Energy or chow, that's a question: "two-bottle" may be more suitable than "one-bottle" in modeling nutritional obesity

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Objective: To model nutritional obesity in rats with two-bottle-feeding method according to human eating feature.

Design: Animals were grouped into control and model randomly according to their initial body weight. The control rats fed with standard chow singly and the model rats fed with the standard chow and a high fat (HF) diet simultaneously for 20 weeks. Lee's index of control was used as the reference of obesity identification. The inducing effect of this method and animals' eating features were studied.

Subjects: 142 weaned male Sprague-Dawley (SD) rats (body weight: 43.95±4.0g).

Measurements: Body weight (BW), body length (BL), and food accumulation were measured weekly, and Lee's index was calculated for obesity evaluation.

Results: This modeling process based on human eating feature facilitated the production and evaluation to HF diet, and showed an effective inducing effect. At the end of 14th week, about 51% rats (by Lee's index) or 70.6% rats (by body weight) developed obesity and all developed fatty liver. Animals' preference to HF diet was proved to be varied greatly. Its influence on obesity and obesity resistance (OR) rats can be balanced by two-bottle-feeding method, p=0.42. Obesity rats ate more HF diet than OR rats, but they ate same amount of chow. On the other hand, Lee's index of the animals was not well related to energy intake, r^2=0.48. Thus, animal's preference to HF diet and their own metabolic feature were potential influence factors, except for these factors, animals' food intake and energy efficiency are key factors deciding obesity.

Conclusions: Two-bottle-feeding method is an effective way to modeling nutritional obesity as well as fatty liver in rats. Food-intake and energy efficiency are inner key factors deciding obesity or obesity resistance.

Keywords:

nutritional obesity; modeling; human; eating behaviors; feeding method

Introduction

In nutritional obesity research, the major question to be answered is “why” bodies can have extra energy ingested despite their accurate regulatory mechanisms,[1] and correspondingly, the major problem to be resolved in its modeling process is “how” to interfere with animals’ food or energy intake regulatory system and have them to take more extra energy [2]. The modeling process reflects the concept and understanding to that question.

Diet is the source of extra energy resulting obesity and obviously takes the main responsibility.

Thus, to increase the energy level of feed is the sure way to model nutritional obesity. People have currently exerted supreme efforts to the production of an ideal high-fat (HF) diet with nutrient balance.[3] However, a model based on not only HF diet but also the eating behaviors which are under strict control of mind and permit extra energy to enter into the body has not been reported yet.

The major difference between human eating behavior and that of animals raised in laboratory is that, for being lack a natural food possessing all needed nutrients, people select different food to meet their nutrient need.[4] This eating pattern had been name as "nutritional wisdom". On the other hand, human have the freedom to evaluate diet and choose what they like or need.[4] When faced on abundant food supplies, people pick up food according to their pleasure rather than need of calories.[5] High energy food (together with instant food or "junk food") is always associated with high palatability, so it was in their choices.[6] If the wisdom was to select different nutrient-rich food to maintain the homeostasis in poor time, with the economic development and society transition from developing to developed ones, the wisdom has developed into the choice of eating delicious high quality food rich in energy for pleasant under the guarantee of high quantity traditional food rich in nutrients for homeostasis.

We thus supposed that these eating behaviors may have special significance in modeling process. so in this article, we designed a "two-bottle-feeding" method, in which animals were provided with palatable HF food and standard rodent chow together, to let animals evaluate and choose diet freely and to balance nutrient and energy actively. The results demonstrated that this modeling process facilitated the production and evaluation to HF diet, and showed an effective inducing effect. It also made the influence from diet palatability clear. By this method, we proved that besides food energy level, animals' food intake and energy efficiency are key factors deciding obesity.

Methods

Animals and feeding methods 142 weaned male SD rats (Charles River, China) with body weight of 43.95±4.0g were used under the instruction of animal's welfare committee of Peking University. Rats were housed individually in SPF environment with 12 hours dark-night cycle. The room temperature was 22±2℃, and humidity was 65±5%. According to their initial body weight, rats were divided randomly into control, n=40, and model, n=102. The control group was fed with standard chow singly as mentioned everywhere and the model group was provided with standard chow and palatable HF diet together. Rats took food and water ad libitum.

Feeds Chow (protein 20%, fat 4%, carbohydrate 50%, 3.16kcal/g, 11.4% from fat) and HF feed (protein 15.8%, fat 41.2%, carbohydrate 30.9%, 5.58kcal/g, 66.5% from fat) were used in this experiment.

HF diet was designed to blend 38.5% of chow with 25.6% lard, 25.6% egg yolk powder (protein 31.6%, fat 55.1%, carbohydrate 5.3%) and 10.3% sucrose. Animal's evaluation and preference to diet was judged by HF/Chow of their food intake.

Before adopting this formula of HF diet, we had exerted many trials including semi-purified HF diet with nutrient well-defined as well as unbalanced HF diet. However, despite the high energy composition, they all failed to cause obesity due to bad taste and thus animals' eating rejection which in some cases even resulted weight loss and growth retardation. When fed rats with those HF food and chow together, animals prefer chow to HF diet remarkably. In this HF diet, egg yolk powder and lard provided pleasant odor while sucrose provided sweet taste besides energy providing. The lard became freezing in SPF environment and made the diet solid. This formula was designed mainly for the considerations of energy and palatability but slightly less for the nutrient balance, see table 1.

These two distinct food, one was prominent with high energy, ordour and sweetness, while the other was marked in hardness, salt and other component rich in protein, promised the rats to select their own pleasure on the premise of health. This method is not a simplified form of "multichoice" feeding manner which indicated animal's "nutritional wisdom",[7] but a choice of pleasure or homeostasis.

Measurements of animals Animal's body weight (BW), body length (BL) and food accumulations were measured and recorded weekly. The BL was from nose tip to anus and measured at their leisure state. 20 weeks later, animals were anesthetized with 3% pentobarbital sodium (5mg per 100g BW) by peritoneal injection and then sacrificed by exsanguinations. The viscera were collected and liver was investigated histologically by heamatoxilin-eosin staining in routine protocol.

Definition of obesity and energy efficiency For the normal distribution of body weight in animals fed with HF diet,[8] the upper border mean+$t_{\nu,\alpha}$·sd of Lee index's (BW(g)$^{1/3}$×1000/BL(cm)) normal value scope from control was taken as the standard of obesity evaluation, individuals with values higher than that were regarded as obesity. The energy efficiency was the BW gain per calorie (BW gain (g) ×1000/energy intake (kcal)).

Data analysis Data are presented here as mean±s.e.m. All data were explored for normal distribution before analysis. They were then compared by independent t test and analysis of variance (ANOVA). A probability of less than 5% (two-tailed) was considered significant.

Statement of Ethics

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research.

Results

The growth and occurrence of obesity

The growth of rats under this feeding method was observed and recorded intensively. Same as rats in control, model rats got BW and BL increased continuously and steadily during experiment. But model rats gained more BW and BL than control significantly and the total cluster drifted rightwards, Fig 1. At the end of 14th week, the BW of model rats was 740.44±7.08g, while that of control was 614.38±7.02g, (20.1% more BW than control, $p<0.0001$). From 0w to 8w of experiment, BL of control and model made no difference, $p>0.05$, while from 9th week, BL of model rats was higher than that of control significantly, $p<0.05$, Tab 2.

We then studied the occurrence of obesity. By Lee's index, at the end of 14th week, the normal value scope mean$\pm t_{\nu,\alpha}\cdot$sd ($\nu=39$, $\alpha=0.05$) of Lee's index from control was 305.29-331.71. The upper border 331.71 was then taken as the standard of obesity. Results indicated that 52 in 102 (51%) animals developed obesity, and the highest value was 359.67. While giving BW singly for consideration, 72 in 102 (70.6%) were obesity, the highest value was 920.9g compared with 791.1g in control. The number of obesity ascertained by BW was more than that from Lee's index remarkably and there were always a few rats were not coincident with those from Lee's index, Fig. 3b. Under this standards of obesity, 1 in 40 (2.5%, according to Lee's index) or 2 in 40 (5%, according to BW) rats in control can also be decided as obesity, see in Tab. 3.

After sacrificed at the end of 20th week, animals' viscera were investigated. The weight of liver and fat tissues (mainly from retroperitoneal fat pad and epididymal fat) of model were higher than control significantly, $p<0.0001$, Tab. 4 and all animals developed fatty liver, Fig 2.

Eating behavior investigation

Totally, the HF diet ingestion was higher than chow significantly ($p<0.0001$), and the ratio of HF/chow was 2.20±1.05 (mean±sd), Fig. 3a, which showed an excellent palatability of HF diet.

However, animals' preference to this HF diet was varied remarkably. According to their preference, obesity or obesity resistance (OR) rats can be further divided into 3 subgroups, very liking HF diet (6 to 8 times more than chow), liking HF diet in average (2 times more than chow) and liking chow much (less than chow), Fig. 3b. There was no difference between obesity and OR rats (obesity: 2.11±1.12; OR: 2.28±0.98; $p=0.42$), Tab. 5. So, this model diminished the influence of animal's special like or dislike on the differentiation of obesity and OR.

Food intake and energy efficiency were analyzed for 6 weeks. Animals ate HF diet as well as chow according to their own choices. Model rats took lower food but higher total energy intake and higher energy efficiency (EE) than that of control remarkably, $p<0.0001$. Compared with OR rats, obesity rats exhibited higher food intake and energy efficiency and took more HF diet, $p<0.0001$. However, they ate same amount of chow, $p=0.50$, Tab. 5.

On the other hand, animals' BW was linearly correlated with energy intake significantly, $r^2=0.84$,

Fig. 3c, but the Lee's index was not well related with their energy intake, r^2=0.48, Fig. 3d. This means some animals possessed special growth status. So, according to this relationship, obesity rats can be further divided into a majority with high food intake, energy efficiency and then BW and a small portion with lower ones, while OR rats can be further classified into a majority with low food intake, energy efficiency and then BW and small portion with higher ones, Fig. 3b.

Discussion

Eating behavior is the process to have food, nutrients entering into the body. This critical link between diet and body is under strict control of hungry/satiety and rewarding center [9,10] and decides what food and how much be permitted and absorbed. It thus takes important responsibility for adequate nutrient supply and nutrient balancing. On the other hand, eating behavior is also a barrier for extra nutrient, such as fat or energy, to enter. Besides over-feeding or damaging the regulatory center by surgery or medicine, for human, the natural way to break this barrier is to have the extra nutrient some special features to stir up extra appetite.[5,11,12] Human take extra food on the premise of nutrient balance to meet their pleasure.[5]

Since long time before, we paid more attention to the unique and standard HF diet in modeling of nutritional obesity. However, we avoided to talk about the varied, fluctuated and unpredicted inducing effects. It will raise this question that this situation was due to the unstandard formulation, which suggested that all research on nutritional obesity use a unique and standard production of HF diet. It sounds rather paradoxical. If obesity depends on HF diet composition so much, the prevalence of it must be a "joke". This "tool's standardization" failed to touch the essence of mechanisms of obesity occurrence. Additionally, traditional "one-bottle-feeding" method ignored human eating behaviors. It homogenized the varied choice to HF diet and thus failed to tell the real process of human obesity occurrence. Moreover, it even failed to tell the influence of animals' special preference on varied biological effects of fat or energy and lost in the seeking of a "standard" HF diet [3, 13-19]. We thus conceived that the standard of obesity modeling should not be put on the ingredients, but the same preference to diet as well as energy level.

"Two-bottle-feeding" method models nutritional obesity by replicating human eating behavior and breaks through the "bottleneck" of HF diet manufacture easily by their ability of self-regulation [20-22]. In this method, HF diet and chow were provided together, and animals selected HF diet by preference and chow/nutrient by need. Nutrient and extra energy were decided and balanced by animals themselves. The results displayed a strong ability from rats to balance nutrients need with extra energy, which facilitated the production of HF diet, and a marvelous modeling results. Besides, it was also testified to be a potent way to model obesity related fatty liver for been able to have energy level elevated to a large extent.

In this experiment, we found that rats' preference to HF diet varied greatly. This disparation cast some influence on the judgement of obesity but can not be identified in traditional way. By two bottles feeding method, this influence from special liking or disgust to diet was visible and can be balanced. Besides, some animals' using of extra energy (fat deposition) was not strictly paralleled with the energy amount being ingested. There may be some different metabolism regulatory mechanisms. So, in this modeling process, animal's preference and inner metabolic direction of fat may be important influence factors. Thus, obesity and OR rats should be grouped according to their diet liking and metabolism features before further research.

When faced on two providings, for rats under same preference to HF diet, the obesity rats ate same amount of chow as OR ones but higher HF diet. This fact demonstrated that they ate chow in the need of physiology, while ate HF diet for special pleasure which amount depended mainly on their food intake, the capacity of stomach. So, food intake was one of the important factors deciding the amount of extra energy entering into the body, and thus deciding obesity or not. Besides food intake, we also found that energy efficiency of obesity rats, which indicated their utility ability to extra energy, was also higher than that of OR rats. Whether this change resulted from mind regulation, or an inherit mechanism, or this method provided some hints for modeling hedonic eating? [5] This still needs a further verification.

In conclusion, energy or chow i.e., pleasure or homeostasis, that's a question. People failed in this choice and thus resulted in an epidemic of obesity. While by two-bottle-feeding method, animals failed also in this choice and brought about obesity prevalence too. It's a wise choice for researchers using this method to model nutritional obesity, however.

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Tables

Table 1 The composition and macronutrients of HF diet (g/100g)

Ingredient	Amount	Protein	Fat	Carbohydrate
Chow	38.5	20.0	4.0	50.0
Egg Yolk Powder	25.6	31.6	55.1	5.3
Lard	25.6	-	99.9	-
Sucrose	10.3	-	-	99.9
Total		15.8	41.2	30.9

Table 2 BW and BL of model and control group (means±s.e.m.)

		0w	4w	6w	8w	12w	14w
Contr	B	43.99±0.6	244.72±4.00	379.45±4.21	467.86±4.86	579.63±6.45	614.38±7.02

ol	W	8					
	BL	11.08±0.09	20.41±0.12	23.03±0.10	24.73±0.10	26.23±0.08	26.68±0.10
Model	B	43.93±0.4	259.46±2.06	394.74±3.02	502.45±4.06	679.59±6.00	740.44±7.08†
	W	0	*	*	†	†	
	BL	11.16±0.06	20.60±0.06	23.08±0.05	24.91±0.06	26.68±0.06†	27.27±0.06†

*: $p<0.05$, compared with control. †: $p<0.0001$, compared with control.

Table 3 The occurrence of obesity in model group at the end of 14th week (n=102)

	mean±$t_{\nu,\alpha}$·sd*	Standard	Number of obesity	Incidence
Lee's index†	305.29-331.71	331.71	52(1/40)	51.0%
Body weight (g)	525.61-703.15	703.15	72(2/40)	70.6%

*: ν=39, α=0.05. †: Lee's index=BW (g) $^{1/3}$×1000/BL (cm).

Values in brackets indicated the obesity identified in control under this standard.

Table 4 The fatty liver and viscera fat (g) (means±s.e.m.)

	N	Retroperitoneal fat	Epdidymal fat	Liver
control	36	24.35±0.94	16.91±0.63	18.24±0.37
model	27	55.44±3.64*	23.34±1.24*	28.55±1.39*
OR	12	42.81±4.07*	24.94±1.93*	24.57±1.83*
Obesity	15	65.54±4.19*†	29.26±1.48*†	31.73±1.64*†

*: compared with control, p<0.0001. †: compared with OR rats, p<0.0001.

Table 5 Food and energy analysis per week (means±s.e.m)

	N	FI (g)	EI (kcal)	EE(g/cal)	HF diet (g)	Chow(g)	HF/Chow
Control	40	228.37±2.74	721.64±8.66	28.87±0.56	--	--	--
Model	102	178.17±1.98[†]	845.97±8.96[†]	41.47±0.47[†]	116.92±1.78	61.25±2.04	2.20±1.05[‡]
Obesity	52	188.25±2.22[*]	898.92±9.98[*]	43.39±0.59[*]	125.65±2.35[*]	62.60±2.84	2.28±0.14
OR	50	167.69±2.61	790.90±10.43	39.47±0.63	107.85±2.03	59.84±2.95	2.11±0.16

*: compared with OR rats, p<0.0001. †: compared with control, p<0.0001. ‡: mean±sd.

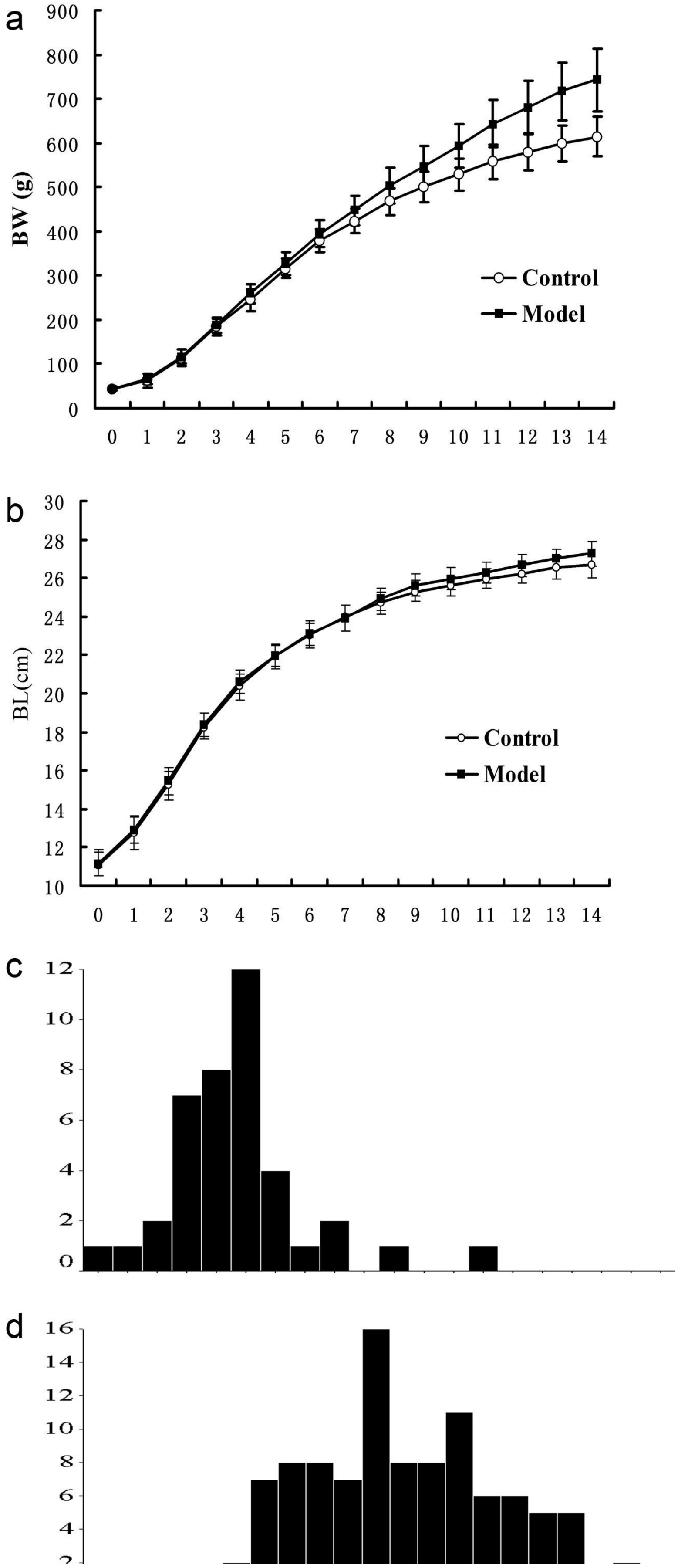
Abbreviations: FI, food intake. EI, energy ingestion. EE, energy efficiency. HF, high-fat diet. OR, obesity resistance.

Titles and legends to figures

Figure 1. Rats' growth curves. The BW (a) and BL (b) of rats fed by two-bottle method exceeded that of control remarkably at 6th and 9th week separately, and this trend was extended later. At the end of 14th week, the BW of model population drift rightwards compared with control and maldeveloped individual was not found. This characteristic was showed in frequency histograms (c) and (d).

Figure 2. Morphology of rats' liver, which showed a typical fatty change resulting from HF diet by two-bottle-feeding method. All rats in model developed fatty liver. Macro morphology appearance of control (a) and model rat (b). Micro morphology of control (c), obese (d), moderate obesity (e), and OR rat with lowest lee's index and BW (f), HE staining, 400×.

Figure 3. Eating feature and obesity development investigation. (a) Diet palatability evaluated by animal themselves. The food accumulations of HF diet was about two times more than that of chow, $p<0.0001$. (b) The scatter plot of 102 rats according to their HF diet and chow ingestion. Rats' distinguished preference to HF diet, different growth status and the relationship between food intake and BW can be visible. Cases were labeled either by lee's index: OR rats, □; obesity rats, ○; or by body weight: OR rats, ×; obesity rats, +. Statistically, obesity rats eat more HF food than OR rats but the chow ingestion made no difference. Food accumulations were gathered for 6 weeks totally. Energy intake was linearly correlated with BW significantly, $r^2=0.84$ (c), but less promised with lee's index, $r^2=0.48$ (d). Some animals exhibited a different growth and energy utility status. OR, ○; obesity, ●.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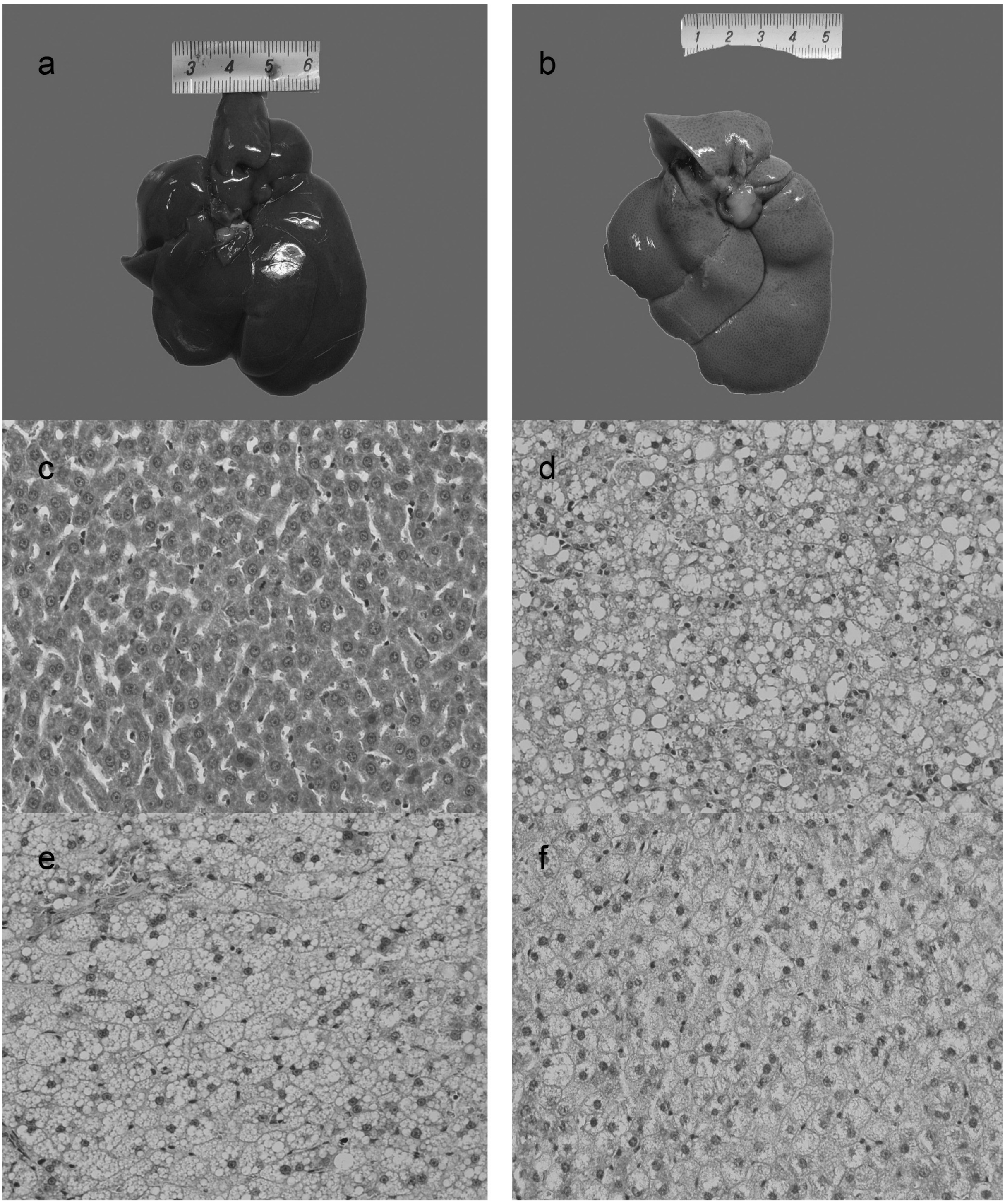

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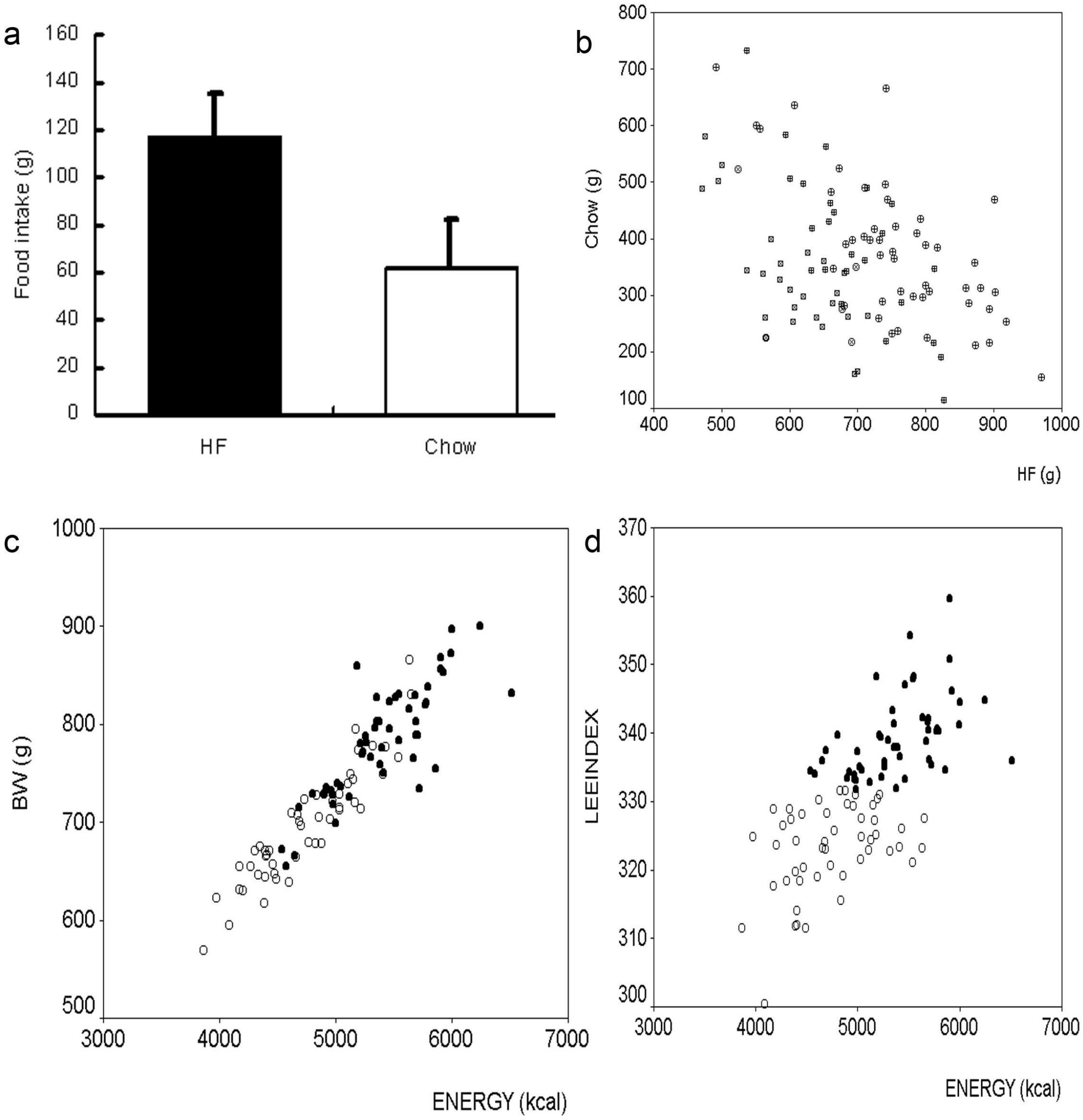

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Food intake (g)
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