Journal of Developmental Origins of Health and Disease (2018), **9**(1), 63–76.

doi:10.1017/S2040174417000617

ORIGINAL ARTICLE

Exercise during pregnancy and its impact on mothers and offspring in humans and mice

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Exercise during pregnancy has beneficial effects on maternal and offspring's health in humans and mice. The underlying mechanisms remain unclear. This comparative study aimed to determine the long-term effects of an exercise program on metabolism, weight gain, body composition and changes in hormones [insulin, leptin, brain-derived neurotrophic factor (BDNF)]. Pregnant women ($n = 34$) and mouse dams ($n = 44$) were subjected to an exercise program compared with matched controls (period I). Follow-up in the offspring was performed over 6 months in humans, corresponding to postnatal day (P) 21 in mice (period II). Half of the mouse offspring was challenged with a high-fat diet (HFD) for 6 weeks between P70 and P112 (period III). In period I, exercise during pregnancy led to 6% lower fat content, 40% lower leptin levels and an increase of 50% BDNF levels in humans compared with controls, which was not observed in mice. After period II in humans and mice, offspring body weight did not differ from that of the controls. Further differences were observed in period III. Offspring of exercising mouse dams had significantly lower fat mass and leptin levels compared with controls. In addition, at P112, BDNF levels in offspring were significantly higher from exercising mothers while this effect was completely blunted by HFD feeding. In this study, we found comparable effects on maternal and offspring's weight gain in humans and mice but different effects in insulin, leptin and BDNF. The long-term potential protective effects of exercise on biomarkers should be examined in human studies.

Received 2 January 2017; Revised 29 June 2017; Accepted 6 July 2017; First published online 7 August 2017

Key words: brain-derived neurotrophic factor, comparative study, high-fat feeding, leptin, physical activity

Introduction

Worldwide, increasing rates of non-communicable diseases (NCD) due to a sedentary lifestyle and high caloric diets have been reported.[1] The importance of factors in preventing NCDs, particularly in the external environment have been reported in early life.[2] A healthy and active lifestyle, which promotes a balance between energy intake and expenditure has several beneficial effects even during pregnancy.[3,4] Several studies have emphasized the benefits of maternal exercise on mothers and their offspring in mice and humans, such as reduced risk of excessive weight gain or improved glucose metabolism.[3,5–7]

However, to date, the underlying mechanisms have not been identified. Key hormones in this context are insulin and leptin as well as brain-derived neurotrophic factor (BDNF), which are involved in the regulation of metabolic homeostasis, food intake control, cognitive performance and also in pathologies such as the development of insulin resistance, metabolic syndrome and gestational diabetes mellitus (GDM).[8] Accordingly, these hormones are affected via lifestyle factors.

Besides reduced maternal leptin and fasting insulin levels,[9,10] regular physical activity during pregnancy increases levels of BDNF, stimulating neurogenesis and improving spatial learning and mental performance in the offspring.[11–17] Furthermore, Aksu *et al.*[16] demonstrated that maternal exercise during gestation might protect the pups from anxiety in later life and resulted in elevated BDNF levels in the cortex. However, the effects of maternal exercise on BDNF serum levels in dams and offspring as well as BDNF levels in the adult offspring after they were exposed to different lifestyle diets have not been reported to date. Little is known in this field regarding humans. Vega *et al.*[13] found that short bouts of high-intensity exercise increased the serum concentration of BDNF during late pregnancy in women. With regard to long-term effects, Clapp[18] demonstrated that 5-year-old children from mothers who exercised regularly during pregnancy had improved intelligence scores. However, BDNF was not measured in that study.

Therefore, the purpose of our study was to examine the influence of exercise during pregnancy on maternal body composition, insulin, leptin and BDNF levels as well as on offspring's body composition up to the end of the weaning period in humans and mice (short-term and mid-term effects).

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Long-term effects in the mouse were monitored to estimate potential effects of maternal exercise on adult offspring's body composition and hormones.

In details, we conducted a comparative study in which human and animal models were synchronized according to study design, age and serum parameters. Our hypotheses were three-fold. First, we hypothesized that maternal exercise during pregnancy exerts similar effects in mice and humans regarding body weight gain and serum levels of insulin, leptin and BDNF (period I). Second, we hypothesized that maternal exercise during pregnancy exerts similar effects in mice and humans regarding body weight gain in the offspring (period II). Third, we hypothesized that sedentary offspring from mothers who exercised are protected from the deleterious effect of high-fat feeding during adulthood (period III).

Materials and methods

The study was carried out by the German Sport University Cologne, the Cologne Center for Prevention in Childhood and Youth and the University Hospital of Cologne. It was approved by the Ethical Committee of the German Sport University and the University Hospital of Cologne and performed according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). Participants in the human study provided informed written consent and they received no financial compensation.

The mouse study was ethically approved by the appropriate governmental authority (institutional protocol number of the animal welfare application: AZ 8.87-50.10.37.09.292, Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Germany) and was in accordance with the German Animal Welfare Law. Animal care and use was performed by qualified individuals and supervised by a veterinarian. The manuscript complies with the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines.[19]

Timeline for the human and mouse studies

The translational approach in this study evaluates different time periods (Fig. 1a and 1b).

Period I: pregnancy (maternal data)

In this study, pregnant women were either subjected (*Maternal-INT$_h$*) or not subjected (*Maternal-CO$_h$*) to an exercise intervention during pregnancy. Human maternal data during pregnancy were evaluated during the 14th week of gestation (T0) and at the 36th week of gestation (T1). Female mice were either subjected (*Maternal-INT$_m$*) or not subjected (*Maternal-CO$_m$*) to an exercise group in which voluntary wheel running intervention was performed during pregnancy. Maternal mice data were collected from gestational day (G)0 (T0) until G18 with serum analysis at G16 (T1).

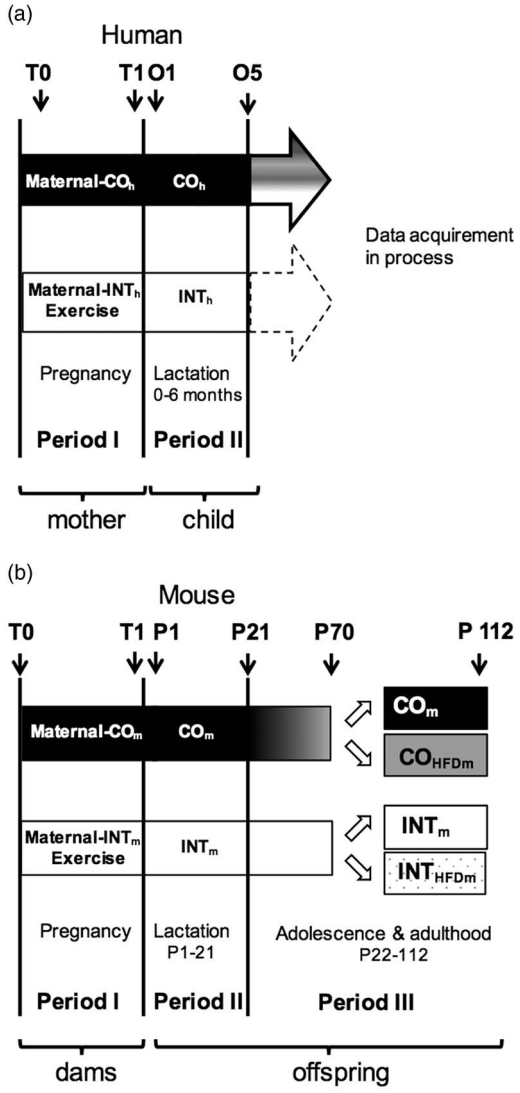

Fig. 1. Experimental design. (*a*) Human study. Period I: pregnant women were either subjected (*Maternal-INT$_h$*) or not subjected (*Maternal-CO$_h$*) to exercise intervention during pregnancy (T0-T1). Period II: offspring were examined five times between birth and 6 months of age (O1–O5). (*b*) Mouse study. Period I: female mice were either subjected (*Maternal-INT$_m$*) or not subjected (*Maternal-CO$_m$*) to an exercise group in which voluntary wheel running intervention was performed during pregnancy (G0–G18). Period II: mothers were fed a standard diet during the lactation period and offspring received same standard diet after weaning; 50% of offspring were sacrificed at P21. Period III: up to P70, offspring continued receiving a standard diet. Afterwards, half of the offspring received HFD over a period of 6 weeks. Notably, only male offspring were taken for analysis. CO, control; INT, running/exercise intervention during pregnancy; HFD, high-fat diet; h, human; m, mouse; P, postnatal day; O, postnatal examination timepoint for children.

Period II: recommended lactation period (offspring data)

Human offspring were examined five times between birth and 6 months of age, since the average lactation period for

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

humans is about 6 months (O1–O5) (Fig. 1a). In the murine model, this period corresponds to 3–4 weeks, that is postnatal day (P) 21 to P28.[20] By calculating 21 days for the weaning period this reveals: 180 days (~6 months): 21 = 8.571 human days = 1 mouse day. For this developmental phase of the lactation period, the timepoint of 6 months (O5) in our human study corresponds with the timepoint P21 in our murine model[20] (Fig. 1a and 1b). During the lactation period, dams were fed a standard diet, which the offspring also received after weaning. At P21, 50% of male offspring were sacrificed.

Period III: adulthood in the mouse study (offspring data)

Our study provides information about long-term effects of maternal exercise on mouse offspring up to P112. To calculate the comparative age from mice to humans, different calculation factors for adolescence and adulthood have to be considered. At P28, first signs of puberty are visible in mice.[20] A total of 3.65 mouse days up to P70 equals 1 human year. At P70, the average mouse is sexually mature. Therefore, P70 is defined as the beginning of adulthood in mice.[20] For the calculation of the comparative age for adult mice, Dutta and Sengupta[20] calculated that 2.6 mouse days corresponds to 1 year in humans. Thus, to calculate the comparative age of P112 in human age, we need to sum:

P1–P21 ~ 6 months
+ P22 to P69 ~ 11.5 years
+ P70 to P112 ~ 16 years
= P112 equals an age of 28 years (adapted according to the recommendations of Dutta and Sengupta[20]).

Up to P70, offspring continued receiving a standard diet. Afterwards, 50% of the offspring received high-fat diet (HFD) over a period of 6 weeks. Notably, only male offspring were taken for analysis.

Study collective and outcome measures: human study

Pregnant women were recruited when they first contacted their gynecologist at the beginning of their pregnancy between March, 2012 and May, 2013. In total, 34 women were enrolled in the study. Women with any contraindication, as described in the American College of Obstetricians and Gynecologists guidelines,[21] the inability to speak German, multiple pregnancy, younger than 18 years and a high-risk pregnancy were excluded. A total of 19 women were enrolled in the intervention group (*Maternal-INT$_h$*) and 15 women served as a control group (*Maternal-CO$_h$*). The changes in anthropometric data of the population at the beginning of pregnancy (14th week of gestation, T0) and at the end of gestation (36th week of gestation, T1) are shown in Table 1.

Detailed descriptions of human anthropometric measurements are shown in Supplemental Material and Methods as well as in Supplementary Tables S1–S3.

Maternal-INT$_h$

From the 14th week up until at least the 30th week of gestation, women carried out the supervised exercise program twice a week for 60 min. The exercise program complied with the international guidelines of physical activity during pregnancy (ACOG[22]), as aerobic and strength-conditioning exercises at a moderate intensity during pregnancy are highly recommended. Therefore, the 1st day consisted of moderate-intensity aerobic exercises such as Nordic walking or walking. The 2nd day consisted of moderate-intensity strength-training on different machines targeting major muscle groups in the upper body, trunk and lower body. In addition, women were encouraged to engage in leisure-time activities to reach the recommended level of 150 min a week. Total physical activity was controlled by pedometer and questionnaires to ensure that women met the recommended level of physical activity (see also Supplementary

Table 1. *Changes (T0–T1) in weight and anthropometric measures between the human intervention group (*Maternal-INT$_h$*) and the human control group (*Maternal-CO$_h$*) during pregnancy*

Group	*n*	Change T0–T1 [mean (S.E.M.)]	Difference (95% CI)	*P*-value[a]
Weight (kg)				
Maternal-CO$_h$	15	10.2 (1.7)	1.0	0.592
Maternal-INT$_h$	19	11.2 (0.6)	(−1.8, 4.4)	
% body fat				
Maternal-CO$_h$	12	3.1 (0.8)	− 9.1	<0.001
Maternal-INT$_h$	19	−6.0 (1.0)	(−11.9, −6.2)	
Upper arm fat mass estimate (cm^2)				
Maternal-CO$_h$	12	2.2 (1.7)	−7.5	0.002
Maternal-INT$_h$	19	−5.3 (0.8)	(−11.5, −4.6)	
Upper arm fat-free mass estimate (cm^2)				
Maternal-CO$_h$	12	3.7 (1.3)	0.4	0.347
Maternal-INT$_h$	19	4.0 (1.3)	(−3.0, 5.1)	

CI, confidence interval.
[a]ANCOVA adjusted for age and baseline data.

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

Table S2). Women in the intervention group participated for a minimum of 16 and a maximum of 21 weeks (mean participation was 18 weeks and 23 sport units). In total, 11 women (57.9%) of the intervention group exercised more than the average sport units.

Based on 7-day dietary records, individual dietary counseling took place at T0 and during pregnancy. General dietary counseling consisted of three sessions for 90 min (16th, 24th and 36th week of gestation) and was based on recommendations from the German Health Infoservice (see also Supplemental Materials).

Maternal-CO_h

Control participants received standard prenatal care. They were neither encouraged nor discouraged from exercising and did not participate in any additional sport or nutrition program. Total physical activity was controlled by pedometer and questionnaires to ensure that participants did not exercise more than the intervention group. A flyer with general nutritional information and recommendations for lactation from the German Health Infoservice was provided. The 7-day dietary records were used to evaluate nutritional intake.

Study collective and outcome measures: mouse study

C57BL/6N mice were bred locally at the animal facility of Pharmacology, Animal Housing Network of the University Hospital of Cologne (Cologne, Germany). Breeding colonies were kept in individually ventilated cages (Blue Line Cages, type II long; Tecniplast, Italy) at a temperature of 20–24°C, humidity of 50–60% and a 12/12-hour light/dark cycle. As bedding, spruce granulate (Lignocel FS 14; Rettenmaier & Söhne GmbH, Germany) was provided. Nestlets, mouse smart home and aspen bricks served as enrichment (Plexx B.V., The Netherlands); 3-week-old female mice were fed a standard diet (R/M-H SSniff, Germany) containing 412 g/kg carbohydrates, 190 g/kg protein and 33 g/kg fat with a total metabolizable energy (ME) of 3220 kcal/kg (9% of total ME originating from fat) for 9–10 weeks pre-conception and during gestation and lactation (*Maternal-CO_m*, $n = 35$). The running intervention group (*Maternal-INT_m*, $n = 9$) was fed the same diet but had continuous access to a running wheel in their home cage during gestation and starting with mating. Running wheels were equipped with speed indicators measuring distance (km), average speed (km/h) and time (h:min), as described previously.[6] Water and diet were available *ad libitum* and male breeders were fed the same diet as females. The body weight of the dams was monitored daily, starting immediately before mating, defined as G0, and up to G18. All studies with offspring were performed with males. At each timepoint, 1–2 pups per litter were studied. The body weight of all pups was monitored daily starting immediately after birth (P1). On P3, litter size was randomly adjusted to 6 for each litter. Two litters with less than 6 pups in the control group mice (CO_m) were excluded from the experiment. A subset of animals was sacrificed at P21 and blood samples were collected and epigonadal white adipose tissue (WAT) was harvested for further analyses. The remaining animals were weaned and kept on a standard diet until P70. From P71 to P112 50% of the CO_m and intervention group mouse (INT_m) offspring were subjected to a HFD (C1057 modified Altromin, Germany) containing 269 g/kg carbohydrates, 208 g/kg protein and 351 g/kg fat with a total ME of 5237 (60% of ME originating from fat) designated control group mouse high-fat diet (CO_{HFDm}) and intervention group mouse high-fat diet (INT_{HFDm}) (Fig. 1b). From P21 to P112, mice were weighed weekly. At P112, the remaining animals were sacrificed, blood samples were taken and epigonadal WAT was dissected, weighed, snap-frozen and stored at −80°C before mRNA and protein analysis.[6,23]

Analytical procedures: human and animal study

In humans, at both timepoints (T0 and T1) venous blood samples (3 × 6 ml) were taken from pregnant women after an overnight fast (>10 h). No blood samples were taken from human offspring. Samples were drawn in serum venipuncture tubes (Becton Dickinson, USA), centrifuged for 10 min at 4000 g and 4°C after blood collection and clotting period over 30 min and stored at −20°C until analyzed.

In mice, blood samples for serum analyses were collected via submandibular puncture at G16, designated T1. In order to minimize stress-induced side effects, dams had no baseline blood collection at the beginning of pregnancy (T0) and both dams and offspring were not fasted. Blood samples from offspring were taken via intracardial puncture at P21 and P112 after offspring were sacrificed. All blood samples were centrifuged for 10 min at 3000 g and 4°C and stored at −20°C until analyzed.

Biomarker analyses

Human samples: human insulin was measured by a radioimmunoassay kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. Human leptin was measured using a direct sandwich ELISA kit (Catalog no. EZHL-80SK, MERCK Millipore, Germany). Human BDNF concentrations (Catalog no. EPX010-12116-901; ProcartaPLEX) were investigated by a multiplex immunoassay from eBioscience (calculated with Bio-Plex Manager 6.1; Bio-Rad Laboratories, Hercules, CA, USA).

Mouse samples: murine serum insulin, leptin and BDNF levels were measured in duplicates in a multiplex analyzer (Bio-Plex 200®; Bio-Rad Laboratories, USA) according to the manufacturer's instructions (Milliplex® MAP, MA, USA). Sera were thawed, centrifuged for 5 min at 10,000 g and 4°C and were added undiluted to the appropriate wells. By using the median fluorescence intensity and the standard curve, the absolute concentration of each cytokine (pg/ml) was calculated (Bio-Plex Manager 6.1; Bio-Rad Laboratories).

Molecular analysis for mouse WAT

See Supplemental Material and Methods.

Statistical analysis

The data are presented as means ± S.E.M. A two-tailed Mann–Whitney test was used to test the significance of differences between *Maternal-CO$_h$* and *Maternal-INT$_h$* as well as *Maternal-CO$_m$* and *Maternal-INT$_m$* at the given timepoints. Statistical significance was defined as $P < 0.05$. In the animal study, we performed a one-way ANOVA for non-parametric tests (Kruskal–Wallis test) followed by pair-wise Mann–Whitney *t*-tests if significance was stated (for non-parametric distribution) for the timepoint P112. For body weight and body fat content at P112, we first tested for normal distribution by D'agostino and Pearson's omnibus normality test and then performed an ordinary one-way ANOVA followed by Holm-Sidak multiple comparisons test. Body weight gain was calculated by a two-way ANOVA test. In addition, statistical analyses of human phenotypical data were performed using IBM SPSS Statistics (SPSS Inc.; vol. 22, IL, USA). Demographic data are given as minimum, maximum, mean values and S.E.M. All confidence intervals were estimated at the 95% level. For comparisons between groups' independent sample, the unpaired *t*-test was used. An ANCOVA served for comparing the differences concerning individual characteristics in the human groups (e.g. leptin concentration in different groups) adjusted for age and baseline data.

The sample size of human participants varies due to unanswered questions in the questionnaire.

Results

Period 1: maternal anthropometric data

In the human study, the participants were 31.7 ± 0.7 years old and had a pre-pregnancy BMI of 22.6 ± 0.5 kg/m^2. There were no significant differences between the *Maternal-CO$_h$* and *Maternal-INT$_h$* except with respect to age (Supplementary Table S3). Weight gain averaged 15.4 ± 1.0 kg during pregnancy but no difference between groups was found (Fig. 2a). At the beginning of the intervention, the *Maternal-CO$_h$* had a significantly lower percent body fat than the *Maternal-INT$_h$*

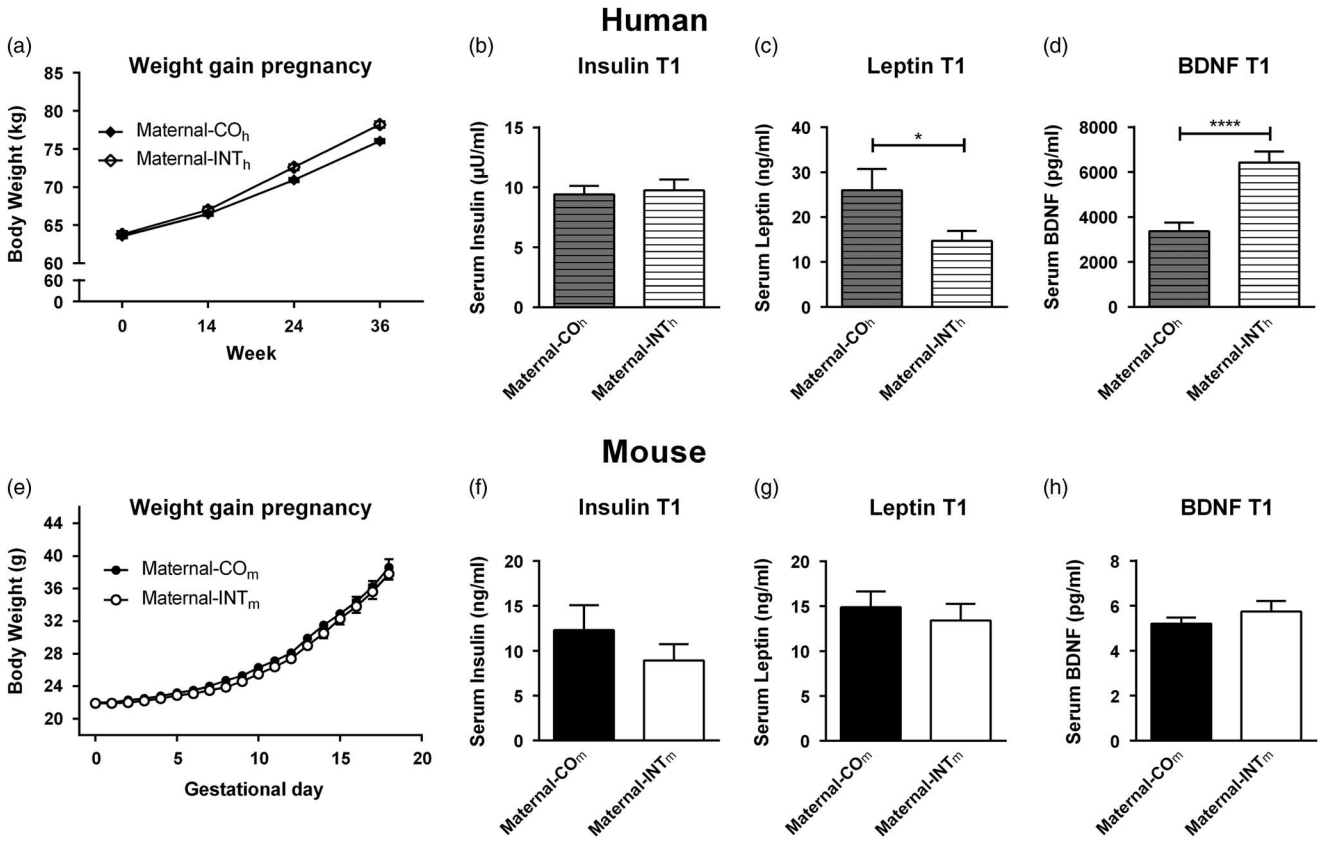

Fig. 2. Maternal parameters. Human: (*a*) body weight gain during pregnancy (*Maternal-CO$_h$*: $n = 15$, *Maternal-INT$_h$*: $n = 19$). (*b–d*) Serum levels at T1, (*b*) insulin (*Maternal-CO$_h$*: $n = 14$, *Maternal-INT$_h$*: $n = 19$), (*c*) leptin (*Maternal-CO$_h$*: $n = 15$, *Maternal-INT$_h$*: $n = 19$), (*d*) brain-derived neurotrophic factor (BDNF) (*Maternal-CO$_h$*: $n = 15$, *Maternal-INT$_h$*: $n = 19$). Mouse: (*e*) body weight gain during pregnancy (*Maternal-CO$_m$*: $n = 35$, *Maternal-INT$_m$*: $n = 9$). (*f–h*) Serum levels at G16 (*Maternal-CO$_m$*: $n = 7$, *Maternal-INT$_m$*: $n = 6$), (*f*) insulin, (*g*) leptin, (*h*) BDNF. Data are presented as mean ± S.E.M.; $^*P < 0.05$, $^{****}P < 0.0001$. CO, control; INT, running/exercise intervention during pregnancy; G, gestational day; h, human; m, mouse.

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($P = 0.043$, Supplementary Table S3). During pregnancy, women in the *Maternal-CO$_h$* increased percent body fat while the *Maternal-INT$_h$* reduced their percent body fat. Similar results were observed in the upper arm fat area, adjusted for age and baseline data ($P = 0.002$, Table 1).

In the animal study, dams had an average pre-pregnancy body weight of 21.6 g in *Maternal-CO$_m$* group and 22.1 g in *Maternal-INT$_m$* group just before mating. There were no significant differences in weight gain during pregnancy (Fig. 2e). As dams were not sacrificed at the end of gestation or lactation, body fat content was not measured. Litter size was larger in *Maternal-INT$_m$* dams than in *Maternal-CO$_m$* dams (Supplementary Fig. S1A). There were more male offspring in *Maternal-INT$_m$* litters. The average distance of voluntary wheel running was around 13–15 km/day for the first days of gestation. At the beginning, the amount of wheel running slightly increased up to G5 and continuously decreased shortly after. As the pregnant mice approached delivery the average distance of voluntary wheel running was around 200 m/day (Supplementary Fig. S1B).

Period I: maternal biomarkers during pregnancy

In the human study, no differences were found between *Maternal-CO$_h$* and *Maternal-INT$_h$* with respect to serum insulin levels during pregnancy at T1 (Fig. 2b). Human leptin levels were significantly higher in the *Maternal-CO$_h$* than in the *Maternal-INT$_h$* at the beginning and at the end of gestation (Table 2 and Fig. 2c, unadjusted means). Regarding changes in levels of human serum leptin from T0 to T1, no differences were found between the *Maternal-CO$_h$* and *Maternal-INT$_h$*, adjusted for baseline data and age (Table 2). Human levels of BDNF were significantly higher at T1 in the *Maternal-INT$_h$* than in the *Maternal-CO$_h$* (Fig. 2d, unadjusted means). There was a significant difference regarding changes in levels of human serum BDNF from T0 to T1 between *Maternal-CO$_h$* and *Maternal-INT$_h$*, adjusted for baseline data and age (Table 2). In the animal study, no differences were found between *Maternal-CO$_m$* and *Maternal-INT$_m$* with respect to serum insulin, leptin and BDNF levels during pregnancy at T1 (Fig. 2f–2h).

Period II: offspring data during recommended lactation period

In the human study, the average infant birth weight was 3377.3 ± 122.5 g in the CO_h and 3466.6 ± 89.4 g in the INT_h. There was no significant difference between the groups ($P = 0.197$, 95% Cl: −210.3, 388.8) when adjusted for maternal weight, age, height and BMI before pregnancy (Fig. 3). No differences were found between the groups for mode of delivery (cesarean *v.* spontaneous), Apgar score at 5 and 10 min, and umbilical blood pH (data not shown). There was no significant difference in body weight gain during the first 6 months of life (Fig. 3). The average weight of babies after 6 months (O5) was 7813.3 ± 306.9 g in the CO_h and 7775.3 ± 198.1 g in the INT_h. There was no significant difference between the groups ($P = 0.483$, 95% Cl: −754.6, −678.6) when adjusted for birth weight, maternal age, maternal pre-pregnancy weight and maternal weight gain during pregnancy (Fig. 3).

In the animal study, body weight gain during the lactation period from P1 to P21 did not reveal significant differences (Fig. 3). Body fat content at P21 was similar in both groups (Supplementary Fig. S2A). Serum taken at P21 revealed a decrease in insulin ($P = 0.07$) and leptin levels ($P = 0.8$) and an increase in BDNF ($P = 0.1$) levels in INT_m offspring (Supplementary Fig. S2B–S2D), but not to a significant extent. Molecular analysis of WAT at P21 revealed a significant increase of peroxisome proliferator-activated receptor γ (Pparγ) and glucose transporter type 4 (Glut4) on the mRNA level in INT_m offspring as well as a significant increase of insulin receptor β (INSRβ) on the protein level, while phosphorylated protein kinase (pAKT) did not show marked differences between both animal groups (Supplementary Fig. S3A–S3F).

Table 2. *Human biomarkers at baseline (T0), at the end of gestation (T1) and change during pregnancy (T0 – T1)*

Group	*n*	T0 [mean (S.E.M.)]	*P*-value[a]	*n*	T1 [mean (S.E.M.)]	*P*-value[a]	*n*	Change T0 – T1 [mean (S.E.M.)]	*P*-value[b]
Insulin (μU/ml)									
Maternal-CO$_h$	14	7.3 (1.9)	0.475	14	9.4 (0.8)	0.820	14	2.2 (2.3)	0.738
Maternal-INT$_h$	19	6.0 (0.5)		19	9.7 (0.8)		19	3.7 (0.8)	
Leptin (ng/ml)									
Maternal-CO$_h$	14	21.0 (3.3)	0.020	15	28.8 (5.0)	0.036	14	10.2 (4.0)	0.353
Maternal-INT$_h$	19	11.8 (1.4)		19	16.2 (2.2)		19	4.4 (1.8)	
BDNF (pg/ml)									
Maternal-CO$_h$	14	3987.1 (377.0)	0.250	15	3371.2 (410.5)	<0.001	14	−691.9 (398.1)	0.001
Maternal-INT$_h$	19	4966.6 (673.6)		19	6540.7 (509.2)		19	1574.1 (587.9)	

Maternal-INT$_h$, human intervention group; *Maternal-CO$_h$*, human control group; BDNF, brain-derived neurotrophic factor.
[a]Unpaired *t*-test.
[b]ANCOVA adjusted for baseline data and age.

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

Period III: mouse offspring data in adulthood

Body weight gain up to P112 in CO_m and INT_m did not differ between the groups (Fig. 3). However, when offspring were given a HFD starting at P71, CO_{HFDm} significantly increased their body weight compared with INT_{HFDm} (Fig. 3). INT_{HFDm} were protected against such a rapid weight gain even though after 6 weeks of high-fat feeding their body weight was significantly higher than CO_m offspring, but not compared with INT_m offspring (Fig. 3). Accordingly, the HFD induced a strong increase of WAT in CO_{HFDm} and INT_{HFDm} compared with CO_m or INT_m (Fig. 4a). However, the amount of WAT was significantly lower in INT_{HFDm} compared with CO_{HFDm} (Fig. 4a).

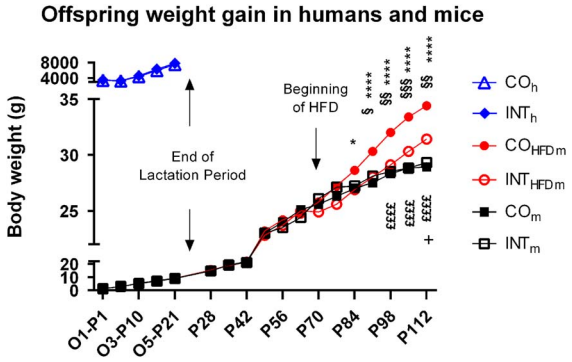

Fig. 3. Offspring body weight gain in humans and mice. CO_h: $n = 15$, INT_h: $n = 19$, CO_{HFDm}: $n = 30–37$, INT_{HFDm}: $n = 9–12$, CO_m: $n = 28–32$, INT_m: $n = 10–14$. *Differences between CO_m and CO_{HFDm}, §differences between CO_{HFDm} and INT_{HFDm}, +differences between INT_{HFDm} and CO_m and £differences between INT_m and CO_{HFDm}. There were no significant differences between INT_m and INT_{HFDm} $^{*/§/+/£}P < 0.05$, $^{§§}P < 0.01$, $^{§§§}P < 0.001$, $^{****/§§§§/££££}P < 0.0001$. CO, control; HFD, high-fat diet; h, human; INT, running/exercise intervention during pregnancy; m, mouse; P, postnatal day for animal model; O, postnatal examination timepoint for children.

Insulin

Serum analyses at P112 revealed a strong increase in serum insulin levels due to HFD feeding when comparing CO_m and CO_{HFDm} as well as INT_m and INT_{HFDm} (Fig. 4b). Maternal exercise decreased serum insulin levels in INT_m and INT_{HFDm} offspring in comparison with CO_m and CO_{HFDm}, but not to a significant extent (Fig. 4b).

Leptin

In line with WAT mass, serum leptin levels showed an eight-fold increase in CO_{HFDm} compared with CO_m and 66-fold increase compared with INT_m (Fig. 4c). Maternal exercise markedly decreased leptin levels eight-fold in INT_m offspring compared with CO_m offspring and again three-fold in INT_{HFDm} offspring compared with CO_{HFDm} offspring (Fig. 4c).

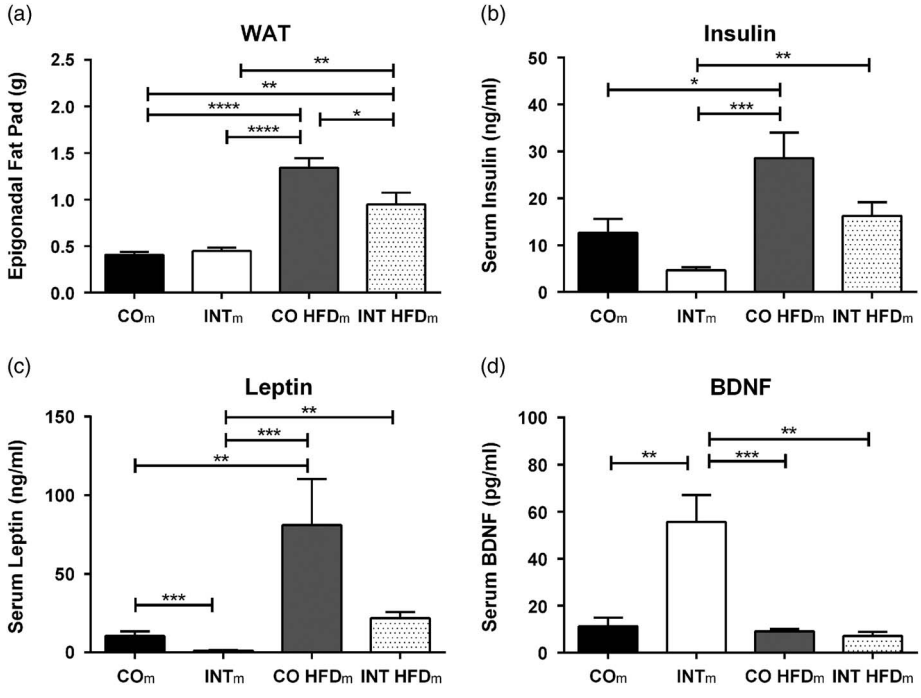

Fig. 4. Phenotype of mouse offspring at P112. (*a*) Epigonadal fat pad weight (CO_m: $n = 28$, INT_m: $n = 14$, CO_{HFDm}: $n = 33$, INT_{HFDm}: $n = 8$). (*b–d*) Serum levels at P112 (CO_m: $n = 8$, INT_m: $n = 7$, CO_{HFDm}: $n = 9$, INT_{HFDm}: $n = 5$). Data are presented as mean ± S.E.M.; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. BDNF, brain-derived neurotrophic factor; CO, control; HFD, high-fat diet; INT, running intervention during pregnancy; m, mouse; WAT, white adipose tissue.

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

BDNF

At P112, serum BDNF levels were elevated three-fold in INT_m offspring (Fig. 4d), while this effect was completely blunted in INT_{HFDm} after 6 weeks of high-fat feeding. There was no difference in serum BDNF levels between CO_m and CO_{HFDm} ($P > 0.05$, Fig. 4d).

Discussion

Period I: maternal effects during pregnancy

We hypothesized that maternal exercise during pregnancy exerts similar effects in mice and humans regarding body weight gain and serum levels of insulin, leptin and BDNF during pregnancy.

Regarding maternal insulin levels during pregnancy, it has been shown in mice that maternal exercise has no impact on glucose tolerance results during pregnancy in lean dams,[24] which was confirmed in the present study. In humans, fasting insulin levels increase during pregnancy and several human studies have shown decreased levels of insulin and incidence of GDM in overweight and obese pregnant women as a result of physical activity.[25] However, we could not confirm these findings, which may be due to two reasons for not finding any differences in insulin levels in our human population. First, the participants in both groups were lean, had normal pre-pregnancy BMI, and were active before pregnancy. Studies reporting effects of exercise on insulin level are often related to overweight and obese pregnant women, due to their higher risk factor for developing GDM. Furthermore, Retnakaran *et al.*[26] already showed that pre-gravid sports is associated with a reduced risk of glucose intolerance in pregnancy, an effect likely mediated by enhanced insulin sensitivity. Taken together, our participants had a low risk for developing gestational diabetes. Second, the exercise program started in the second trimester (14th week of gestation), which is in line with almost every published human physical activity intervention. However, Tobias *et al.*[27] demonstrated in their meta-analysis that higher levels of physical activity in early pregnancy are associated with a significantly lower risk of developing GDM during pregnancy. Although our participants were active during the first trimester, both groups did not meet the recommended physical activity level. Song *et al.*[28] concluded in their review that lifestyle modification before the 15th gestational week can reduce the risk of GDM. Possibly, our intervention started too late during pregnancy to achieve an effect on insulin level.

Human maternal leptin levels and body fat mass increase over the course of pregnancy.[29] We observed lower maternal body fat at the end of gestation compared with the control group in the human population. Since fat mass influences hormone levels, maternal body composition and lifestyle are considered to play a key role within perinatal programming.[30] Serum concentrations of leptin correlate with total body fat content during pregnancy.[29] Consistent with our result of reduced body fat content, maternal levels of leptin were markedly lower in the human intervention group. Leptin is predominantly secreted from WAT[31] but other tissues such as the trophoblast cells in human placenta synthesize and secrete the protein as well.[32] Bouassida *et al.*[33] showed in a non-pregnant population that leptin levels decrease after long-term exercise (⩾60 min) stimulating free fatty acid release. Van der Wijden *et al.*[34] investigated whether moderate-to-vigorous intensity physical activity (MVPA) was associated with insulin sensitivity, the insulin-like growth factor 1 system, leptin levels and weight change in healthy pregnant women. At three timepoints (15th, 25th and 35th weeks of gestation) levels of leptin were lower in women with MVPA above the median than those below although these differences were not statistically significant. We confirmed the results of Clapp and Kiess,[30] concluding that changes in leptin levels during pregnancy may reflect the concomitant change in fat mass.[30] However, the question arises whether maternal exercise also influences placental-derived leptin. Contrary to our hypothesis, serum leptin levels in exercised dams were not significantly decreased in the animal group. In contrast to humans, where synthesis of leptin by the human placenta is established, it remains controversial whether the murine placenta synthesizes leptin[35] or not.[36] Therefore, human and mice placenta should be examined for leptin metabolism in future studies of our working group. For GDM, maternal serum leptin has been examined regarding potential effects on inflammatory processes in the placenta and differential effects on humans and mice have been shown.[37] The present study is the first to evaluate the effects of maternal exercise in lean dams on maternal leptin levels. Since we did not measure body fat composition of mouse dams at G16 we cannot conclude whether leptin levels are associated with changes in body fat composition in our mice dams as was observed for human pregnancies. The literature indicates that serum leptin levels during pregnancy originate from different sources and have different functions in humans and mice.[35,37,38] Taken together, further studies examining the effects of maternal exercise on murine and human placenta and leptin levels during pregnancy are recommended for advancing our understanding of the mechanisms behind the beneficial effects of exercise.

Regarding the effects of physical activity on BDNF, it has been shown that single bouts of physical activity and training can up-regulate serum BDNF levels.[39–43] BDNF seems to be important for brain function, mood, neuroplasticity and mental performance.[12] However, little is known about the effects of physical activity during pregnancy on maternal BDNF concentrations and its role on metabolic regulation. In a single bout of high-intensity exercise, Vega *et al.*[13] showed that physical activity increases the serum concentration of BDNF during late pregnancy. To the best of our knowledge, the present study is the first to demonstrate that regular moderate physical activity results in significantly elevated maternal BDNF serum levels during pregnancy in humans.

Contrary to our hypothesis, these findings were not confirmed in the mouse study. During human brain development,

both neurogenesis and the formation of functional connectivity occur during gestation. However, in rodents, full synapse connectivity is not established until the end of the postnatal period with a brain spurt at P7.[44] Therefore, it is possible that BDNF serum levels still increase after G16 in mice and that this increase continues up to P21. Thus, the corresponding maternal BDNF serum elevation in comparison to our human data could be possible during the lactation period and not at G16 as chosen in this study. For future analysis, it would therefore be worthwhile to analyze serum parameters and body composition in dams until P7 when brain growth peaks and at the end of the lactation period to fully cover brain maturation in mice. Thus, contrary to our hypothesis, these findings give first indications that regular moderate physical activity during pregnancy can significantly elevate maternal levels of BDNF in human pregnancies but not in mice dams up to G16.

Period II: effects of maternal exercise on young offspring

We hypothesized that maternal exercise during pregnancy exerts similar effects in mice and humans regarding body weight gain in offspring. In both humans and mice, physical activity during pregnancy had no effect on offspring birth weight, confirming our hypothesis. These findings are in line with previous studies for humans and mice.[7,45,46] Based on current evidence in humans, the effects of exercise on offspring birth weight may occur primarily at the upper end of the birth weight range.[47] Since both of our sample sizes were normal weight and the women were active before pregnancy (Supplementary Table S2), no significant differences in birth weight were expected. In animal models, different activity regimes need to be taken into consideration. Swimming exercise is known to be more stressful for animals than voluntary running exercise.[48,49] Lower birth weight in offspring up to P21 was found after swimming exercise during pregnancy,[50] while in a study by Carter *et al.*[7] who also investigated voluntary wheel running, no effect on the offspring's body weight was observed.

Up to the end of the lactation period there were no significant effects on the offspring's body weight and fat content between the intervention and control groups in humans and mice (Fig. 3, Supplementary Fig. S2A). Although little information is available on long-term effects of maternal exercise on human offspring, comparable results were reported by Clapp[18] who evaluated the effect of regular physical activity during pregnancy on morphometric outcomes after 1 year. Unlike our results, children of active mothers had lower birth weight compared with the control group but no significant effects were detected after 1 year.[18] In line with our results from mice, Carter *et al.*[7] showed that maternal exercise in lean dams did not produce differences in offspring body weight until week 76 of life, indicating that under normal lifestyle conditions (standard died) maternal exercise has no significant impact on the offspring body weight during childhood and later life. It should be noted that the evaluated time span in human offspring in the present study is short. Differences in human offspring body weight will perhaps be observed in later life such as in puberty or adulthood, once again underlining the urgent need for long-term follow-up studies.

Period III: effects of maternal exercise on adult offspring

We hypothesized that sedentary offspring from exercised mothers could be protected from the deleterious effect of high-fat feeding during adulthood.

Effects of maternal exercise on adult offspring without HFD

Up to P112, there were no significant effects on the offspring's body weight in CO_m and INT_m group. In line with previous studies, insulin levels were clearly lowered in the INT_m offspring at P112 but not to a significant extent.[7] While body fat content at P112 was similar in the CO_m and INT_m offspring, serum leptin levels were significantly lower in the INT_m group. In a non-pregnant animal population, Jen *et al.*[14] showed that leptin levels in the exercise groups were reduced by 55% and that these results were independent of the reduction of body fat. Stanford *et al.*[51] already revealed in a mouse model that only 11 days of exercise training causes multiple functional and molecular adaptations to WAT without affecting the amount of WAT. Furthermore, these adaptations lead to metabolic improvements in WAT and other tissues.[51,52] The extreme low levels of leptin in the INT_m group in our study could be an indication of a different WAT homeostasis and function at P112 independent of the WAT amount.

Consistent with our study, Dayi *et al.*[53] discussed that maternal exercise during pregnancy has beneficial effects on the generation and/or secretion of neurotrophic factors for the brain development of the rat offspring. BDNF has an important role in regulating maintenance, growth and survival of neurons. The brain is a major contributor to the circulation of BDNF both at rest and during exercise.[42,54] Although it is still unknown where exactly BDNF is produced peripherally, BDNF level in the brain seems to correlate with serum BDNF concentration.[55] Lommatzsch *et al.*[56] suggested that the blood level of the BDNF may reflect the brain level and vice versa. Moreover, it has been shown that there is a bi-directional transport from the brain to the extra-cerebral venous blood in mice.[57] Accordingly, we demonstrated in a previous human study that umbilical cord BDNF is correlated with maternal BDNF, although the correlation was weak.[58]

Animal models using different exercise protocols already demonstrated that maternal exercise during pregnancy has beneficial effects on the development and cognitive function of the offspring brain.[11,59] Our study observed three-fold higher serum BDNF levels in the offspring of exercised mothers (INT_m) compared with CO_m. This finding supports previous work showing that exercise during pregnancy is able to elevate BDNF mRNA expression in the hippocampal formation of offspring at P0, P28/29 and P60.[11,17,59] Nevertheless, there is also a certain dynamic within the hippocampal BDNF expression, as BDNF levels were significantly up-regulated at

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

P0 in offspring of exercised dams but again down-regulated at P28.[11] In line with these findings, we did not find any significant difference in serum BDNF levels at P21 in our study.

As maternal exercise is able to induce epigenetic changes in different organs in the offspring, the question arises whether epigenetic changes are a possible explanation for the BDNF levels found in our study. Yet, our results demonstrate that maternal exercise during pregnancy causes long-term elevation of BDNF serum levels in the young adult offspring of mice even though serum BDNF levels in dams were not influenced by exercise. In addition, BDNF levels in pregnant women were significantly increased by moderate-intensity exercise in the present study. Taking into account that the timepoint of measuring BDNF serum levels in the mice at G16 was not adequately chosen, this leads to the assumption that the effect of serum BDNF levels measured in adult mouse offspring should also be visible in human offspring. Thus, one underlying mechanism for the amelioration in neurodevelopmental outcome observed in children of physically active women at age 4 to 5 years might be related to differences in serum BDNF levels.[18,60] Altogether, our findings indicate the need for human long-term follow-up studies on the influence of controlled exercise programs during pregnancy on BDNF levels and associated neurodevelopmental outcome in young adults.

Effects of maternal exercise on adult offspring with HFD

Current Western lifestyle is characterized by a lack of exercise and high caloric intake. To evaluate the effect of such an unhealthy lifestyle, mouse offspring were fed a HFD starting at P70 (Fig. 3). Offspring of mothers who exercised during pregnancy were protected against rapid body weight and body fat gain. Comparably, Wasinski *et al.*[49] showed that forced swimming during pregnancy can protect the offspring from the deleterious effect of a HFD during adulthood. Thus, these results point to the conclusion that maternal exercise is beneficial in protecting the offspring from developing obesity regardless of level of offspring physical activity and dietary habits. Consistent with body fat content, insulin serum levels were clearly decreased in INT_{HFDm} compared with CO_{HFDm} offspring at P112. This is in line with Stanford *et al.*,[24] who already showed that perinatal exercise in chow-fed dams before and throughout pregnancy as well as during lactation significantly decreases insulin levels in the offspring at age 52 weeks with ameliorated glucose metabolism. Wasinski *et al.*[49] indicated insulin-sensitizing effects of maternal exercise on the offspring by ameliorated insulin tolerance tests in offspring of exercised mothers. However, the potential molecular mechanism remains to be elucidated. By analyzing WAT at P21, which is supposed to be a vulnerable timepoint in the field of perinatal programming,[61] we found that Pparγ and Glut4 as well as INSRβ were significantly increased in offspring of exercised mothers. Elevation in Glut4 mRNA level, which is responsible for the insulin-dependent uptake of glucose to WAT, and elevation in the protein amount of the insulin receptor itself, are indicators for a better insulin signal transduction and a better glucose disposal in WAT.[62–64] Furthermore, Pparγ is known to cause enhanced insulin sensitivity and improves glucose metabolism in WAT and Pparγ agonists have been used in the treatment of dyslipidemia and diabetes.[65] Regular physical exercise is known to cause adaptions to WAT including decreases in cell size and releasing adipokines influencing whole body metabolism.[51,52] Thus, we speculate that exercise by lean dams influences offspring's glucose metabolism in WAT at P21 with long-lasting functional adaptions to offspring's WAT[51,52] and protection against obesity and diabetes in later life.

Consistent with WAT and insulin levels, leptin serum levels were decreased in INT_{HFDm} compared with CO_{HFDm} offspring at P112, although this was not significant. Our results confirm those of Wasinski *et al.*[49] who found no differences in leptin levels, but lower insulin levels following HFD in adult offspring from swimming mothers. They concluded that maternal exercise leads to long-term changes in adult offspring with benefits for basal metabolism that protects from the influences of HFD.

In contrast to previous studies, our study also evaluated the effect of HFD on BDNF, which resulted in completely blunted BDNF serum levels in INT_{HFDm} compared with INT_{m}. This is of major interest as it seems that the positive short[11] and long-term effects of maternal exercise on the offspring's BDNF level, as described in our results, can clearly be influenced by an unhealthy lifestyle of the offspring itself.

Strengths and limitations

One strength of our study is the comparative assessment of humans and mice as to whether similar effects of maternal exercise can be observed in two different species. Comparative studies in this field of research are still scarce as they are time-consuming, more expensive and difficult to assess. To date, the main focus of human maternal exercise interventional studies during pregnancy is on maternal and obstetrical outcome parameters disregarding long-term effects over several years on the offspring[25] as it is costly and time-consuming. Therefore, another strength of our study is the use of fast and accessible mouse model to predict promising future examination timepoints and target tissues in humans. Due to our parallel design we can conclude that effects of maternal exercise on offspring's body weight are most likely to occur in adulthood and not at birth or up to the end of the weaning period, where both of our models consistently showed no changes.

One particular strength of the human study is the well-guided, controlled exercise regime, as most human intervention studies during pregnancy consist of only consultations, explanation meetings or the distribution of informational materials[66,67] and last only for several weeks.[68,69] Due to variations in substantive and methodological refinements, results of various studies are inconsistent.[70] The strength of our human study lies in the duration of the exercise program, which

https://doi.org/10.1017/S2040174417000617 Published online by Cambridge University Press

was conducted twice a week over more than 16 weeks to reach the recommended level of 150 min a week. This could, therefore, be responsible for the observed effect in body fat content, leptin and BDNF levels.

However, there are some limitations to this study. First, in the human study, women were not randomized, which could have resulted in greater baseline differences between the groups. Second, our exercise regime did not align with the end of our last blood collection (around 30 weeks of gestation *v.* 36 weeks of gestation). However, several other studies have also used this timetable and therefore, our data is comparable with other studies.[68,71] Due to the fact that we did not find effects of our human exercise intervention on insulin level at T1, we should consider starting our intervention in the first trimester of pregnancy in future studies. Third, a comparison between animal and human data needs to be interpreted with caution even though the murine model has already been widely used in biomedical research due to its cost-effectiveness. The gene pool of the mice in the murine model has a homology of 99% with that of humans. In addition, the mice resemble humans in physiology and disease pathogenesis.[20] Despite the similarities, mice have a diminutive lifespan compared with humans, but in accordance with the literature, we assessed parallel points in the lifespans of humans and mice.

In order to minimize stress-induced side effects, dams had no baseline blood collection prior to the gestational period. Although we compared offspring's body weight gain up to P21 in mice and corresponding 1 year postpartum in humans, we were not able to match blood parameters. Another limitation of the study is that only male offspring were analyzed in the animal model. Some effects of developmental programming on long-term offspring health can be sex specific[7] but since recently published studies examining the effects of maternal exercise on the offspring used only male offspring,[17,24,50] our data are comparable with those studies.

Conclusion

NCDs, in particular diabetes, obesity and cardiovascular diseases are increasing worldwide due to an overall unhealthy lifestyle including sedentary behavior and high caloric diet.[1,2] Here, we demonstrate that maternal exercise during pregnancy has positive short-term and long-term effects on both mother and offspring in mice and humans. Among the most promising findings, the protective effect of maternal exercise on body weight gain and elevated serum hormone levels in HFD-challenged mice is conspicuous. Our approach is novel in that it directly compares human and murine outcome parameters in aligned models. However, our results revealed profound differences between murine and human effects during observation periods I and II. Hence, the translational value of the beneficial long-term effects observed in period III in our mouse model should be interpreted with care. Notably, our data indicate a further need for comprehensive translational studies with an emphasis on long-term offspring outcome. The overall goal must be to develop powerful preventive strategies to effectively reduce any offspring predisposition for diseases such as obesity, diabetes or cognitive impairment.

Acknowledgments

The authors gratefully appreciate the Förderverein des Herzzentrums Köln for their support. The authors thank the women who participated in the study. The autors also like to thank Erica and Daniel Landerson for critically reviewing the manuscript.

Financial Support

This study was supported by an internal grant from the German Sport University Cologne to N.F. and C.G., by a grant from the University Hospital of Cologne to I.B.-G., R.J. and E.H.R., and intramural funding to E.M.

Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (German Sport University). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (AZ 8.87-50.10.37.09.292, Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Germany) and has been approved by the institutional committee (University Hospital of Cologne).

Authors' Contributions

N.F., I.B.-G., R.J., J.D., C.G. and E.H.R. designed the study; N.F., I.B.-G., C.B., R.J., C.G. and E.H.R. conducted the study and collected data; N.F., I.B.-G., C.B., R.J., C.G. and E. H.R. analysed the data; I.K. and E.M. conducted ELISA measurements, N.G. and C.V. performed the Western Blot and qPCR analyses, C.B., R.J., I.K., E.M., S.A., M.A.A.A., N. G., K.B. and J.D. contributed to the discussion and reviewed the manuscript; N.F., I.B.-G., C.G. and E.H.R. wrote the manuscript; N.F., I.B.-G., C.G. and E.H.R. have primary responsibility for the final content.

Statement: Nina Ferrari and Inga Bae-Gartz are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174417000617

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