whereas MAT fat in particular is suspected to have a role in the aetiology of metabolic diseases⁽⁴¹⁾, the

BAT has been shown to play a significant role in energy balance via non-shivering thermogenesis⁽⁴²⁾.

Serum leptin concentration was significantly elevated in all obese mice, and there was no detectable effect of the diet. Since leptin concentration correlates with body fat mass and adipocyte size⁽⁴³⁾, this was not surprising, based on the increase in body fat mass in all mice, except for the C-H diet. Similarly, the mRNA expression level of leptin was higher in obese mice in EAT as well as MAT depots with no effect of the diet. Resistin showed significantly elevated mRNA expression levels in MAT depots when compared with mice given a C pellet diet, and displayed a marginally significant increase when compared with mice given a C powder diet. Resistin, at least in rodents, has been shown to counteract insulin activity⁽⁴⁴⁾, and therefore the finding that adipose tissue depots increase resistin expression may result from an adaptation to the elevated serum insulin, which could additionally enhance insulin resistance, in particular in mice fed pellets. Interestingly, in mice fed the powder diets, a HF diet induced a significant increase in circulating resistin concentration, although mice displayed similar insulin concentrations. The mRNA expression level of *11-β-hsd-1* decreased significantly in all obese mice and more so in mice fed the HF and W powder diets. This suggests that any expansion of fat depots leads to a reduced mRNA expression level of this enzyme. *11-β-hsd-1* is involved in glucocorticoid synthesis in adipose tissue and has been implicated in the pathology of the metabolic syndrome⁽⁴⁵⁾. The present results are in accordance with those of Morton *et al.*⁽⁴⁵⁾ who proposed that the decrease in *11-β-hsd-1* expression might also represent a protective mechanism during chronic high fat feeding. Taken together, changes in gene expression observed in the different fat depots are mainly a measure of fat mass expansion and only subtle effects of diet composition are detectable.

In summary, we observed a very interesting phenomenon when inducing obesity in C57BL/6N mice with two types of high-energy diets of identical macronutrient composition, provided either as pellet or in powder form. Regardless of the source of energy – whether based on a high-fat diet, or a W diet with lower fat but higher sucrose/dextrin content, or a starch-based C diet – all mice, when fed the powder diets, became obese with roughly the same weight gain. Although food intake rates were different, based on almost identical daily energy intake rates and identical feed efficiency values, the powder-fed mice displayed essentially the same proportional expansion of WAT depots and, as a consequence, possessed similar serum leptin concentrations. The only difference found between the diets was an increase in liver weight (absolute and relative to body mass) and IHTG concentrations in mice fed the W diet, which in this respect seems to be more deleterious to the liver than a pure HF diet providing 60% energy as fat. In mice fed the pellet diets, IHTG concentration was similarly elevated in the HF and W groups, although the latter displayed decreased body weight and WAT depot expansion compared with mice fed the HF diet. Most interestingly, both liver weight and IHTG concentrations increased proportionally to body mass in all mice after a threshold level of approximately 32 g body weight had been reached.

Finally, we would like to critically ask whether a pellet-based, high-carbohydrate/starch diet is a proper C diet when used for comparison with HF diets. Feeding diets with >45% energy as fat is meanwhile accepted as a ‘gold standard’ to induce obesity in normal or transgenic mice models. The pellets of this C diet have an exceptionally hard texture and are therefore difficult to chew, to swallow and may need huge amounts of energy for handling in the gastrointestinal tract. They may also cause a loss of energy by the delivery of larger amounts of undigested starch to the microbiota and may after all produce an artificially ‘lean phenotype’.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114512003340>

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