Early and Late Onset of Voluntary Exercise Have Differential Effects on the Metabolic Syndrome in an Obese Mouse Model

Authors
A. Wagener[1], A. O. Schmitt[1,2], G. A. Brockmann[1]

Affiliations
[1] Department for Crop and Animal Sciences, Humboldt-Universität zu Berlin Berlin Germany
[2] Free University of Bozen/Bolzano Bozen/Bolzano Italy

Key words
- adiposity
- glucose homeostasis
- insulin resistance
- prevention
- running wheel

Abstract

In a mouse model for juvenile obesity, we investigated how the age of onset of voluntary exercise affects factors of the metabolic syndrome. One exercise group had access to running wheels from 3 weeks (representing childhood) and another one from 9 weeks on (early adulthood). Both groups were compared to mice without exercise. The investigations were performed under 2 diets (standard maintenance and high-fat diet). Average daily running activity was independent of diet and exercise. On both diets, mice with exercise from 3 weeks on gained 10 g body weight and 5 g fat mass less than mice without exercise. The highest body weight difference between mice on HFD without exercise and mice on standard maintenance diet with exercise was 24 g. Despite the higher energy expenditure during exercise, young mice did not increase their energy intake adjusted for lean mass, while mice with exercise from 9 weeks had an increased energy intake of 6 kJ per day and therefore could not reduce fat mass on both diets. However, mice with exercise from 9 weeks had better glucose tolerance at 20 weeks than mice with exercise from childhood on. Independently of the age of exercise onset, triglycerides were reduced from 2.4 to 1.7 mmol/l on both diets and insulin levels from 1.5 to 0.3 and 4.5 to 1.8 µg/ml on standard maintenance and high-fat diet, respectively, which represents a considerable improvement. Physical activity seems to have long-lasting effects on body composition and health, but they are different depending on when exercise has begun.

received 27.03.2012
first decision 12.06.2012
accepted 20.06.2012

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1321727
Published online: July 31, 2012
Exp Clin Endocrinol Diabetes

ISSN 0947-7349

Correspondence
G. A. Brockmann
Department for Crop and Animal Sciences Breeding biology and molecular genetics
Humboldt-Universität zu Berlin
Invalidenstraße 42
10115 Berlin
Germany
Tel.: +49/30/2093 6449
Direct Tel.: +49/30/2093 6089
Fax: +49/30/2093 6397
gudrun.brockmann@agrar.hu-berlin.de

Introduction

One strategy to treat obesity and its associated disorders is a low calorie diet in combination with exercise. In general, there is a positive correlation between the fat content of the diet and the body fat content and a negative correlation between the level of physical activity and the body fat content [1]. Meta-analyses of randomized trials have shown that a reduction in dietary fat may cause weight loss [2], whereas increased levels of physical activity have been associated with reduced body fatness [3,4]. Although still controversially discussed, exercise seems to reduce the risk of type 2 diabetes and other features of the metabolic syndrome [5–9]. This further indicates that insufficient physical activity is an important factor for the development of obesity and demonstrates that exercise could contribute to the prevention of the development of metabolic syndrome.

Although early childhood has been considered to be the key time for educating healthy behaviour, most studies addressing physical activity in the context of obesity and diabetes were performed with adult men or laboratory animals. Little is known about the lasting effects of exercise in the childhood on health in adulthood. Studies during the childhood were mainly focused on obesity interventions targeting school-aged children, but they comprised only a specific age and not the lasting effects of physical activity from childhood to adulthood [10–12]. Few studies indicate that childhood physical activity patterns may influence adult physical activity and health [13]. Studies in rat models showed that early-onset exercise is able to prolong obesity resistance [14,15] indicating that exercise could be a helpful instrument for the prevention of obesity and related disorders.

The intention of this study was to compare the effects of exercise on body composition and metabolic syndrome when starting at childhood, as a

prevention strategy, and in adulthood, as a therapeutic strategy. As most types of obesity are genetically influenced [16] we used the Berlin Fat Mouse inbred (BFMI) line as a model that develops obesity and the metabolic syndrome because of its genetic predisposition. BFMI mice are obese as a result of repeated selection for high fatness over many generations. Therefore, they harbour natural mutations causing a higher fat percentage due to hyperphagia, an altered lipid metabolism and impaired insulin sensitivity [17–20]. In this study, we examined the effects of running activity starting at different time points on body weight, food intake, changes in body composition, serum lipids and glucose tolerance. This study is a contribution to evaluate strategies against the development of health risk factors in the light of genetic predisposition for obesity.

Materials and Methods

Animals and diet

All animal treatments were in accordance with the German Animal Welfare Legislation (approval no. G0301/08). After weaning at 3 weeks, males of the BFMI860 line were randomly chosen and fed with either a standard maintenance diet (SMD) or a high-fat diet (HFD). The SMD contained 12.8 MJ/kg metabolizable energy with 9 % of its energy from fat, 33 % from protein content and 58 % from carbohydrates (V1534-000 ssniff R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany), whereas the HFD contained 19.1 MJ/kg metabolizable energy with 45 % of its energy from fat, 24 % from protein content and 31 % from carbohydrates (S8074-E010 ssniff EF R/M, ssniff Spezialdiäten GmbH, Soest, Germany). Animals were single housed and had *ad libitum* access to water and diets. Mice were kept at room temperature (22 °C–24 °C) with a lightdark-cycle of 12 h.

Food intake was estimated as the difference between the offered and the remnant amount based on weekly measurements during the whole experimental period. Food pellets were specifically pressed for low spillage by the food providing company and possible residual spillage was not considered. Total energy intake was determined from the energy content in each diet. Energy intake adjusted for lean mass was taken to compare feed efficiency.

Food and energy intake were calculated on a per-day basis. For the comparison of food and energy intake between different groups, weekly measurements of food intake were averaged over the period between 4 and 9 weeks as well as between 10 and 20 weeks.

Voluntary running activity

Each diet group was divided in 2 exercise groups and a control group. The first group was offered running wheels from 3 and the second group from 9 weeks on. Mice had unrestricted access to the running wheel during the whole experiment until 20 weeks. The control group had no running wheels. Voluntary running activity was recorded with the automatic running wheel system TSE Labmaster (TSE Systems GmbH, Bad Homburg, Germany). Data were collected every 30 min for a period of 48 h from mice at the age of 4, 6, 8, 10, 12, 18 and 20 weeks. Run distance was transformed into km per day (km/d). For the comparison of the running activity between different groups, measurements of the daily run distance (km/d) per mouse were averaged over the period between 4 and 9 weeks as well as between 10 and 20 weeks. The total exercise activity was calculated based upon the average distances of 4–8 and 10–20 weeks multiplied by the number of days with access to the running wheel.

Body composition and serum parameters

To characterize obesity and the metabolic syndrome in BFMI mice under different diet and exercise conditions, we compared body weight, body composition, energy intake, serum lipids and glucose tolerance. To distinguish between the effect of exercise in childhood and adulthood, we divided the investigation period into 2 phases: the growth phase encompassing childhood and adolescence between 3 and 9 weeks and the adult phase from 10 to 20 weeks.

Animals were weighed weekly and subsequently body fat and lean mass were determined in non-anesthetized animals by quantitative magnetic resonance analysis using the EchoMRI whole body composition analyser (Echo Medical Systems, Houston, Texas, USA) [21]. Fat content was calculated as the ratio of body fat mass to body lean mass. At 20 weeks, mice were fasted for 2 h and sacrified to obtain blood, various white adipose tissues, and organs.

Blood glucose concentration was measured at 10 and 20 weeks after a fasting period of 2 h between 6–9 a.m. using the glucose analyser Ascensia Elite (Bayer HealthCare AG, Leverkusen, Germany). Serum triglycerides and total cholesterol were determined at 20 weeks using the Fluitest TG and Fluitest Chol kits (both Analyticon Biotechnologies AG, Lichtenfels, Germany), respectively, and non-esterified free fatty acids were measured using the NEFAHR(2) kit (Wako Chemicals GmbH, Neuss, Germany).

Glucose tolerance was determined with an intraperitoneal (ip) glucose tolerance test after overnight fasting (12–14 h) in week 20. Each animal received a single ip injection of glucose (B. Braun AG, Melsungen, Germany) at a dose of 2 g/kg body weight. For determining glucose concentrations, blood was collected from the tail vein immediately before (0 min), and at 15, 30, 60, and 120 min after ip injection of glucose. Glucose tolerance is expressed as the area under the curve (AUC) of the glucose concentrations between 0–120 min in the glucose tolerance test. At 0 min an additional blood sample was collected to determine fasting insulin. Insulin was measured in 4–5 µl serum samples using a commercial Insulin Mouse Ultrasensitive ELISA Kit (DRG Instruments GmbH, Marburg, Germany). Values below or above the detection level of the standard curve were set to the detection level.

Statistical analyses

ANOVA was performed to evaluate differences between (i) the diets and (ii) between the exercise groups. Longitudinal data for body weight, fat mass and lean mass were subjected to ANOVA with repeated measurements. For the comparison of body composition, serum parameters, food intake and running wheel parameters, the Wilcoxon rank sum test was used. P-values smaller than 0.05 were considered statistically significant. For correlation analyses, Spearman's rank correlation coefficient was applied. The calculations were performed using the R statistical software package [22].

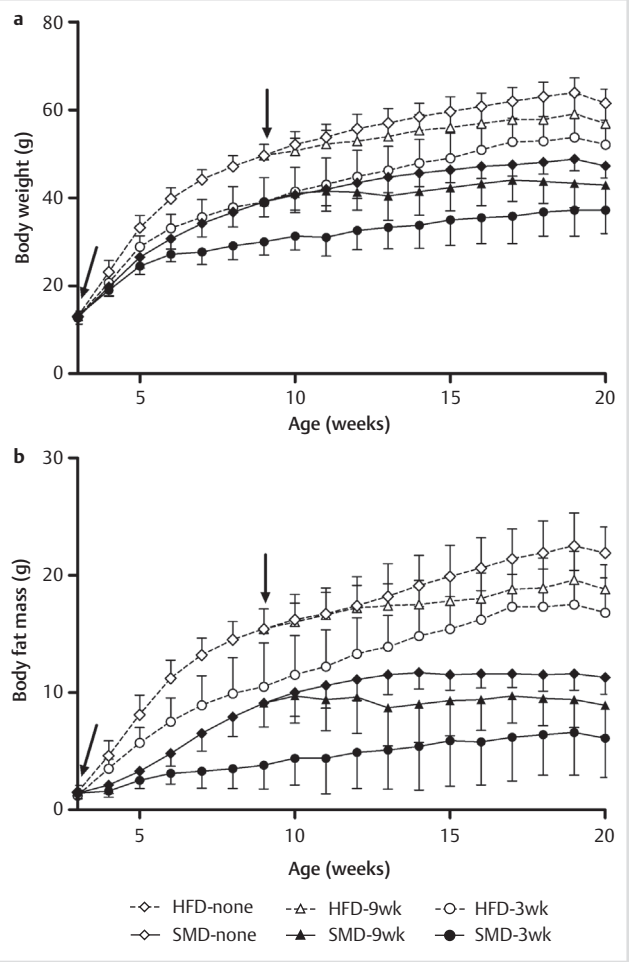

Fig. 1 Body weight **a** and body fat mass **b** of BFMI mice in different diet and exercise regimes from 3–20 weeks of age. Each point represents the mean weight with standard deviation (n = 15–25 per group). The arrows indicate the time points when the running wheel was made available.

Results

Effect of diet on features of the metabolic syndrome

Without exercise BFMI mice developed a marked obesity with the greatest gain of body weight and fat mass in the growth phase between 3 and 9 weeks (**Fig. 1**). On HFD, obesity was even more pronounced than on SMD due to higher energy intake during the whole experimental period ($p < 0.001$). Accordingly, HFD led to increased mass of all white adipose tissues, muscles and most organs and to significantly elevated total serum cholesterol and fasting insulin concentrations at 20 weeks and blood glucose concentrations at 10 weeks (**Table 1**). Serum triglycerides, free fatty acids and blood glucose concentrations at 20 weeks and glucose tolerance were not affected by diet in our model.

Effects of exercise on mice fed a standard maintenance diet

Diet and age of onset of voluntary exercise had no influence on the average daily running activity which was about 2 km per day. In general, exercise from 3 weeks on reduced body weight and fat content compared to no exercise. During the growth phase, mice with exercise from 3 weeks on gained about 10 g less body weight and about 5 g less body fat mass than control mice. In the adult phase, mice with and without exercise from 3 weeks on gained the same weight, thus the differences in body weight and fat mass were maintained until the age of 20 weeks (**Fig. 2**). Voluntary exercise from 9 weeks on stabilized body weight and body fat mass and prevented further increase, but did neither reduce body weight nor body fat mass. Mice with exercise from 9 weeks on gained 5 g less body weight and 3 g less body fat mass than control mice and mice with exercise from 3 weeks on. Likewise, there was a negative correlation between the total run distance and body weight gain ($r = -0.76$; $p < 0.001$) and fat mass gain ($r = -0.71$; $p < 0.001$), respectively, in the growth phase but not in the adult phase. The effect of exercise on lower fat deposition was seen in all white adipose tissues, in particular in subcutaneous, mesenteric and renal, but also in lower organ weights (kidney, liver, pancreas and spleen) (**Table 1**). Weights of the 2 muscle groups, heart and quadriceps, were not affected by exercise. The effect of exercise on total energy intake depended on the age of onset of exercise and differed between the growth and the adult phase. In the growth phase, exercise led to a reduced total energy intake in mice of about 8 kJ per day (correlation between total run distance and total energy intake: $r = -0.44$; $p < 0.01$). Energy intake adjusted for lean mass was the same in the exercise group as in the control group in the growth phase (**Fig. 2**). In the adult phase, exercise from 3 weeks on had no influence on energy intake compared to the control group whereas mice with exercise from 9 weeks on consumed more energy than control mice. In this phase, a weak positive correlation between total run distance and total energy intake was found ($r = 0.32$; $p < 0.01$). Adjusted for lean mass, however, energy intake was increased by 13.9 kJ/d in mice with exercise from 3 weeks on and by 7.6 kJ/d in mice with exercise from 9 weeks on compared to the control group (correlation between total run distance and adjusted energy intake: $r = 0.77$; $p < 0.0001$).

On serum parameters, exercise from 3 weeks on contributed to slightly reduced blood glucose concentrations at 10 weeks and to reduced total serum cholesterol, triglyceride and fasting insulin concentrations of 20 week old mice, but glucose tolerance was not improved. Free fatty acids were not affected by exercise. Exercise from 9 weeks on improved serum triglycerides, fasting insulin and blood glucose concentrations at 20 weeks and glucose tolerance (**Table 1**, **Fig. 3**). Likewise, insulin concentrations were positively correlated with body weight gain ($r = 0.63$; $p < 0.001$) and fat mass gain in the growth phase ($r = 0.61$; $p < 0.001$), whereas blood glucose concentrations at 20 weeks and glucose tolerance (AUC) were only correlated with body weight gain ($r = 0.42$ and $r = 0.46$, respectively; $p < 0.001$) and fat mass gain ($r = 0.36$ and $r = 0.40$, respectively; $p < 0.01$) in the adult phase.

Effects of exercise on mice fed a high-fat diet

Although mice on a HFD were heavier than mice on SMD, the effect of exercise on reduced body weight gain was similar in both regimes. HFD-fed mice with exercise from 3 weeks on gained less body weight and body fat mass than control mice (Δ HFD ~ Δ SMD) in the growth phase, which also had no further effect on weight gain in the adult phase. Likewise, there was a negative correlation between the total run distance and body weight gain ($r = -0.76$; $p < 0.001$) and fat mass gain ($r = -0.46$; $p < 0.001$) in this phase. Again voluntary exercise from 9 weeks on only stabilized body weight. Unlike SMD-fed mice, the body fat content of HFD-fed mice was reduced only until 15 weeks after access to running wheels at 3 weeks. Afterwards, it slowly

Table 1 Body composition in BFMI mice under different conditions of exercise and diet at 20 weeks.

	SMD			HFD			ANOVA effects of		
	3 wk	9 wk	none	3 wk	9 wk	none	exercise	diet	exercise*diet
body weight (g)	37.2 (5.27)^{c}	42.9 (5.58)^{b}	47.2 (2.70)^{a}	52.1 (5.57)^{c*}	56.9 (4.33)^{b*}	61.5 (3.22)^{a*}	<0.001	<0.001	ns
body fat mass (g)	6.1 (3.35)^{c}	8.9 (2.62)^{b}	11.3 (1.45)^{a}	16.8 (2.98)^{b*}	18.8 (2.11)^{b*}	21.9 (2.22)^{a*}	<0.001	<0.001	ns
body lean mass (g)	27.6 (1.97)^{c}	30.5 (2.69)^{b}	32.1 (1.97)^{a}	31.5 (2.76)^{c*}	34.1 (2.24)^{b*}	35.2 (1.77)^{a*}	<0.001	<0.001	ns
total energy intake (kJ/d)	73.6 (5.38)^{b}	79.0 (5.83)^{a}	74.8 (4.66)^{b}	93.3 (7.30)^{a*}	95.0 (7.88)^{a*}	98.2 (9.67)^{a*}	ns	<0.001	ns
adjusted energy intake (kJ/d)	86.7 (5.51)^{a}	82.7 (5.18)$^{(b)}$	78.5 (3.66)^{c}	98.7 (8.56)^{a*}	90.7 (8.33)^{b*}	91.1 (7.82)^{b*}	<0.001	<0.001	ns
weights of adipose tissues									
reproductive adipose tissue (g)	1.34 (0.65)^{b}	1.61 (0.25)^{ab}	1.70 (0.34)^{a}	2.05 (0.50)^{b*}	2.00 (0.33)^{b*}	2.36 (0.36)^{a*}	<0.001	<0.001	ns
renal adipose tissue (g)	0.65 (0.36)^{c}	0.90 (0.27)^{b}	1.20 (0.25)^{a}	1.89 (0.60)^{c*}	2.57 (0.50)^{b*}	3.24 (0.56)^{a*}	<0.001	<0.001	<0.001
subcutaneous adipose tissue (g)	1.23 (0.74)^{c}	1.70 (0.48)^{b}	2.16 (0.54)^{a}	3.64 (0.90)^{c*}	4.36 (0.85)^{b*}	4.86 (0.90)^{a*}	<0.001	<0.001	ns
mesenteric adipose tissue (g)	0.78 (0.59)^{c}	1.22 (0.49)^{b}	1.60 (0.19)^{a}	1.70 (0.31)^{b*}	1.76 (0.26)^{b*}	1.95 (0.29)^{a*}	<0.001	<0.001	<0.01
weights of organs/muscle tissues									
kidney (g)	0.45 (0.04)^{b}	0.49 (0.05)^{ab}	0.52 (0.06)^{a}	0.58 (0.09)^{b*}	0.63 (0.08)^{ab*}	0.67 (0.13)^{a*}	<0.001	<0.001	ns
liver (g)	1.67 (0.25)^{c}	1.94 (0.27)^{b}	2.21 (0.28)^{a}	2.97 (0.77)^{b*}	3.43 (0.72)^{b*}	4.36 (0.82)^{a*}	<0.001	<0.001	0.01
lung (g)	0.17 (0.01)^{a}	0.18 (0.03)^{a}	0.19 (0.03)^{a}	0.19 (0.03)^{a*}	0.21 (0.02)^{a*}	0.21 (0.01)^{a*}	<0.05	<0.001	ns
pancreas (g)	0.35 (0.05)^{b}	0.39 (0.09)^{ab}	0.41 (0.09)^{a}	0.38 (0.06)^{a}	0.43 (0.07)^{a}	0.40 (0.08)^{a}	<0.01	ns	ns
spleen (g)	0.11 (0.05)^{b}	0.13 (0.07)^{ab}	0.16 (0.08)^{a}	0.15 (0.07)^{b*}	0.21 (0.08)^{ab*}	0.21 (0.12)^{a*}	<0.01	<0.001	ns
heart (g)	0.15 (0.02)^{a}	0.15 (0.02)^{a}	0.16 (0.02)^{a}	0.17 (0.02)^{a*}	0.18 (0.02)^{a*}	0.19 (0.02)^{a*}	ns	<0.001	ns
quadriceps (g)	0.38 (0.03)^{a}	0.40 (0.03)^{a}	0.38 (0.03)^{a}	0.44 (0.05)^{a*}	0.42 (0.04)$^{a(*)}$	0.43 (0.03)^{a*}	ns	<0.001	ns
serum parameters									
triglycerides (mmol/l)	1.48 (0.55)^{b}	1.85 (0.78)^{b}	2.49 (0.79)^{a}	1.87 (1.28)$^{(b)}$	1.71 (1.01)$^{(b)}$	2.31 (0.92)^{a}	<0.05	ns	ns
total cholesterol (mmol/l)	2.64 (0.20)^{b}	2.98 (0.45)^{a}	2.93 (0.32)^{a}	4.68 (0.37)^{b*}	4.64 (0.72)^{b*}	5.26 (0.48)^{a*}	<0.001	<0.001	ns
free fatty acids (mmol/l)	0.74 (0.22)^{a}	0.66 (0.15)^{a}	0.69 (0.21)^{a}	0.67 (0.15)^{a}	0.72 (0.09)^{a}	0.76 (0.23)^{a}	ns	ns	ns
glucose at 10 wk (mmol/l)	5.81 (0.97)^{b}	–	6.62 (1.78)^{a}	7.90 (3.75)^{b*}	–	12.2 (5.59)^{a*}	<0.001	<0.001	<0.05
glucose at 20 wk (mmol/l)	6.39 (1.64)^{ab}	5.87 (1.72)^{b}	8.23 (3.87)^{a}	7.87 (2.18)^{a*}	6.53 (2.13)$^{(b)}$	8.62 (3.83)^{a}	<0.01	ns	ns
AUC (mol/l*120 min)	1.78 (0.83)^{a}	1.12 (0.58)^{b}	2.02 (0.65)^{a}	1.98 (0.65)^{a}	1.23 (0.60)^{b}	2.02 (0.62)^{a}	<0.001	ns	ns
fasting insulin (µg/ml)	0.3 (0.4)^{b}	0.4 (0.2)^{b}	1.5 (0.6)^{a}	1.6 (1.1)^{b*}	2.0 (1.2)^{b*}	4.5 (1.9)^{a*}	<0.001	<0.001	<0.05

Values of the traits are means with standard deviations in parentheses. Means of exercise groups within each diet sharing a common character in their superscript are not significantly different ($p > 0.05$). * indicates significant differences between standard and high-fat diet within each exercise group. (*) $P = 0.07$ between SMD and HFD. (b) $P = 0.08$ between exercise groups. Serum concentrations were obtained after a fasting period of 2 h (n = 11–13 per group for triglycerides, total cholesterol and non-esterified free fatty acids, n = 15–25 per group for glucose concentrations), except for insulin and the AUC which were measured after overnight fasting (n = 11–22 per group). AUC from glucose concentrations in the glucose tolerance tests between 0–120 min. SMD – standard maintenance diet; HFD – high-fat diet; AUC – area under the curve; ns – not statistically significant

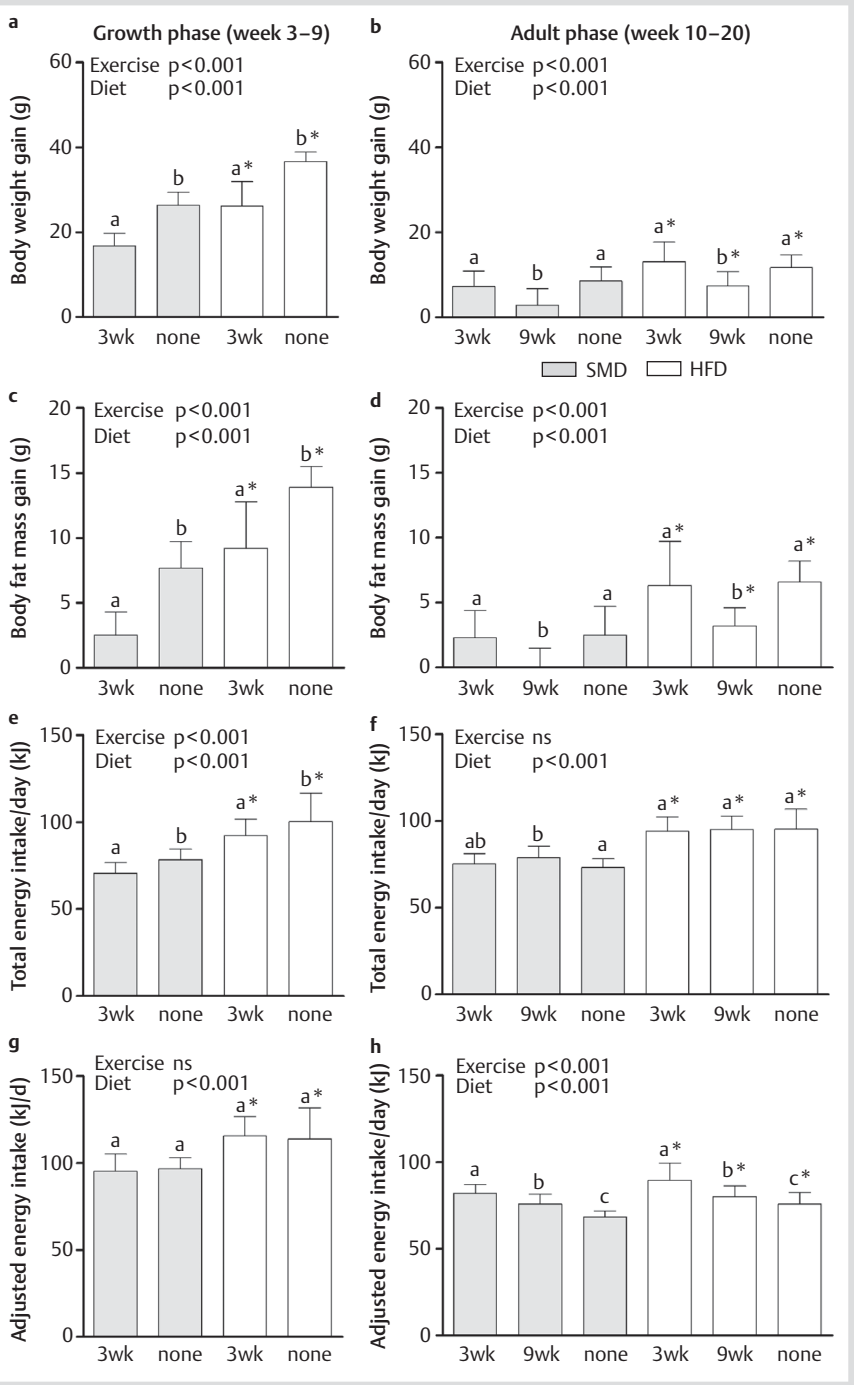

Fig. 2 Body weight gain **a/b**, body fat mass gain **c/d**, total energy intake **e/f** and energy intake adjusted for lean mass **g/h** of BFMI mice under different diet and exercise conditions during the growth phase encompassing weeks 3–9 **a, c, e, g** and adult phase encompassing weeks 10–20 **b, d, f, h**.

increased until it reached the same fat content as mice with exercise from 9 weeks on (**Fig. 1**). Although organ weights and concentrations of serum parameters were generally higher in HFD-fed mice, subcutaneous and renal adipose tissue, kidney and liver weights profited from exercise as in SMD-fed mice. Pancreas, heart and quadriceps were not affected by exercise in HFD-fed mice.

As on SMD, exercise led to reduced total energy intake in the growth phase (Δ HFD ~ Δ SMD) whereas energy intake adjusted for lean mass did not differ (**Fig. 2**). In the adult phase, the exercise groups did not differ in their total energy intake, whereas energy intake adjusted for lean mass was increased in mice with exercise from 3 weeks on by 13.7 kJ/d and in mice with exercise from 9 weeks by 4.5 kJ/d compared to the control group (correlation between total run distance and adjusted energy intake: $r = 0.54$; $p < 0.0001$).

On serum parameters, exercise from 3 weeks on and from 9 weeks on contributed to reduced total serum cholesterol, triglyceride and fasting insulin concentrations at 20 weeks, whereas free fatty acids were not affected. As in SMD-mice, glucose concentrations at 10 weeks and insulin concentrations were significantly improved by exercise (**Table 1**). In particular, the insulin concentrations were half as high with exercise as without. Glucose clearance was similar in all exercise groups on HFD as on SMD, with an improved glucose clearance only in mice exercising from 9 weeks on. But fasting serum insulin concentrations on HFD were 3–5 times as high as on SMD. As in SMD-fed mice, insulin concentrations were positively correlated with body

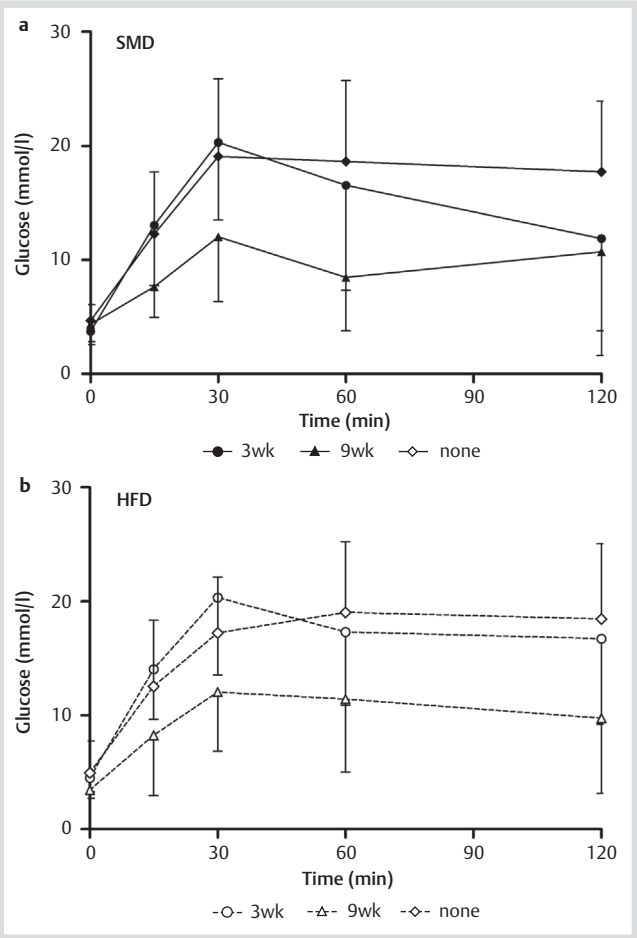

Fig. 3 Glucose concentrations over 120 min from a intraperitoneal glucose tolerance tests in overnight fasted BFMI mice on a standard maintainance (SMD) **a** and high-fat diet (HFD) **b** in week 20.

weight gain ($r = 0.67$; $p < 0.001$) and fat mass gain in the growth phase ($r = 0.72$; $p < 0.001$), while blood glucose concentrations at 20 weeks and glucose tolerance (AUC) were correlated with gains of body weight ($r = 0.44$ and $r = 0.38$; $p < 0.01$) and fat mass ($r = 0.42$ and $r = 0.49$; $p < 0.001$) in the adult phase.

Discussion

The purpose of this study was to test the impact of the age of onset of voluntary exercise as a strategy to prevent or to improve features of the metabolic syndrome. As a model the high-fatness selected BFMI line was used, which shows juvenile obesity, reduced insulin sensitivity, and impaired fat metabolism as components of the metabolic syndrome on SMD [17,18,20]. On HFD, BFMI mice gained additional weight and features of the metabolic syndrome were even more pronounced.

Although the daily running activity of BFMI mice was relatively low compared to many other mouse lines [23–27], exercise led to reduction of weight gain; and features of the metabolic syndrome were improved on both diets. The earlier the exercise started the lower the body weight was at 20 weeks of age. Exercise did not necessarily reduce body weight, but could stop further fat mass gain. Consistent with exercise effects in agouti and MC4R KO mice, most fat was reduced in subcutaneous adipose tissue [23,28]. The best outcome was observed, if voluntary exercise started in childhood in combination with SMD. Mice on HFD without running wheels and mice on SMD with running wheels from 3 weeks on had 61.5 ± 3.22 g and 37.2 ± 5.27 g body weights, respectively, which is a huge difference of 24.3 g, although even mice on SMD and running wheels were still obese. For comparison, C57BL/6NCrl males weighed 27.5 g ± 1.6 g on SMD and 33.5 g ± 2.7 g on HFD at 20 week in our experimental animal unit (unpublished data). 28 mouse strains that were maintained under a standard diet (4.4% fat) had an averaged body weights of 30.0 ± 5.4 g at the age of 28 weeks [29].

Interestingly, exercise of BFMI mice during the major growth phase between 3 and 9 weeks reduced food intake, while exercise beginning in early adulthood was accompanied by higher food intake compared to control mice without exercise. This observation is in line with a study with genetically obese rats, where young exercising animals ate less than controls [15], and studies with exercising adult mice whose food intake was increased in response to running wheel activity [23,27,28,30]. Our data suggest that energy intake adjusted for lean mass was independent of exercise in young BFMI mice, while in the older mice energy intake adjusted for lean mass was increased with higher energy expenditure due to more running wheel activity. In BFMI mice on HFD, exercise from childhood on could slow down the genetically determined juvenile obesity, in particular until 10 weeks, but afterwards weight gain could not be stopped. In contrast, running activity from 9 weeks on prevented further body weight gain. As a result, mice on HFD with exercise either from childhood or from early adulthood on had the same fat content at 20 weeks, and, albeit their fat contents were still high, exercise could improve fasting insulin concentrations in both groups.

Exercise from childhood on could not only dramatically reduce the excessive fat mass gain but also suppress lipid dysregulation and hyperinsulinemia. However, the positive effects on health dependent on the diet. Mice on running wheels had improved serum triglyceride and cholesterol concentrations compared to diet matched controls. These findings are consistent with exercise effects in agouti and MC4R KO mice [23,27,28].

The reduced fat mass gain in exercising BFMI mice was associated with normal circulating insulin concentrations. These effects were also observed in genetically and diet-induced obese rat models [14,15]. However, in other studies with obese mouse lines, where exercise began after puberty, the genetic effect for obesity could only be overridden in MC4R KO mice, which showed reduced fat mass gain as well as insulin, glucose and cholesterol concentrations in response to exercise [23,28,30].

Surprisingly, glucose homeostasis in BFMI mice was not directly linked to energy expenditure. Glucose homeostasis in BFMI mice depended on both age of onset of exercise and duration of exercise. Albeit on both diets, mice had lower insulin concentrations the more they ran, glucose clearance at 20 weeks was only improved in mice that started exercise at 9 but not at 3 weeks. Even mice on HFD with exercise from 9 weeks on, which had much higher fat deposition than SMD-fed mice, showed a better glucose clearance at 20 weeks than mice on SMD and exercise from childhood on. These data suggest that glucose clearance that was improved by exercise during childhood could disappear at a higher age. Causes that could account for the loss of positive effect of exercise on glucose homeostasis that mice have during the young age could include (1) adaptation to the higher energy expenditure during exercise or (2) independent regulatory pathways that affect glucose homeostasis during different phases of

aging. For example, prolonged exercise leads to an adaptation response in muscle which involves morphological changes, metabolic responses as well as an increase in the oxidative capacity [31]. Since oxidative stress stimulates muscle glucose uptake [32] it could be speculated that an increase in oxidative stress capacity could contribute to the adaptation of the body to exercise followed by a reduced glucose tolerance. In BFMI mice, different genes are responsible for obesity in early and later age. Linkage analysis provided evidence that juvenile obesity is the result of a major gene defect while weight gain in early adulthood is caused by additional genetic factors [19]. Thus, the genetic predisposition of the body to adapt to different exercise and diet conditions changes with age.

In summary, our study provides a complex picture of different effects on the metabolic syndrome as a result of voluntary exercise from either childhood or early adulthood on. Our data suggest that energy intake in response to energy expenditure by exercise is differently regulated in the juvenile growth and the adult phase and may depend on the developmental stage of the organism and the age dependent action of genetic factors. Mechanisms that regulate glucose homeostasis act in part independently of the body fat content. Basal fasting serum insulin concentrations seem to depend on adaptations or previous body weight changes. Thus, physical activity in childhood has longlasting effects on body composition and health in adulthood, but they differ from those of exercise beginning at adulthood.

Acknowledgement

This research was supported by grants from the German National Genome Research Network (NGFN: 01GS0829).

Conflict of Interest: The authors declare that they have no conflict of interests.

References

1 *Astrup A*. Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. Public Health Nutr 2001; 4: 499–515
2 *Astrup A*, *Grunwald GK*, *Melanson EL et al.* The role of low-fat diets in body weight control: a meta-analysis of ad libitum dietary intervention studies. Int J Obes Relat Metab Disord 2000; 24: 1545–1552
3 *Leskinen T*, *Sipilä S*, *Alen M et al.* Leisure-time physical activity and high-risk fat: a longitudinal population-based twin study. Int J Obes (Lond) 2009; 33: 1211–1218
4 *Waller K*, *Kaprio J*, *Kujala UM*. Associations between long-term physical activity, waist circumference and weight gain: a 30-year longitudinal twin study. Int J Obes (Lond) 2008; 32: 353–361
5 *Carnethon MR*, *Gidding SS*, *Nehgme R et al.* Cardiorespiratory fitness in young adulthood and the development of cardiovascular disease risk factors. JAMA 2003; 290: 3092–3100
6 *Ekelund U*, *Franks PW*, *Sharp S et al.* Increase in physical activity energy expenditure is associated with reduced metabolic risk independent of change in fatness and fitness. Diabetes Care 2007; 30: 2101–2106
7 *Laaksonen DE*, *Lindström J*, *Tuomilehto J et al.* Increased physical activity is a cornerstone in the prevention of type 2 diabetes in high-risk individuals. Diabetologia 2007; 50: 2607–2608
8 *Lakka TA*, *Laaksonen DE*. Physical activity in prevention and treatment of the metabolic syndrome. Appl Physiol Nutr Metab 2007; 32: 76–88
9 *Yates T*, *Khunti K*, *Bull F et al.* The role of physical activity in the management of impaired glucose tolerance: a systematic review. Diabetologia 2007; 50: 1116–1126
10 *Campbell KJ*, *Hesketh KD*. Strategies which aim to positively impact on weight, physical activity, diet and sedentary behaviours in children from zero to five years. A systematic review of the literature. Obes Rev 2007; 8: 327–338
11 *Hesketh KD*, *Campbell KJ*. Interventions to prevent obesity in 0-5 year olds: an updated systematic review of the literature. Obesity (Silver Spring) 2010; 18 (Suppl 1): S27–S35
12 *Olstad DL*, *McCargar L*. Prevention of overweight and obesity in children under the age of 6 years. Appl Physiol Nutr Metab 2009; 34: 551–570
13 *Leonard WR*. Assessing the influence of physical activity on health and fitness. Am J Hum Biol 2001; 13: 159–161
14 *Patterson CM*, *Dunn-Meynell AA*, *Levin BE*. Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. Am J Physiol Regul Integr Comp Physiol 2008; 294: R290–R301
15 *Schroeder M*, *Shbiro L*, *Gelber V et al.* Post-weaning voluntary exercise exerts long-term moderation of adiposity in males but not in females in an animal model of early-onset obesity. Hormones and Behavior 2010; 57: 496–505
16 *Barsh GS*, *Farooqi IS*, *O'Rahilly S*. Genetics of body-weight regulation. Nature 2000; 404: 644–651
17 *Wagener A*, *Schmitt AO*, *Aksu S et al.* Genetic, sex, and diet effects on body weight and obesity in the Berlin Fat Mouse Inbred lines. Physiol Genomics 2006; 27: 264–270
18 *Hantschel C*, *Wagener A*, *Neuschl C et al.* Features of the metabolic syndrome in the Berlin Fat Mouse as a model for human obesity. Obes Facts 2011; 4: 270–277
19 *Neuschl C*, *Hantschel C*, *Wagener A et al.* A unique genetic defect on chromosome 3 is responsible for juvenile obesity in the Berlin Fat Mouse. Int J Obes (Lond) 2010; 34: 1706–1714
20 *Meyer CW*, *Wagener A*, *Rink N et al.* High energy digestion efficiency and altered lipid metabolism contribute to obesity in BFMI mice. Obesity (Silver Spring) 2009; 17: 1988–1993
21 *Tinsley FC*, *Taicher GZ*, *Heiman ML*. Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. Obes Res 2004; 12: 150–160
22 *RDC Team*. R: A Language and Environment for Statistical Computing. In: Vienna, Austria: R Foundation for Statistical Computing, 2011
23 *Haskell-Luevano C*, *Schaub JW*, *Andreasen A et al.* Voluntary exercise prevents the obese and diabetic metabolic syndrome of the melanocortin-4 receptor knockout mouse. FASEB J 2009; 23: 642–655
24 *Leamy LJ*, *Pomp D*, *Lightfoot JT*. Genetic variation for body weight change in mice in response to physical exercise. BMC Genet 2009; 10: 58
25 *Lightfoot JT*, *Turner MJ*, *Daves M et al.* Genetic influence on daily wheel running activity level. Physiol Genomics 2004; 19: 270–276
26 *Nehrenberg DL*, *Hua K*, *Estrada-Smith D et al.* Voluntary exercise and its effects on body composition depend on genetic selection history. Obesity (Silver Spring) 2009; 17: 1402–1409
27 *Simoncic M*, *Horvat S*, *Stevenson PL et al.* Divergent physical activity and novel alternative responses to high fat feeding in polygenic fat and lean mice. Behav Genet 2008; 38: 292–300
28 *Chiu S*, *Fisler JS*, *Espinal GM et al.* The yellow agouti mutation alters some but not all responses to diet and exercise. Obes Res 2004; 12: 1243–1255
29 *Bachmanov AA*, *Reed DR*, *Beauchamp GK et al.* Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav Genet 2002; 32: 435–443
30 *Mayer J*, *Marshall NB*, *Vitale JJ et al.* Exercise, food intake and body weight in normal rats and genetically obese adult mice. Am J Physiol 1954; 177: 544–548
31 *Yan Z*, *Okutsu M*, *Akhtar YN et al.* Regulation of exercise-induced fiber type transformation, mitochondrial biogenesis, and angiogenesis in skeletal muscle. J Appl Physiol 2011; 110: 264–274
32 *Higaki Y*, *Mikami T*, *Fujii N et al.* Oxidative stress stimulates skeletal muscle glucose uptake through a phosphatidylinositol 3-kinase-dependent pathway. Am J Physiol Endocrinol Metab 2008; 294: E889–E897