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Mice long-term selected for high body mass are more susceptible to body fat deposition in response to a high fat diet due to insufficient increase in heat production

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Abstract

Using a mouse model long-term selected for high body mass (DU6i), we investigated if their higher degree of body fat as compared to unselected controls (DUKsi) was due to a greater fat accumulation, attributable to differences in substrate oxidation in response to a higher fat intake.

We measured energy expenditure (EE) and substrate oxidation by indirect calorimetry at the ages of 42 d and 98 d in response to a fat rich diet compared to a standard diet (F, 20 %; C, 5 % fat) introduced at weaning (21 d). The EE to food energy intake ratio (Q) was calculated and uncoupling protein (UCP1) mRNA expression was analysed in brown adipose tissue in male mice of both strains. The F diet increased body and fat mass in DU6i ($P<0.05$) but not in DUKsi. Energy intake was not influenced by diet in both strains, but EE was lower in DU6i than in controls ($P<0.05$). In contrast to DU6i, fat oxidation was higher in DUKsi mice fed the F diet until the age of 42 d ($P<0.05$). At the age of 42 d, the Q value was lower in DU6i, and higher with F diet irrespective of strain. UCP1 mRNA expression was twice as high in DUKsi as in DU6i ($P<0.05$).

Between 42 d and 98 d of age, DU6i mice were more susceptible to body mass gain and fat deposition in response to the F diet due to insufficient increase in fat oxidation and energy expenditure possibly related to lower UCP1 mRNA expression.

Keywords: body fat, fat diet, high body mass, mouse, indirect calorimetry, UCP1

Introduction

Gain in body weight or body fat might be a consequence of increased food intake, a decline in resting metabolic rate or energy expenditure (EE), lower physical activity, and/or a decreased capacity to oxidise fat. One possible mechanism for high EE is the uncoupling of respiration rate from ATP production, which leads to decreased efficiency in food energy utilisation due to an increased expression of uncoupling protein 1 (UCP1), a protein expressed in brown adipose tissue (BAT) (Klaus 1991, Dulloo & Samec 2001). In mice fed a high fat diet, an increased UCP1 gene expression as compared to low fat controls was observed (Surwit *et al.* 1998, Rippe *et al.* 2000, Hagemann *et al.* 2010). Whether EE is concomitantly altered under

this condition is not clear. Experimental evidence in rats and humans suggests that increased fat intake does not stimulate fat oxidation, and changes in fat oxidation in response to a higher fat intake occur slowly (Schrauwen & Westerterp 2000, Ji & Friedman 2007).

Various polygenic models of obesity in mice such as the New Zealand Obese (NZO) mouse (Crofford *et al.* 1965), and other mouse strains display different susceptibility to body fat deposition in response to high fat diets (fat content 40 % and higher) (West *et al.* 1992, Surwit *et al.* 1995, Tschöp *et al.* 2001, Hu *et al.* 2004, Wagener *et al.* 2006). This indicates the presence of a certain genetic background as well as gene-environmental interactions regulating growth and differentiation, which determine whether an individual becomes large or small, lean or fat (Marti *et al.* 2008). In order to predict long-term breeding responses for polygenic traits in livestock, a mouse strain (DU6i) was developed through long-term selection for high body mass at the Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany (Bünger *et al.* 2001). Its phenotype is characterised by a body weight and a body fat mass of about 2.5 times that of the unselected control strain (Bevova *et al.* 2006, Bünger *et al.* 2001). In addition, these animals also have a greater body length (Langhammer, personal communication). It is currently unknown how sensitive this mouse strain is to a diet rich in fat.

Therefore, the purpose of the present study was to measure body fat mass in this growth-selected mouse model in response to a diet with a moderately high fat content providing 20 % more energy as compared to a control diet. In order to understand the underlying mechanisms for potential differences in body fat we analysed energy intake and expenditure, fat and carbohydrate oxidation, metabolic efficiency and flexibility, as well as gene expression of UCP1 in BAT.

Material and methods

Mouse models, housing and diets

The study was performed in a total n of 64 mice, 8 males and 8 females each of two different mouse strains fed 2 different diets. Mice were investigated at an age of 42 d (n=64) and 98 d (n=56). During the experiment 7 animals died and one became ill. We used mouse strain DU6i which is an inbred derivate from the selection strain DU6 which was selected for high body mass at an age of 42 d for 115 generations, and compared it to its unselected inbred control strain DUKsi. The strains DU6i and DUKsi are bred at the Leibniz Institute for Farm Animal Biology (FBN). The initial population was derived from an original crossing of four outbred (NMRI orig., Han:NMRI, CFW, CF1) and four inbred (CBA/Bln, AB/Bln, C57BL/Bln, XVII/Bln) populations (Schüler 1985, Dietl *et al.* 2004). During the selection the population size in these strains was 80 pairs per generation, and the litter size was standardised at birth to 9 pups and weaning occurred at 21 d.

In the present experiment at weaning age, average body mass of males was 8.4±1.2, and 17.9±0.9 g in DUKsi and DU6i strains, respectively, whereas it was 7.6±2.0, and 18.2±2.7 g in females of DUKsi and DU6i. Mice were housed in Makrolon-cages Type II (Ebeco, Castrop-Rauxel, Germany) (2-4 animals per cage), with wood shavings for bedding, in a semi-barrier system under environmentally controlled conditions with a 12 h light: dark cycle (room temperature 22 °C; humidity 50-60 %). The animals had unlimited access to food and water.

The experimental diets were provided from the day after weaning and composed to contain 50 (control, C) or 200 g fat/kg diet (fat, F) at the expense of starch. Relative protein content was unchanged (Table 1). Diets were provided in pellet form. In the C and F diets, 11.5 % and 37.8 % energy was supplied as fat, respectively. Average apparent digestibilities were determined previously in a balance trial and amounted to 91.3±1.0 % crude protein, 99.3±0.4 % sugar, 99.8±0.3 % starch, and 96.9±0.2 % fat, respectively, and did not differ between strains.

Table 1
Composition and calculated energy content of control or fat diet

Items	Control	Fat
	g/kg dry matter	
Casein	218	218
L-Methionine[1]	5	5
Wheat starch	547	397
Sucrose	50	50
Soy oil	20	80
Coconut fat	30	120
Cellulose	50	50
Vitamin premix[2]	20	20
Mineral premix[3]	60	60
Metabolisable energy (MJ/kg dry matter)	16.3	19.6

[1]L-Methionine (S. A. Ajinomoto, Louvain-la-Neuve, Belgium), [2]Vitamin premix (Altromin, Laage, Germany) provided per kg of diets: Vitamin A: 750 000 IU, vitamin D3: 25 000 IU, vitamin E: 7 500 mg, vitamin K3: 500 mg, vitamin B1: 1 000 mg, vitamin B2: 1 000 mg, vitamin B6: 750 mg, vitamin B12: 1.5 mg, nicotinic acid: 2 500 mg, pantothenic acid: 2 500 mg, folic acid: 500 mg, biotin: 10 mg, cholinchloride: 50 000 mg, P-aminobenzoic acid: 5 000 mg, inosit: 5 000 mg, vitamin C: 1 000 mg, methionine: 173 250 mg, Ca: 503 mg, P: 148 mg, dig. P: 82 mg, Mg: 121 mg, Na: 116 mg, K: 99 mg, S: 37 200 mg, Fe: 2 mg, Mn: 0.3 mg, Zn: 0.9 mg, Cu: 0.3 mg, Se: 0.03 mg, Co: 0.03 mg, Al: 2.6 mg. [3]Mineral premix (Altromin, Laage, Germany) provided per kg of diets: Ca: 146 088 mg, P: 97 356 mg, dig. P: 97 352 mg, Mg: 8 788 mg, Na: 39 232 mg, K: 116 495 mg, S: 10 541 mg, Cl: 63 510 mg, Fe: 2 931 mg, Mn: 1 734 mg, Zn: 388 mg, Cu: 85 mg, I: 7.5 mg, Mo: 3.3 mg, F: 70 mg, Se: 3.8 mg, Co: 2.1 mg, Al: 0.1 mg.

The study was conducted with approval of the Animal Care Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, State Mecklenburg-Vorpommern, Germany (LVL-MV/310-4/7221.3-1.1-018/03).

Body mass and length, and body fatness

Body mass (BM), body fat mass, and body length (nose-anus) were measured at ages of 42 d (end of juvenile phase) and 98 d (fully mature). Body fat mass (expressed as % body mass) was determined by dual-energy x-ray absorption (DEXA) (PIXIMUS, GE Luna Corporation, Madison, WI, USA).

Components of energy expenditure

Energy expenditure of individual mice of the two strains was measured by open-circuit indirect calorimetry at an age of 42 d (n=64) and 98 d (n=56) with ad libitum feed supply. The respiration chambers consisted of transparent plastic cylinders with wire mesh bottoms. The chamber was equipped with a water flask and a hanging basket for feed pellets. Airflow through the chamber was 2.4 l/h, which corresponds to a 3-fold air change per hour. Gas

exchange was measured continuously at 15 min intervals, by infrared absorption based CO_2 and paramagnetic based O_2 gas analysers (Maihak AG, Hamburg, Germany), respectively. Data were collected using Simatic hardware and Win CCâ software (Siemens AG, München, Germany). The respiration chambers were placed inside a climate controlled closet kept at 22 °C. The dark phase was from 18:00 to 06:00.

After recording the body mass at 08:00 mice were placed in the respiration chamber with 15 g feed pellets of the respective diet and 50 ml of water. Gas exchange measurements started at 08:15 h and were terminated at 07:30 the next day. Feed intake in the respiration chamber during the period of EE measurement was recorded as the difference between feed weight before and after the measurement period. EE was calculated according to Brouwer (1965) as

$$EE\,(kJ) = 16.18 \times VO_2 + 5.16 \times VCO_2 - 5.90 \times N_{ex} \quad (1)$$

where VO_2 is the oxygen consumption (l/d), VCO_2 is the CO_2 production (l/d), and N_{ex} is the urinary nitrogen excretion (g/d). Nitrogen excretion was determined previously (Schadereit *et al.* 1997) and set to be 0.06 g/d as an overall mean. EE was calculated as a daily mean.

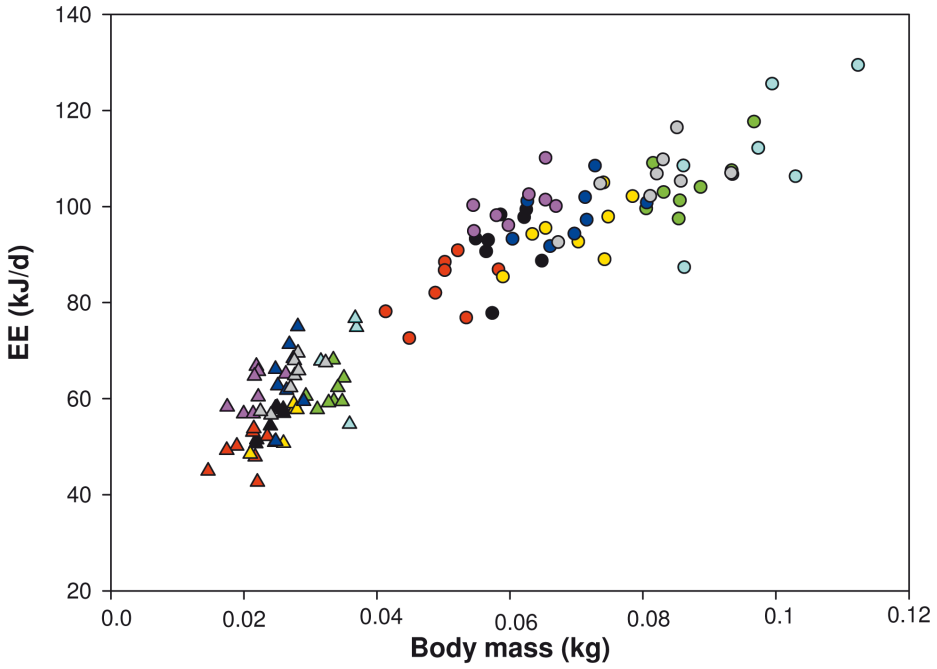

Figure 1
Relationship between energy expenditure (EE) and body mass (BM) for all individual mice fed control (C) or fat diet (F) investigated in this study. Energy expenditure (EE) is a function of body mass and thus independent of body composition. Regression equation is $EE=350\times BM^{0.48}$, $P<0.0001$, $R^2=0.91$.
Triangles represent the DUKsi strain whereas circles indicate the DU6i strain.
Animals fed the C diet: Red: female (f), age 42 d; black: male (m), age 42 d; yellow: f, age 98 d; green: m, age 98 d. Animals fed the F diet: Pink: f, age 42 d; dark blue: m, age 42 d; grey: f, age 98 d; light blue: m, age 98 d.

In order to determine how much food energy is converted to heat, a food energy efficiency ratio relating EE and energy intake (EI) was calculated as Q=EE/EI. The higher the Q value, the higher the relative heat loss and the less energy retained as body mass (protein accretion and/or fat deposition). For Q to be valid, the regression exponent between the dependent variable EI or EE and body mass is required to be in the same order of magnitude, and thus independent of body mass. As expected, EE as well as EI depended on BM (Figure 1). This is reflected by a nonlinear regression of EI or EE on BM.

$$EI = 495.8 \times BM^{0.497} \quad (R^2 = 0.48)$$
$$EE = 350.6 \times BM^{0.482} \quad (R^2 = 0.91) \qquad (2)$$

Net oxidation rates of fat and carbohydrate were calculated in g/d, kJ/(kg$^{0.48}$ × d) as well as kJ/(kg × d) according to Simonson & DeFronzo (1990) as:

$$Fat\ oxidation = 1.69 \times (VO_2 - VCO_2)$$
$$Carbohydrate\ oxidation = 4.57 \times VCO_2 - 3.23 \times VO_2 \qquad (3)$$

Metabolic flexibility, which is defined as the ability of a system to adjust fuel oxidation to fuel availability (Galgani *et al.* 2008), was analysed by the Percentage Relative Cumulative Frequency (PRCF) method as described (Riachi *et al.* 2004). The PRCF indicates how frequent specific respiration exchange ratio (RER=l CO_2/l O_2) values occur in a certain group of individuals.

UCP1 gene expression

Brown adipose tissue (BAT) of male mice was excised at an age of 100 d. Total RNA from subscapular brown fat was extracted using a single-step acid phenol-guanidine protocol (Boeuf *et al.* 2001). In brief, 10 µg of total RNA was separated by electrophoresis in a 1 % agarose gel containing formaldehyde and blotted by capillary transfer to a nylon membrane (Hybond N, Amersham Biosciences, Freiburg, Germany). The blots were probed with ^{32}P labelled probes in a hybridisation solution containing sodium phosphate (0.5 M), EDTA (1 mmol/l), sodium dodecyl sulfate (SDS) (7 %) and bovine serum albumin (1 %) at 63 °C over night, and washed twice with saline sodium citrate (SSC) 2 × SSC–0.1 % SDS for 20 min at room temperature, twice with 0.1 × SSC–0.1 % SDS for 20 min at 42 °C and twice with 0.1 × SSC–0.1 % SDS for 20 min at 63 °C. Samples of all groups were studied on the same gel, and measurements were made in duplicate. An Instant Imager (A202401, Canberra Packard GmbH, Dreieich, Germany) was used for analysis and quantification of radiolabeled signals. For hybridisation complete cDNA probes for rat UCP1 were kindly provided by Daniel Ricquier (CNRS, Paris, France). The molecular weight of the transcript was 1.5 kb.

Statistical analysis

The experiment design was a completely randomised 2 × 2 × 2 factorial arrangement with 2 strains of mice (DU6i, DUKsi), 2 diets (C, F), and 2 sexes (male, female). Data were calculated for groups at ages 42 d and 98 d separately, and results were expressed as least square means LSM±SE. Phenotypic differences among strains and treatments were evaluated using the PROC GLM procedure of SAS (SAS Institute, Inc., Cary, NC, USA). Comparison of means was performed using the Tukey-Kramer test. The significance level was set at $P<0.05$.

Results

Body mass and length, and body fatness

Males and females of the DU6i strain on the C diet were ~2.5 times heavier than the corresponding unselected control strain DUKsi irrespective of age (Table 2). Feeding F diet in DU6i mice resulted in a higher body mass than in corresponding C fed animals ($P<0.05$) at an age of 42 d, whereas body mass in the DUKsi control did not differ (Table 2). In the DU6i strain at an age of 98 d male and female mice consuming the F diet as compared to those on the C diet were 11 and 16 % heavier, respectively ($P<0.05$). Body length did not differ due to diet. However, DU6i compared to DUKsi mice at the age of 42 d and 98 d were longer, respectively (11.1 vs. 8.8 cm; 12.2 vs. 10.5 cm) ($P<0.05$).

Table 2
Body mass (g) and body fat mass (% of body mass) at the age of 42 and 98 d in male and female mice of DUKsi and DU6i strains fed control or fat diet 1-3

	Male			Female		
	Control	Fat		Control	Fat	SE
			Body mass, g			
DUKsi	24.2^{B}	26.6^{B}	42 d	20.1^{B}	21.6^{B}	1.6
DU6i	$59.3^{b,A}$	$69.5^{a,A}$		$50.0^{b,A}$	$61.0^{a,A}$	
DUKsi	33.0^{B}	35.1^{B} (5)	98 d	25.7^{B} (5)	27.2^{B}	2.3
DU6i	$87.0^{b,A}$	$97.0^{a,A}$ (7)		$70.0^{b,A}$ (7)	$81.5^{a,A}$	
			Body fat mass, % of body mass			
DUKsi	6.7^{A}	10.5^{A}	42 d	7.8^{A}	6.9^{A}	1.4
DU6i	$11.0^{a,B}$	$23.6^{b,B}$		$12.4^{a,B}$	$26.4^{b,B}$	
DUKsi	11.6^{A}	15.0^{A} (5)	98 d	6.5^{A} (5)	10.7^{A}	1.6
DU6i	$22.3^{a,B}$	$30.9^{b,B}$ (7)		$23.4^{a,B}$ (7)	$36.0^{b,B}$	

[1]Values are LSM±pooled SE, n=8 per group if not given otherwise in parentheses. Body mass: 42 d: Effect of main factors Strain, Diet and Sex, and interactions ST × D, ST × S were significant ($P<0.05$). 98 d: Effects of main factors Strain, Diet, and interactions ST × D, ST × S were significant ($P<0.05$). Body fat mass, % of body mass: 42 d: Effects of main factors Strain, Diet, and interactions ST × D, ST × S, S × D × S were significant ($P<0.05$). 98 d: Effect of main factors Strain, Diet, and interactions ST × D, ST × S were significant ($P<0.05$). [2]Values in one row within Sex with different superscript lower case letters differ ($P<0.05$). [3]Values in one column within age with different superscript capital letters differ ($P<0.05$).

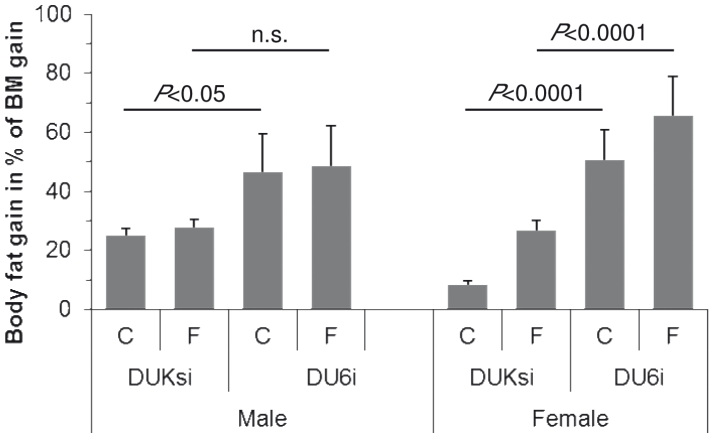

Figure 2
Body fat gain (in % body mass gain) between the age of 42 d and 98 d in DUKsi and DU6i males and females fed control (C) or fat (F) diet (within strain and sex differences between mice fed C and F diets were not significant). Reading example: In male DUKsi mice fed C diet 25 % of the body mass gained between the age of 42 d and 98 d was body fat.

The DU6i strain was always fatter (Table 2). The consumption of F diet between the ages of 21 and 42 d resulted in more than twice the body fat proportion in DU6i males and females, with no such effect in the control strain DUKsi (Table 2). Body mass gain differed among strains between the age of 42 d and 98 d. In the DU6i mice on any diet, about 50 % of the body mass gain was associated to fat whereas in the unselected DUKsi mice body mass gain as fat was between 5-30 % ($P<0.001$) (Figure 2). Female DU6i mice gained more body mass as fat on any diet as compared to DUKsi females ($P<0.05$).

Dietary intake, components of energy expenditure, and UCP1 expression

At an age of 42 d DU6i mice fed the C diet consumed more than twice as much food than the DUKsi strain, whereas at an age of 98 d food intake was about 1.6 fold in DU6i compared to DUKsi (Table 3). We could not find differences in food intake and energy intake between the C and the F diet despite a 20 % higher energy density of the F diet (Table 3). Interestingly, water intake was always lower when mice were fed the F diet (DU6i males, 42 d, $P<0.05$) (Table 3).

Table 3
Food (g), water (ml), energy intake and energy expenditure ($kJ \times d^{-1}$) at the age of 42 and 98 d in male and female mice of DUKsi and DU6i strains fed control or a fat rich diet 1-5

	Male			Female		
	Control	Fat		Control	Fat	SE
			Food intake, $g \times d^{-1}$			
DUKsi	4.0^{A}	3.8^{A}	42 d	3.7^{A}	3.6^{A}	0.3
DU6i	9.3^{B}	8.3^{B}		8.6^{B}	7.8^{B}	
DUKsi	4.2^{A}	4.5 (5)	98 d	4.4^{A} (5)	4.3	0.6
DU6i	7.4^{B}	6.0 (7)		7.9 (7)	7.6	
			Water intake, $ml \times d^{-1}$			
DUKsi	6.6^{A}	4.1	42 d	4.6^{A}	3.9	1.1
DU6i	13.3^{a,B}	7.6^{b}		11.0^{B}	7.4	
DUKsi	6.6	2.8 (5)	98 d	5.6 (5)	4.9	1.0
DU6i	6.9	3.8 (7)		7.9 (7)	4.9	
			Energy intake, $kJ \times d^{-1}$			
DUKsi	66.5^{A}	74.3^{A}	42 d	61.3^{A}	70.1^{A}	5.0
DU6i	153.3^{B}	164.0^{B}		141.8^{B}	153.0^{B}	
DUKsi	68.8^{A}	89.1 (5)	98 d	72.0 (5)	84.2^{A}	10.3
DU6i	121.8^{B}	117.2 (7)		114.2 (7)	149.3^{B}	
			Energy expenditure, $kJ \times d^{-1}$			
DUKsi	54.8^{a,A}	64.5^{b,A}	42 d	49.3^{a,A}	61.9^{b,A}	2.0
DU6i	92.2^{B}	98.5^{B}		82.7^{B}	100.3^{B}	
DUKsi	61.4^{A}	68.5^{A} (5)	98 d	54.8^{A} (5)	64.0^{A}	3.2
DU6i	104.8^{B}	110.7^{B} (7)		95.1^{B} (7)	105.5^{B}	

[1]Values are LSM±pooled SE, n=8 per group if not given otherwise in parentheses. Food intake: 42 d: Effects of main factors Strain, Diet and Sex were significant ($P<0.05$). 98 d: Effect of Strain was significant ($P<0.05$). [2]Energy intake: 42 d: Effects of main factors Strain, Diet and Sex were significant ($P<0.05$). 98 d: Effect of Strain was significant ($P<0.05$). [3]Energy expenditure: 42 d: Effects of main factors Strain, Diet and Sex and interaction D × S were significant ($P<0.05$). 98 d: Effects of main factors Strain, Diet and Sex were significant ($P<0.05$). [4]Values in one row within Sex with different superscript lower case letters differ ($P<0.05$). [5]Values in one column within age with different superscript capital letters differ ($P<0.05$).

As expected, EE was always higher in DU6i mice as compared to the smaller DUKsi controls ($P<0.05$, Table 3). At an age of 42 d DUKsi males and females fed the F diet showed higher EE although energy intake and body mass did not differ (Tables 2 and 3).

Body mass specific fat oxidation (kJ/(kg × d) at an age of 42 d was lower in the larger DU6i mice with the exception of females fed C diet (Table 4). At an age of 98 d only female DU6i mice fed C diet had a lower fat oxidation as compared to DUKi females. In the 42 d-old DUKsi mice, F diet was related to higher fat oxidation per unit body mass but not in DU6i mice ($P<0.05$). At an age of 98 d, females of both strains showed a higher fat oxidation when receiving F diet ($P<0.05$) (Table 4). When fat oxidation was related to body mass raised to 0.48 also in DU6i mice, fat oxidation was higher with F diet than C diet at 42 d. At an age of 42 d carbohydrate oxidation related to body mass (kJ/(kg × d) was lower in DU6i than in DUKsi mice (Table 4).

Table 4
Fat and carbohydrate oxidation rates at the age of 42 and 98 d in male and female mice of DUKsi and DU6i strains fed control or a fat rich diet 1-7

	Male			Female		
	Control	Fat		Control	Fat	SE
			Fat oxidation, kJ/(kg × d)			
DUKsi	479.3^{a,A}	801.2^{b,A}	42 d	364.5^{a}	963.4^{b,A}	47.7
DU6i	202.3^{B}	369.3^{B}		187.1	441.8^{B}	
DUKsi	400.0	498.0 (5)	98 d	274.1^{a,A} (5)	562.3^{b}	53.5
DU6i	241.5	380.0 (7)		75.3^{a,B} (7)	345.9^{b}	
			Carbohydrate oxidation, kJ/(kg × d)			
DUKsi	1 956.2^{A}	1 750.6^{A}	42 d	2 328.7^{A}	2 106.8^{A}	73.3
DU6i	1 494.6^{a,B}	1 148.4^{b,B}		1 440.8^{B}	1 311.0^{B}	
DUKsi	1 664.5^{A}	1 633.8 (5)	98 d	2 058.9^{A} (5)	2 027.7^{A}	70.0
DU6i	1 091.3^{B}	843.6 (7)		1 421.9^{B} (7)	1 065.6^{B}	
			Fat oxidation, kJ/(kg$^{0.48}$ × d)			
DUKsi	69.3^{a}	121.2^{b}	42 d	47.6^{a}	129.9^{b}	9.1
DU6i	46.3^{a}	92.2^{b}		51.4^{a}	103.2^{b}	
DUKsi	68.0	87.5 (5)	98 d	39.6^{a,A} (5)	85.6^{b}	11.0
DU6i	67.4	113.1 (7)		18.0^{a,B} (7)	94.2^{b}	
			Carbohydrate oxidation, kJ/(kg$^{0.48}$ × d)			
DUKsi	282.4^{A}	265.3	42 d	301.8	286.1	9.9
DU6i	343.6^{a,B}	285.6^{b}		302.7	305.1	
DUKsi	281.7	286.3 (5)	98 d	309.2 (5)	310.5	15.6
DU6i	306.4	249.7 (7)		356.4 (7)	287.6	

[1]Values are LSM±pooled SE, n=8 per group if not given otherwise in parentheses. [2]Fat oxidation related to BM: 42 d: Effects of main factors Strain, Diet, and interaction L × D and D × S were significant ($P<0.05$). 98 d: Effects of main factors Strain and Diet and interaction S × D were significant ($P<0.05$). [3]Carbohydrate oxidation related to BM: 42 day: Effects of main factors Strain, Diet and Sex and interactions ST × D were significant ($P<0.05$). 98 d: Effects of main factors Diet, Sex and interactions ST × D were significant ($P<0.05$). [4]Fat oxidation related to BM0.48: 42 d: Effects of main factors Strain, Diet, and interaction S × D were significant ($P<0.05$). 98 d: Effects of main factors Sex and Diet were significant ($P<0.05$). [5]Carbohydrate oxidation related to BM$^{0.48}$: 42 d: Effects of main factor Strain, Diet and interactions ST × S, D × S, ST × D × S were significant ($P<0.05$). 98 d: Effects of main factors Diet, Sex and interactions ST × D were significant ($P<0.05$). 6 Values in one row within Sex with different superscript lower case letters differ ($P<0.05$). [7]Values in one column within age with different superscript capital letters differ ($P<0.05$).

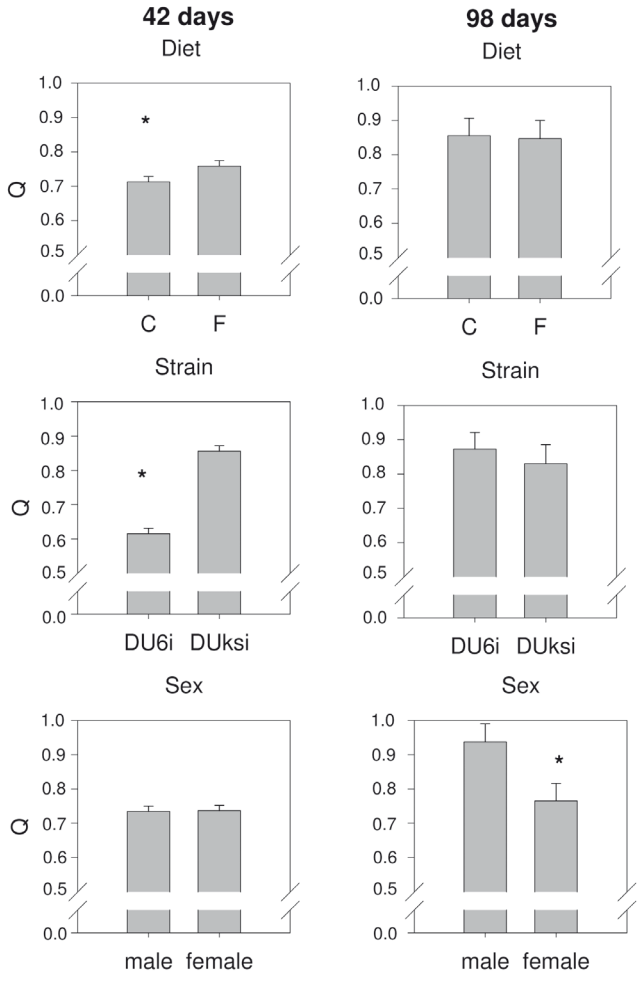

Figure 3
Ratio of energy expenditure relative to the dietary energy intake (Q) of DUKsi and DU6i mice at the age of 42 d and 98 d fed the control (C) or the fat (F) diet. The higher the Q value the higher the relative heat loss. Reading example: In the DUKsi mice at the age of 42 d relatively more energy is lost as heat than in the DU6i mice.

The proportion of food energy converted to heat was expressed by the Q value. At an age of 42 d, Q was higher with the F diet (P=0.045) and in the DUKsi strain (P<0.0001), whereas Q did not differ among sexes and interactions were not significant (Figure 3). At an age of 98 d, we observed a higher Q value for male than female mice (P=0.024). No differences in Q were found between diets and strains at the age of 98 d (Figure 3).

Figure 4 shows PRCF regressed to the RER in mice of both strains fed the C and F diets. The steepness of the curve at the 50th percentile is a measure of metabolic flexibility to switch between fat and carbohydrate oxidation when dietary macronutrient composition is changed. Feeding the F diet resulted in a higher slope in both strains (DUKsi 24.8 (C), 31.6 (F); DU6i 27.6 (C), 45.8 (F); P<0.05) whereby the rise is more pronounced in the fatter animals.

UCP1 mRNA expression in BAT was lower in DU6i than in DUKsi (1.19±0.14 vs. 2.07±0.09 AU; P<0.001) and tended to be higher in DU6i fed F compared to C diet (1.44±0.23 vs. 0.94±0.08; P<0.1) (Figure 5).

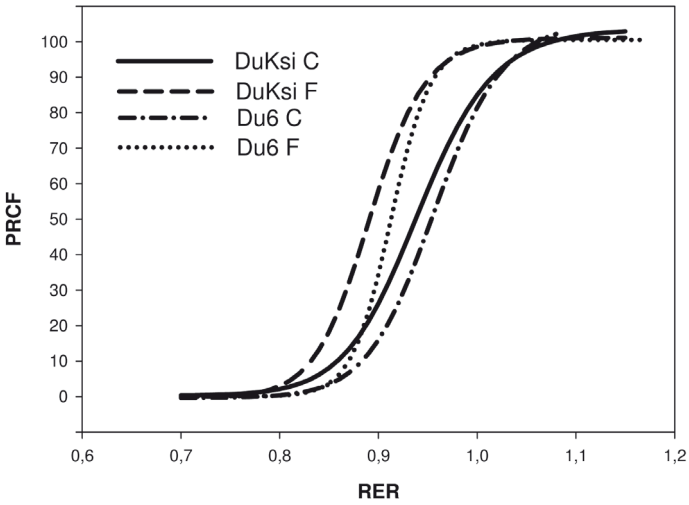

Figure 4
Metabolic flexibility depicted as Percentage Relative Cumulative Frequency (PRCF) values regressed against RER (respiration exchange ratio) of DUKsi and DU6i mice at the age of 42 d fed the control (C) or the fat (F) diet. The steeper the curve at 50 PRCF the lower is the metabolic flexibility. When the level of dietary fat ingested is altered DU6i mice fed the F diet show the least capacity to switch from oxidizing fat to oxidize carbohydrates and vice versa. This was associated to an increased body fat deposition in this group. The greatest metabolic flexibility was observed in DUKsi mice fed C diet.

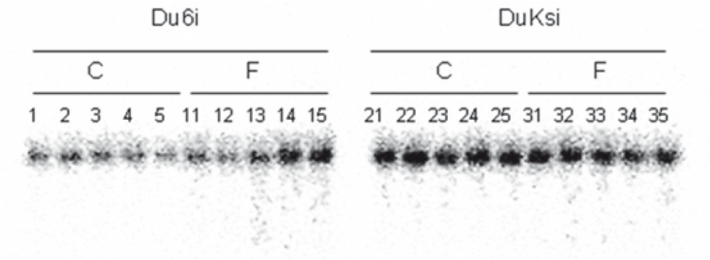

Figure 5
UCP1 mRNA expression (transcript molecular weight 1.5 kb) in brown adipose tissue of DUKsi and DU6i males at the age of 100 d fed the control (C) or the fat (F) diet (LSM±SE: DU6i mice fed C diet: 1.19±0.14; DUKsi mice fed C diet: 2.07±0.09; arbitrary units; $P<0.001$).

Discussion

High fat diets fed to mice have been shown to lead to a higher total energy intake (Lin *et al.* 1979, Rippe *et al.* 2000, Meyer *et al.* 2009). In contrast, our results show that the fat rich diet used here did not lead to hyperphagia within strains, and mice fed F diet adjusted their energy intake to the same level as observed in the C fed mice. This might be related to the only moderately high fat content in our study. In both mouse strains studied here, energy intake with the F diet was not associated to the development of obesity. This is in accordance with observations in human subjects, where increased fat content of the diet showed a consequent change in body fat mass only when total energy intake was increased as well

(Westerterp *et al.* 1996). Interestingly, water intake was lower in mice fed F diet. Since the sodium content in the diets was the same, this could be explained by the known fact that fat oxidation is related to the generation of metabolic water. The complete oxidation of one mole of fatty acid results in the production of 146 moles of metabolic H_2O. Thus, F diet mice partly fuel their water needs from metabolic water produced during fat oxidation.

According to our indirect calorimetry results (Table 3) the reason why DU6i mice are fatter on F diet than on C diet (Table 2) is likely because they are not sufficiently able to increase fat oxidation in adolescent age in response to the higher amount of fat intake. This contrasts with the situation in the unselected control mice (DUKsi) which do not respond to the fat challenge with an increase in body mass and fat proportion due to an increase in both EE and fat oxidation at an age of 42 d. Thus, it appears that a larger fraction of the extra fat consumed is directly incorporated into body fat in DU6i mice. When mice were mature (98 d), the higher fat intake with the F diet led to a higher fat oxidation only in female mice irrespective of mouse strain, which might explain the observation that body fat accretion did not differ between females fed C and F diets.

To compare EE in animals differing in body size or composition, the food energy utilisation ratio Q removes the necessity to normalise EI and EE to body mass with different exponents. The Q value provides information on how much food energy is converted to heat and thus how much energy is available for body mass gain. At an age of 42 d, mice had a slightly higher Q value (Δ=0.046) in response to the F diet indicating a higher EE per unit ingested food energy irrespective of mouse strain and sex. This suggests that a higher fat intake provokes mechanisms preventing excessive deposition of dietary fat during adolescent age. On the other hand, the difference in Q between strains is much larger (Δ=0.24) indicating that the genetic effect due to the selection is greater than the dietary influence.

The lower Q value in DU6i is a possible reason for the different growth rate in the two strains, i.e. a lower relative heat loss, and consequently more energy available for body mass gain. For example, the long-term selected DU6i males were found to grow about 3 times as fast (age 42 d: 1.63 g/d vs. 0.48 g/d) than their unselected counterparts (Renne *et al.* 2003).

Even with the control diet (5 % dietary fat) DU6i mice were already 1.7 times fatter than the unselected control strain at the end of the juvenile phase (Table 2). At this age, body protein content in DU6i was already about twice that of DUKsi (Timtchenko *et al.* 1999) which coheres with the larger body size of these animals. At an age of 98 d, when mice are fully mature, body fat proportion in DU6i males and females was 2.1 and 4.3 times that of the control strain, respectively. Possible reasons for the higher body fat content might be a lower fat oxidation, together with a higher lipogenesis from carbohydrates, since carbohydrate oxidation was found to be lower than in the unselected controls.

UCP1 uncouples activity of oxidative phosphorylation by generation of a proton leak (Nicholls 1984, Klaus *et al.* 1991). As a consequence, respiration rate relative to ATP production can increase, resulting in elevated thermogenesis. Thus, higher UCP1 expression in DUKsi mice coincides with higher Q and fat oxidation values and lower body fat proportion (Figure 2, Table 2). In the DU6i strain, BAT UCP1 mRNA expression tended to be higher with the F diet (Figure 5). This is in accordance with findings in BAT of mice where a dietary fat challenge was associated to a 2-4-fold increase in the expression of UCP1 (Surwit *et al.* 1998, Rippe *et al.* 2000). However, we could not observe that DU6i mice responded to a higher fat intake with

an increase of Q (0.9 vs. 1.1, P=0.73). This might be explained by the fact that UCP1 mRNA expression is not solely responsible for heat loss.

An analysis of RER by the PRCF method (Riachi *et al.* 2004) suggests a lower metabolic flexibility in mice fed the fat-rich diet in both strains (Figure 4). This might be related to the observation that the F diet did not result in a significantly increased UCP1 mRNA level. This counters the proposition that UCP1 plays a role in thermogenic response to a higher fat intake. Also the UCP1 homologues UCP2 and UCP3 have been demonstrated to be higher expressed in white adipose tissue of mice due to high fat feeding but not in skeletal muscle (Surwit *et al.* 1998, Rippe *et al.* 2000, Hagemann *et al.* 2010). Studies with UCP1 ablated mice suggest that UCP1 functions as the only »true« uncoupling protein *in vivo* (Nedergaard *et al.* 2005). Whether UCP2 and UCP3 have an uncoupling effect in the sense of opposing energy accumulation is under discussion (Yonezawa *et al.* 2009) and remains to be investigated in the present mouse model. However, genetic analyses in chromosomal regions harbouring the genes UCP1, UCP2 and UCP3 in crossbred populations with DU6 mice have not shown significant effects on fat deposition (Brockmann *et al.* 1998, Brockmann *et al.* 2000).

In conclusion, we demonstrated here that a mouse strain long-term selected for high body mass (DU6i) develops a high degree of body fat under standard low fat dietary conditions, leading to a 2-3 times higher level of body fat than in their unselected counterparts. In addition, in DU6i mice as compared to the unselected control strain, the intake of a diet moderately higher in fat resulted in higher body fatness due to lower energy expenditure and a lower relative heat loss (not, however, hyperphagia). Underlying mechanisms could be associated with the generally lower UCP1 expression in BAT of DU6i mice and a lower fat oxidation.

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