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RESEARCH

Diabetes Research and Metabolism

# Metabolic programming of adipose tissue in female C57Bl/6 mice offspring by maternal high fat feeding and obesity results in greater adaptability to dietary fat content and resistance to high fat diet-induced glucose intolerance compared to male mice

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## Abstract

*Maternal lifestyle influence may be a factor in the worldwide prevalence of obesity and its complications, including diabetes. Studies investigating the effect of the perinatal maternal environment have produced a range of results, sometimes diametrically opposite. The present study was designed to investigate how obesity and weight gain in pregnant mice affects energy balance, body composition and glucose homeostasis in their offspring, both at a young age on standard diet and when older and fed a high-fat diet. At six weeks of age both male and female offspring from mothers fed a high fat diet had a shorter body length than those from mothers fed standard chow. In contrast to males, female offspring also contained a higher proportion of fat and had elevated circulating leptin and adiponectin. Their gonadal fat pads were heavier and contained larger adipocytes, whereas male offspring had proportionally more smaller adipocytes. Six-week-old female, but not male, offspring had increased gonadal fat gene expression of acetyl CoA carboxylase 1, the rate-limiting step in lipid biosynthesis, and decreased gene expression of carnitine palmitoyl transferase 1, the rate-limiting step in fatty acid oxidation. Maternal high fat diet had no effect on glucose tolerance in six-week-old mice, but this was achieved with higher insulin levels in females. Contrastingly, when the offspring were fed a high fat diet for three months, female, but not male, offspring were leaner than those from mothers fed standard chow. Their gonadal fat depots were lighter and the adipocytes were smaller. Female, but not male, offspring fed high fat diet had decreased gonadal fat gene expression of acetyl CoA carboxylase 1, and increased gene expression of carnitine palmitoyl transferase 1. High fat diet-induced glucose intolerance and elevated plasma insulin concentration were improved in female, but not male, offspring. Plasma leptin and adiponectin remained higher in female offspring on high fat diet with resistin levels being lower. These results suggest that the gonadal fat of female offspring is more adaptable to different levels of dietary fat exposure, increasing storage when levels are low and increasing oxidation when levels are high. This may help female offspring be more resistant to the detrimental effects of high fat diet than male mice.*

**Keywords:** *diabetes, obesity, maternal environment, perinatal programming, high fat diet*

## Introduction

Twin and adoption studies have found that 40-90% of the variance in BMI within the populations studied is due to genetic variation [1,2]. Type 2 diabetes is also strongly heritable (25-80%), the highest estimates coming from studies with the longest follow-up periods [3]. Genome-wide association and polygenic studies are gradually identifying the very many DNA variants that mostly make tiny contributions to the overall variance [4-6]. Genetic changes cannot, however, explain the close to doubling in the prevalence of obesity in the past 35 years [7] or the large increase in the prevalence of Type 2 diabetes [8-9].

Increased affluence and availability of palatable and high energy foods, coupled with more sedentary lifestyles, must be partly responsible for these long-term changes, but there is also evidence that intergenerational mechanisms exacerbate these environmental influences. Specifically, there is evidence that weight gain, obesity and inactivity in pregnant mothers increases the susceptibility of her offspring to obesity and diabetes [10-12] and this can be improved by exercise [13,14]. Some studies have found that susceptibility to obesity is influenced more by the mother's pre-pregnancy body mass index (BMI) than by the father's BMI, which suggests that shared genetic factors between parent and child do not explain all of the association between the child's BMI and the mother's BMI [15,16]. However, others have found no greater association of the child's BMI with that of the mother than that of the father [17].

To aid the understanding of how obesity and weight gain in pregnant women might affect her offspring, there have been many studies in which high fat diets have been fed to rat or mouse dams during pregnancy. Some have looked no further than birth weight, with reduced, increased or no effect on foetal growth. These differing findings may depend on the proportion of fat in the diet, the timing of the intervention, and whether rats or mice were used

[18-19]. Sex differences between offspring, and differences in fetal gene expression have also been reported [20]. Birth weight influences susceptibility to later metabolic disease [21], which provides a link to studies that have investigated the effect of feeding high fat diets to dams on susceptibility to obesity and type 2 diabetes in their offspring. In some of these studies the offspring have also been fed on a high fat diet. Results have been mixed [22]. Some have found that feeding the dams on a high fat diet increases the susceptibility of their offspring to the adverse effects of a high fat diet [23-26]. However, one study found little influence of the post-natal diet on the adverse effects in offspring of feeding dams a high fat diet [27], and another surprisingly found that the offspring of high-fat/high-sugar, or 'cafeteria', diet-fed dams have reduced obesity and improved glucose tolerance when they were fed on chow from weaning. When the offspring in this study were fed on the cafeteria diet, there was no influence of maternal diet on gonadal fat weight, but glucose intolerance developed in offspring from some groups of cafeteria-fed dams by comparison with chow-fed dams [28].

In the present study, we have employed a novel design of switching offspring from a chow to a high fat diet. Thus, pregnant mice were fed on chow or high fat diets and their offspring on a chow diet between weaning and 12 weeks of age and then on a high fat diet to 6 months. This is an efficient design for focussing on the effects of the dams' diet on male and female offspring, though, of course, it does not address differences between chow- and high fat diet-fed offspring of the same age. We have investigated energy balance, body composition and glucose homeostasis in both male and female offspring.

## Materials and Methods

Reagents were obtained from Sigma-Aldrich, Poole, UK, unless otherwise stated.

### Animals

Animal procedures were conducted in accordance with University of Buckingham project licence, under the UK Animals (Scientific Procedures Act (1986)) and as approved by the University's Ethical Review Board.

Thirty female C57Bl/6NCrl mice, age 5-6 weeks (Charles River, Margate, UK) were fed on a chow (CH, n=15) (10% fat, 70% carbohydrate and 20% protein by energy, Beekay Feed, B&K Universal Ltd., UK) or a high fat diet (HF, n = 15) (42% fat, 43% carbohydrate and 15% protein by energy, Western Rodent 829100, SDS diet, UK) for 10 weeks before mating with male C57Bl/6NCrl mice, 15-16 weeks of age (Charles River, cage ratio 1 male to 1 female) and throughout pregnancy and lactation. The male mice were also fed the same chow diet. Within the first 4 days after birth, litter size was adjusted down to 6 pups per litter, which was the smallest litter size. At 4 weeks of age, the offspring of chow and high fat-fed dams were separated according to sex (n=30 per group, two from each litter – M CH, M HF, F CH, F HF (M = male, F = female, CH = dams on standard rodent chow, HF = dams on high fat diet), and were housed at 21-23°C with lights on from 07:00 to 19:00h in groups of 3 mice per cage. All offspring were maintained standard chow diet from weaning until 12 weeks of age (or until culled at 6 weeks of age). At 12 weeks of age they were transferred to the high fat diet until 6 months of age. A schematic diagram of the feeding regime for dams and pups is presented in figure 1.

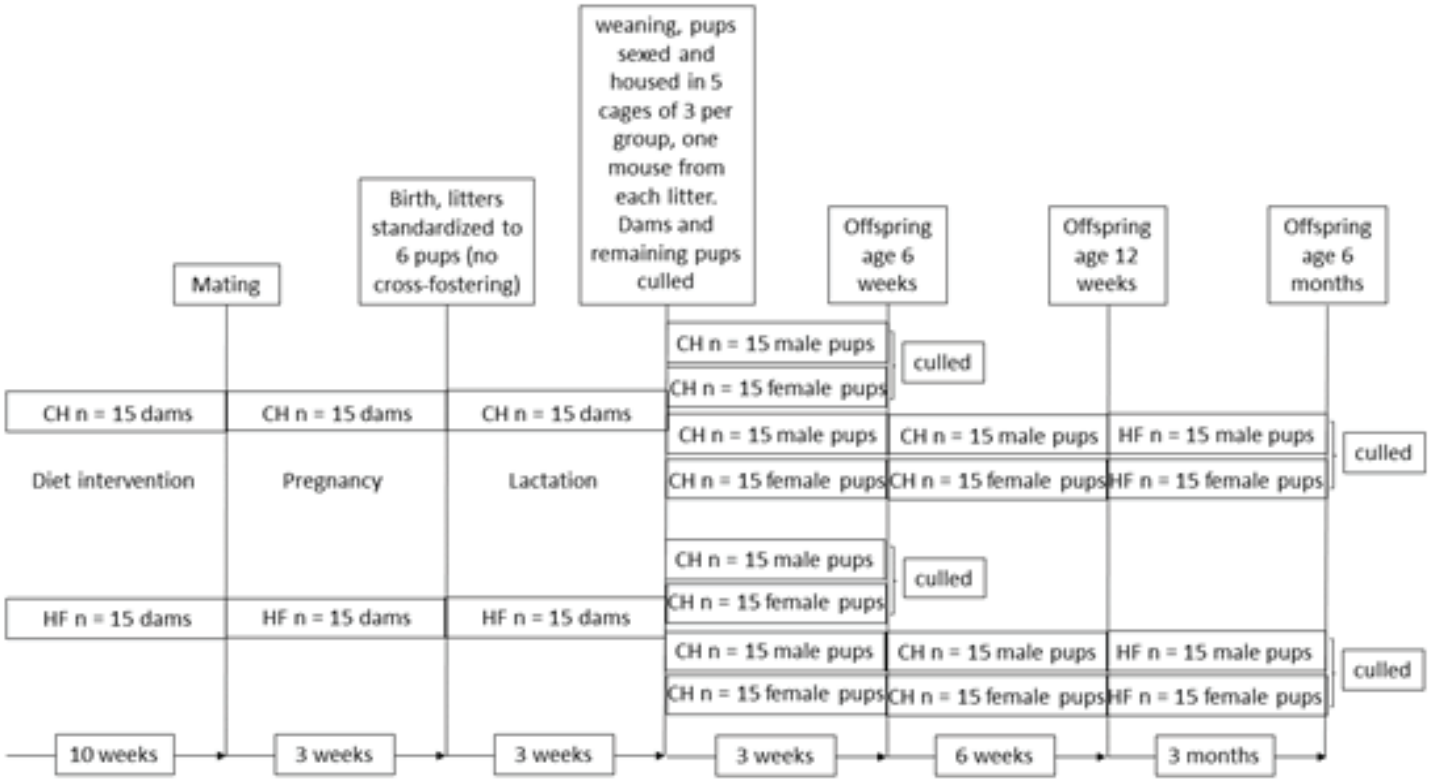


***Figure 1.*** *Schematic representation of the stages of dietary intervention (CH = chow and HF = high fat diet) to dams and offspring with N numbers.*

### Body parameters

Mice were weighed at ages 6 weeks, 12 weeks and 6 months. Body fat and lean content was measured at ages 6 weeks and 6 months using a Minispec LF90II Nuclear Magnetic Resonance (Bruker Corporation, Germany). Measurement of body length was performed dorsally from the tip of the nose to the base of the tail. The tibia length was measured at age 6 weeks after culling. Food was weighed and consumption calculated daily for 4 consecutive days at ages 6 weeks, 10 weeks and 6 months. This was converted to metabolisable energy consumption using data provided by the suppliers of the diets. Energy expenditure was calculated from oxygen consumption as measured by open circuit indirect calorimetry with mice in their home cages at ages 6 weeks, 11 weeks and 6 months [29,30].

### Blood and pancreatic measurements

An oral glucose tolerance test was performed on the offspring at 6 weeks, 12 weeks and 6 months of age. After fasting for five hours, mice were dosed with glucose (3 $g.kg^{-1}$, body weight PO by gavage). Blood samples were collected from the tail at -30, 0, +30, +60, +90 and +120 minutes, relative to glucose dosing. Blood glucose was measured using a glucose oxidase reagent kit (Gluc-PAP, GL2623; Randox, Crumlin, UK). Plasma hormones were measured by ELISA: insulin (Ultra-Sensitive Mouse Insulin ELISA kit, Catalog #: 90080; Crystal Chem, Downers Grove, IL, USA); leptin (Catalog #: 90030; Chrystal Chem); adiponectin (Catalog # MRP300; R&D Systems, Minneapolis, MN, USA), and resistin (Catalog # MRSN00; R&D Systems). Plasma triglycerides were measured by colorimetric assay (Catalog # TR210; Randox). Pancreases from 6-week-old mice were weighed and total pancreatic insulin content was measured using an insulin radioimmunoassay kit (Linco Research, St. Charles, USA).

### Histological methods

At 6 weeks and 6 months of age, 15 mice per group were culled, and the gonadal fat depots were removed and weighed to study their morphology and gene expression. One gonadal fat depot per animal was fixed in 10% neutral buffered formalin for 6 hours and cleared and dehydrated in a tissue processor (TP1020, Leica Microsystems, Germany). The samples were paraffin-embedded and sectioned (4 µm) using a rotary microtome (RM2125, Leica Microsystems, Wetzlar, Germany). This was followed by staining with Harris's haematoxylin (Sigma-Aldrich, Dorset, UK) and eosin Y (Sigma-Aldrich, Dorset, UK). Two sections, at the recommended minimum of 100 µm apart [31], were cut from the middle of the entire gonadal fat depot were collected from each animal.

### Morphometric analysis

Morphometric analysis was performed using ImageScopeTM software (CS2, Aperio, Vista, USA). All whole-slide adipose tissue sections stained in Harris's haematoxylin and eosin Y were scanned by a digital scanner (CS2, Aperio, Vista, USA) under x 40 magnification. The area ($\mu m^2$) and number of adipocytes within representative 5-10 non-overlapping representative areas (400 x 400µm) in two gonadal fat sections per each animal were calculated as described in our earlier report [32].

### Microarray analysis and selection of the genes for validation

Raw image data files were converted to *CEL* and pivot files using Affymetrix GeneChip Operating Software (GCOS). All downstream analysis of microarray data was performed using GeneSpring GX 12.0 (Agilent). The *CEL* files were used for the Robust Multi-array Averaging (RMA) [33] and GeneChip RMA (GC-RMA) (Wu *et al*, 2004) analyses, while the pivot files were used for GCOS analysis. After importing the data, each chip was normalized to the 50th centile of the measurement taken from that chip and all gene expression data reported as a fold-change from the control state. Genes were considered to be up- or downregulated if the genes had differential expression of $P<0.05$ in comparison to the maternal chow-fed group.

### Validation of microarray data using Taqman RT-PCR

Selection of genes for validation was based on the function of the gene and the availability of suitable primers for validation. Validation of the microarray data was carried out using Micro Fluidic Cards (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocol (Applied Biosystems). The reactions were performed in duplicate for each sample using an ABI 7900HT (Applied Biosystems). A standard curve was constructed for each gene using a serial dilution of cDNA from samples. The mean $C_T$ values of the experimental samples were then used to calculate the relative expression for each sample and data were normalized to *Ppia (cyclophilin)*, expression of which did not change between maternal treatment groups. RNA from the was used for gene expression analyses with ATPAF1 as an internal control (Almeida-Oliveira *et al,* 2017). Real time PCR (StepOne™, Applied Biosystems) was carried out using Assay on Demand pre-designed primer and probe sets (Applied Biosystems) (n=15). Data were analysed using the comparative $\Delta C_t$ method.

### Statistical analysis

A priori power analysis was conducted using G*Power3 [34]. All data was analysed by Prism and presented as mean ± SEM values and statistical comparisons are by 2-way ANOVA. All data sets passed the Anderson-Darling test for normality of distribution (alpha of 0.05). ROUT was used to analyse data sets for outliers with no outliers being identified.

## Results

### Dams' bodyweight

*One week before mating, the dams fed on the high fat diet were heavier than those fed on chow*

At 5-6 weeks of age, before dividing the female mice into those fed on the control and high fat diets, there was no difference in bodyweight between female mice subsequently fed on the chow or high fat diets (CH: 15.3 ± 0.3 g vs. HF: 15.3 ± 0.3 g). After 9 weeks on their respective diets (one week prior to mating), dams fed on the high fat diet were significantly heavier than dams fed on the standard chow (CH: 21.2 ± 0.2 g vs. HF: 25.9 ± 0.5 g, $P < 0.0001$).

### Parameters in 6-week-old offspring

At 6 weeks of age, female offspring from mothers fed a high fat diet had a shorter body length and contained a higher percentage of fat than those from mothers fed standard chow

Six-week-old female offspring of dams fed either a standard chow or a high fat diet were of similar bodyweight (F CH: 17.2 ± 0.3 g vs. F HF: 16.9 ± 0.4 g, n =15, figure 2A). The body length of female mice from mothers fed high fat diet was significantly less than that of mice from mothers on chow (F CH: 8.6 ± 0.1 cm vs. F HF: 8.3 ± 0.1 cm, $P < 0.01$, figure 3A). Similarly, tibia length of female mice from mothers fed high fat diet was significantly less than that of mice from mothers on chow (F CH: 2.27 ± 0.01 cm vs. F HF: 2.19 ± 0.01 cm, $P < 0.001$, figure 3B). However, the lean mass of female mice from mothers fed on the high fat diet was not significantly lower than that of mice from mothers fed on chow (F CH: 14.4 ± 0.2 g vs. F HF: 13.7 ± 0.3 g, $P = 0.35$, figure 2A).

The percentage of fat in 6-week-old female mice from mothers fed high fat diet was significantly greater than that of mice from mothers on chow (F CH: 16.9 ± 0.9 % vs. F HF: 18.8 ± 0.8 %, P <

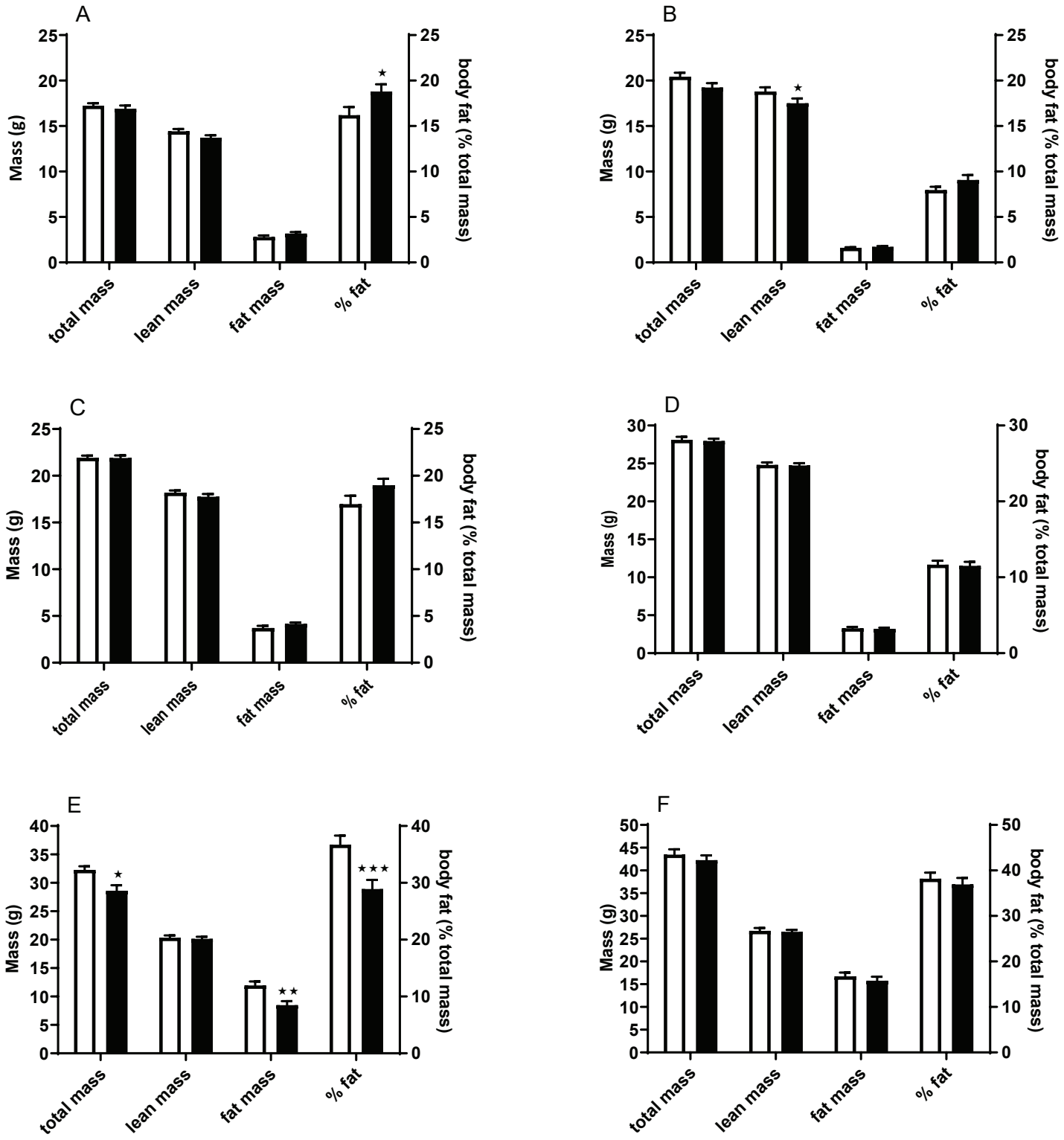


***Figure 2.*** *Body weight and body composition in male and female offspring from dams fed standard diet (unshaded bars) or high fat diet (shaded bars) at age 6 weeks (A females, B males), 12 weeks (C females, D males) and 6 months (E females, F males). N = 15 per group). *P<0.05; **P<0.01; ***P<0.001. Values are expressed as means ± S.E.M.*

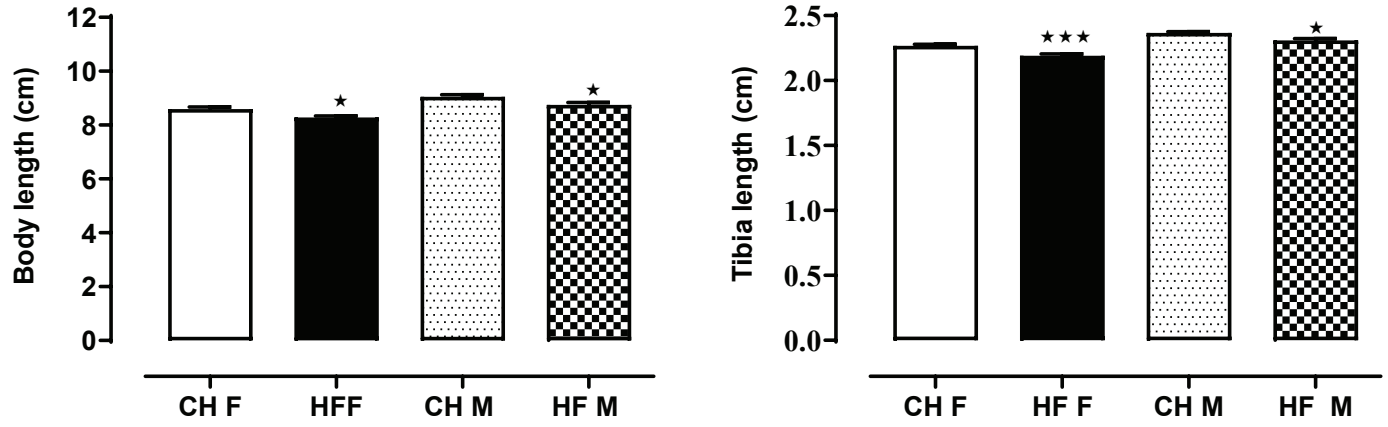


***Figure 3.*** *Body length and tibia length in 6-week-old male (M) and female (F) offspring from mothers fed a standard chow (CH) or 42% fat diet (HF), (n = 15 per group). *P<0.05; ***P<0.001. Values are expressed as means ± S.E.M.*

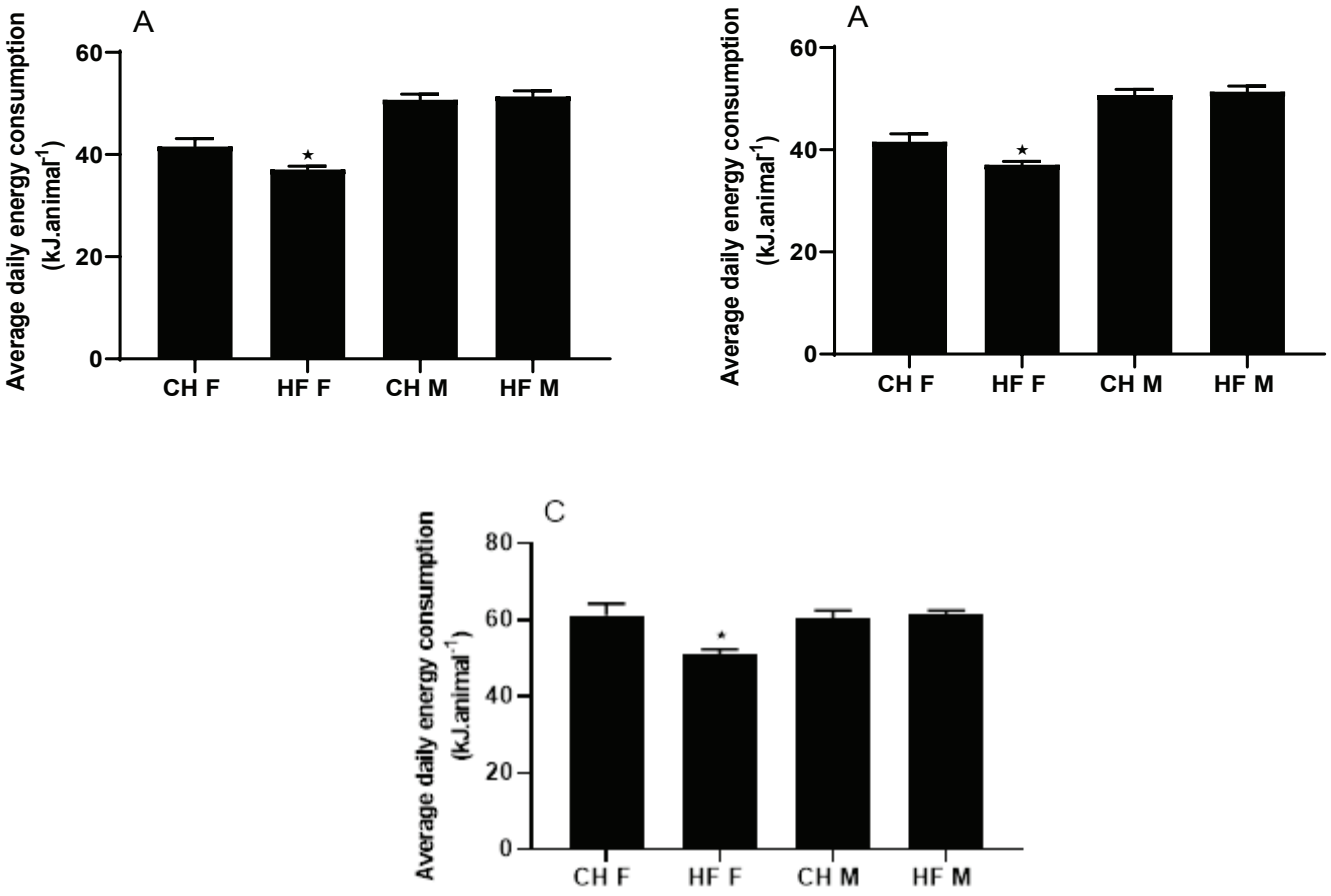


***Figure 4.*** *Average daily energy consumption measured over 4 consecutive days at ages 6 weeks (A), 10 weeks (B) and 6 months (C) in male (M) and female (F) offspring from mothers fed a standard chow (CH) or high fat (HF) diet, (n = 5 per group). *P<0.05. Values are expressed as means ± S.E.M.*

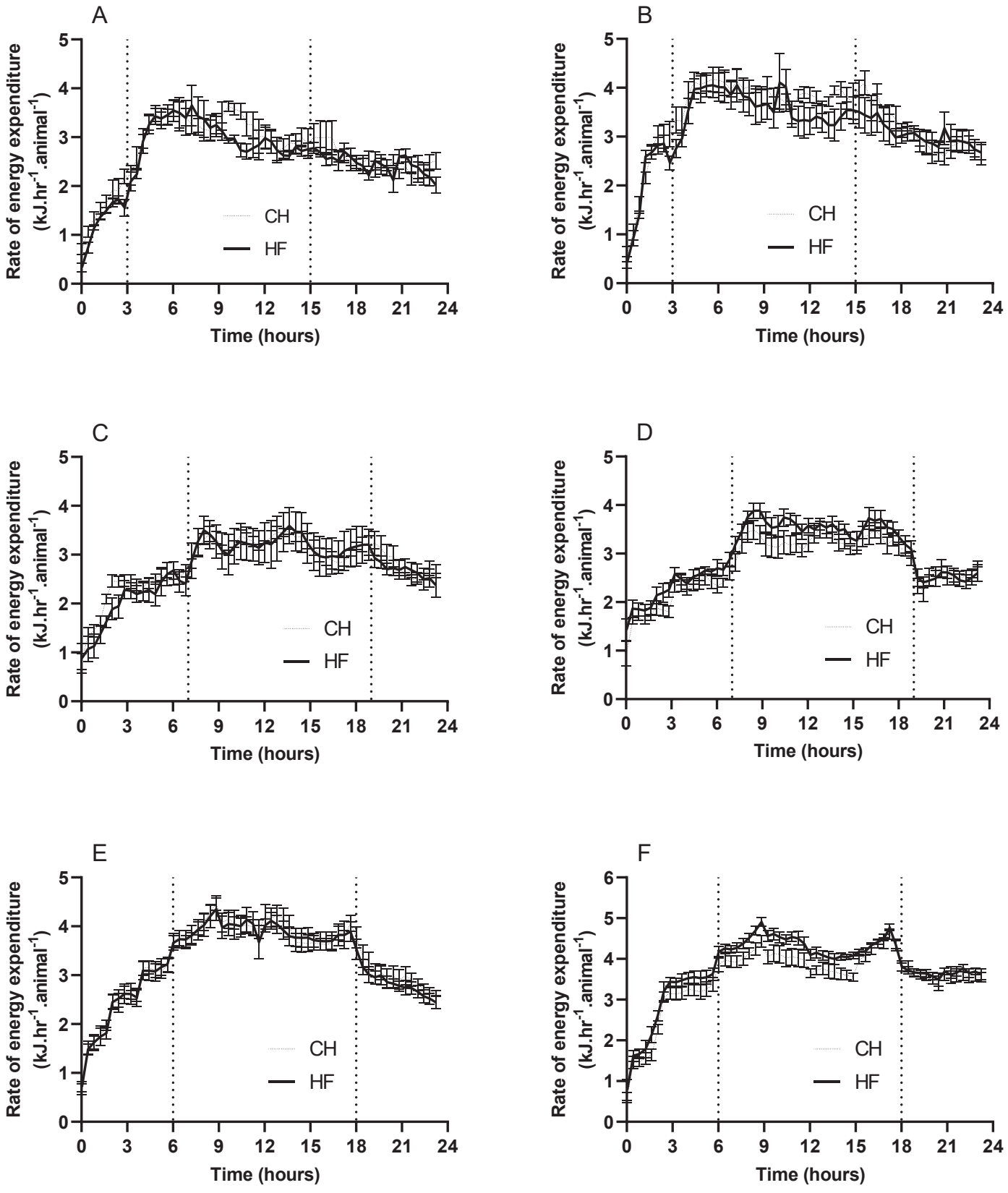


***Figure 5.*** *Energy expenditure in male and female offspring at age 6 weeks (A females, B males), 11 weeks (C females, D males) and 6 months (E females, F males) from mothers fed either a standard chow (CH) or high fat (HF) diet, (n = 5 per group). Values are expressed as means ± S.E.M.*

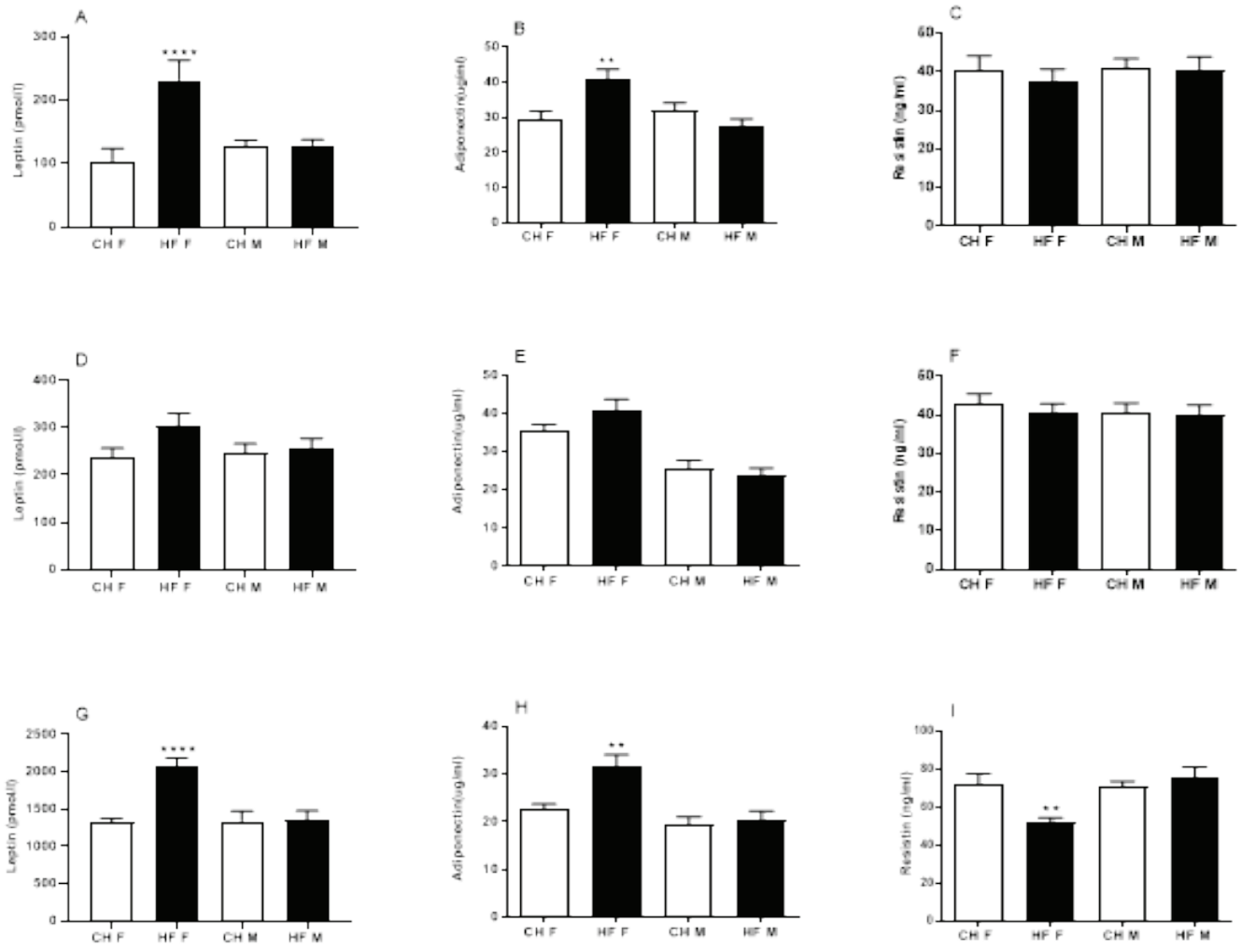


***Figure 6.*** *Plasma leptin, adiponectin and resistin at age 6 weeks (A leptin, B adiponectin, C resistin), 12 weeks (D leptin, E adiponectin, F resistin) and 6 months (G leptin, H adiponectin, I resistin), in male (M) and female (F) offspring from mothers fed a standard chow (CH) or 42% fat diet (HF), (n = 15 per group). **P<0.01; *** P<0.0001. Values are expressed as means ± S.E.M..*

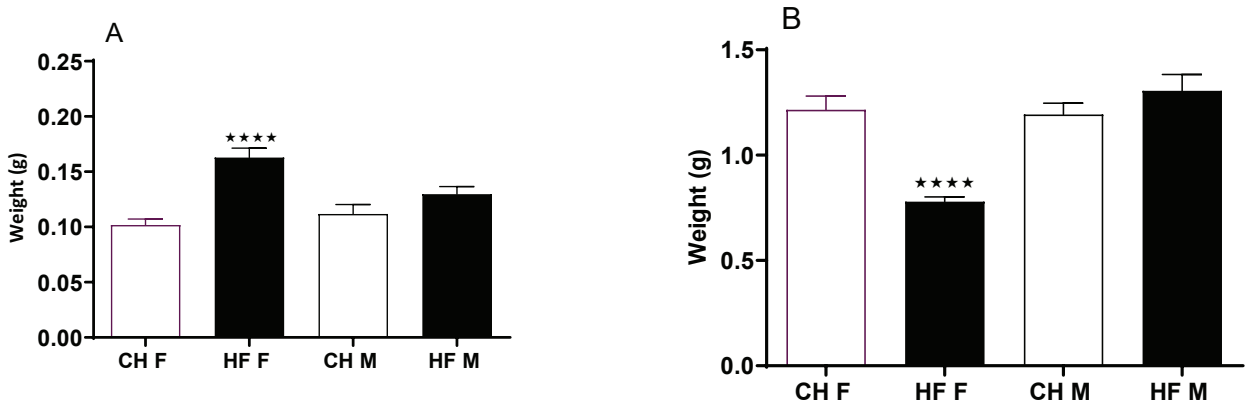


***Figure 7.*** *Gonadal WAT weights (average weights of both pads from each mouse) in 6-week-old (A) and 6-month-old (B) male (M) and female (F) offspring from mothers fed a standard chow (CH) or 42% fat diet (HF), (n = 15 per group).****P<0.0001. Values are expressed as means ± S.E.M.*

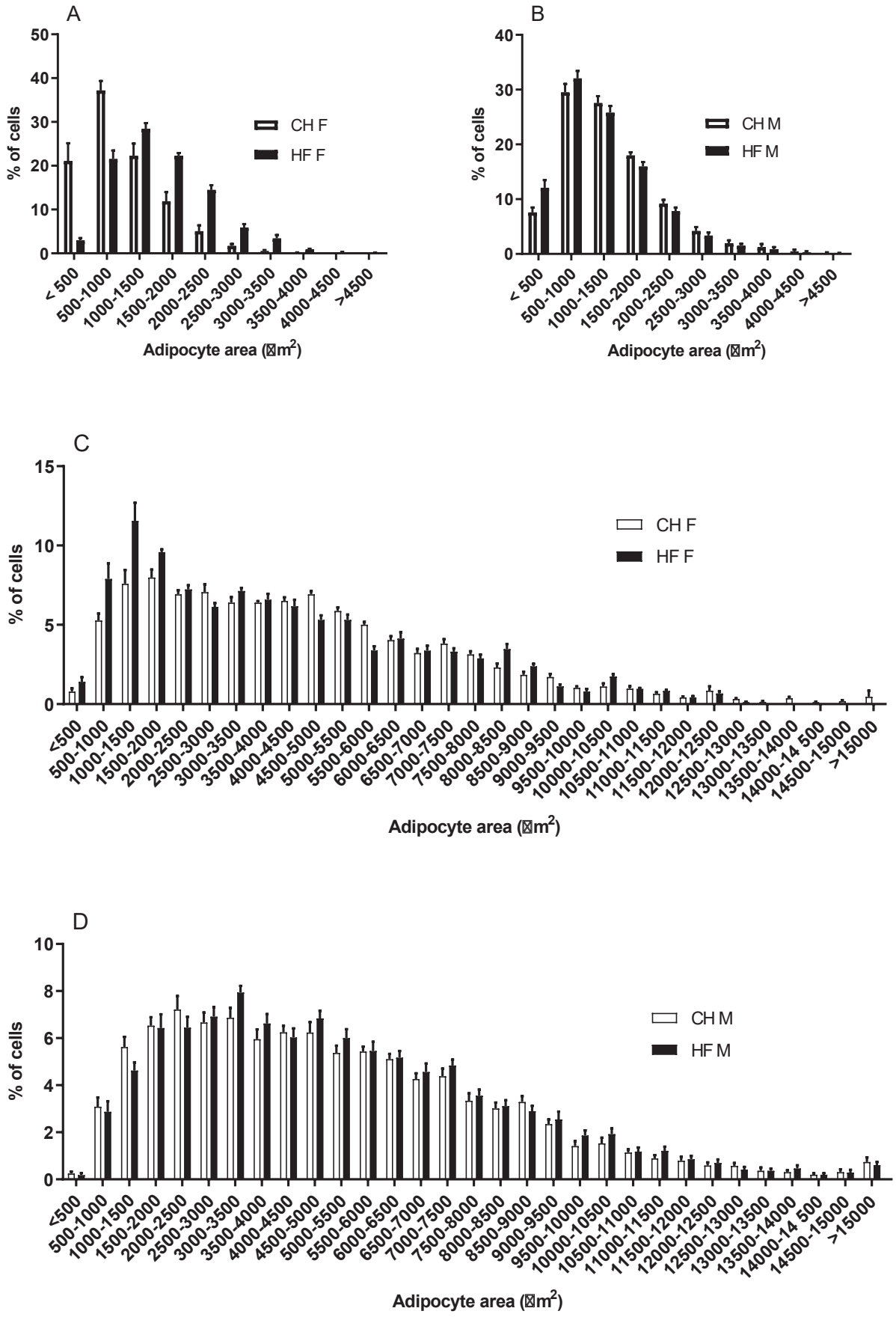


***Figure 8.*** *Gonadal adipocyte size in 6-week-old (A female, B male) and 6-month-old (C female, D male) offspring from mothers fed a standard chow (CH) or 42% fat diet (HF), (n = 15 per group). *P<0.05, **P<0.01, **** P<0.0001. Values are expressed as means ± S.E.M.*

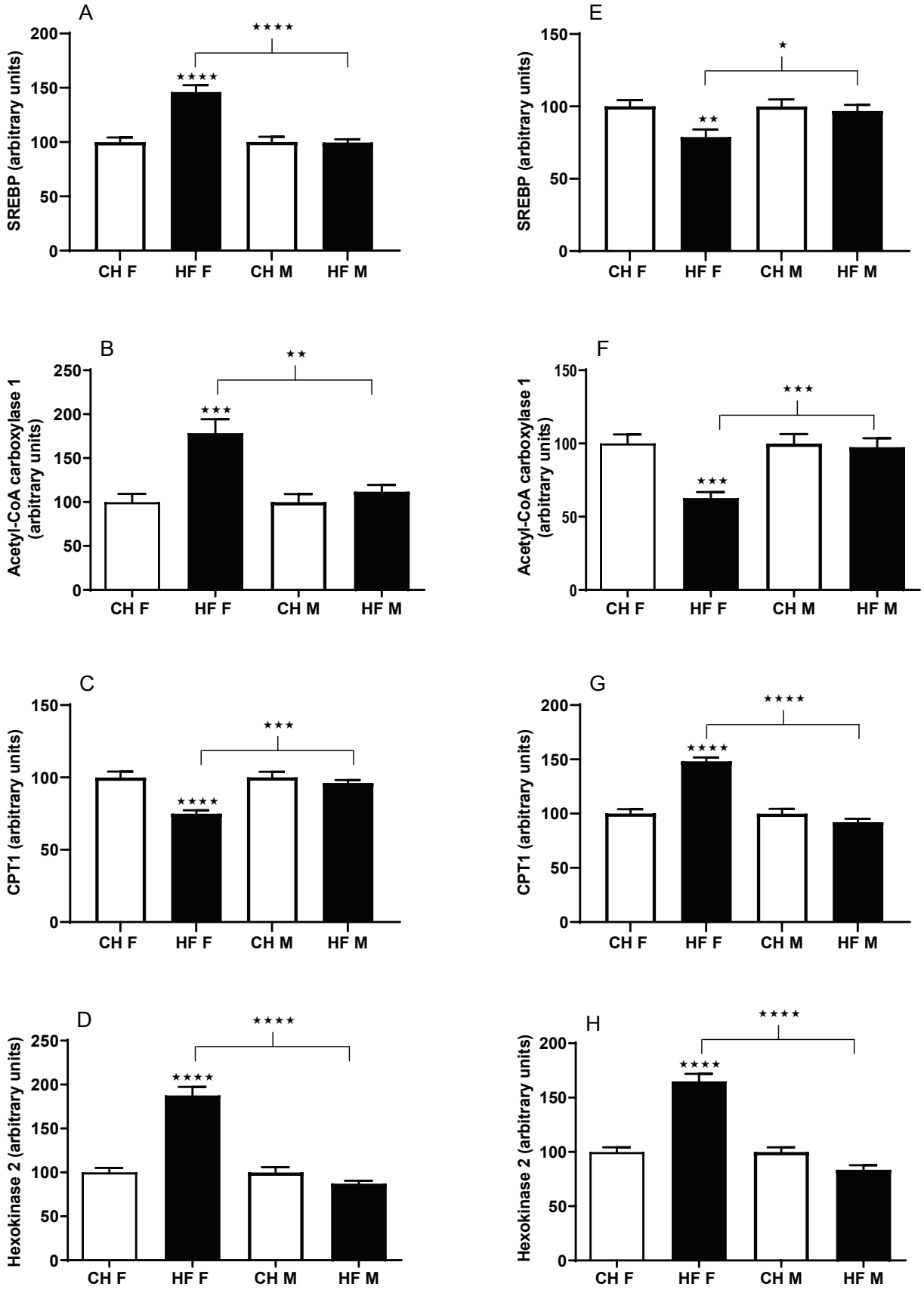


***Figure 9.*** *RT-PCR validation of the differentially expressed genes in the gonadal fat pads of male and female offspring at age 6 weeks (A - D ) and 6 months (E - H) from mothers fed either a standard chow (CH) or high fat (HF) diet, (n = 15 per group). A and E: SREBP, B and F: acetyl-CoA carboxylase, C and G: carnitine palmitoyl transferase 1, D and H: hexokinase 2. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Values are expressed as means ± S.E.M.*

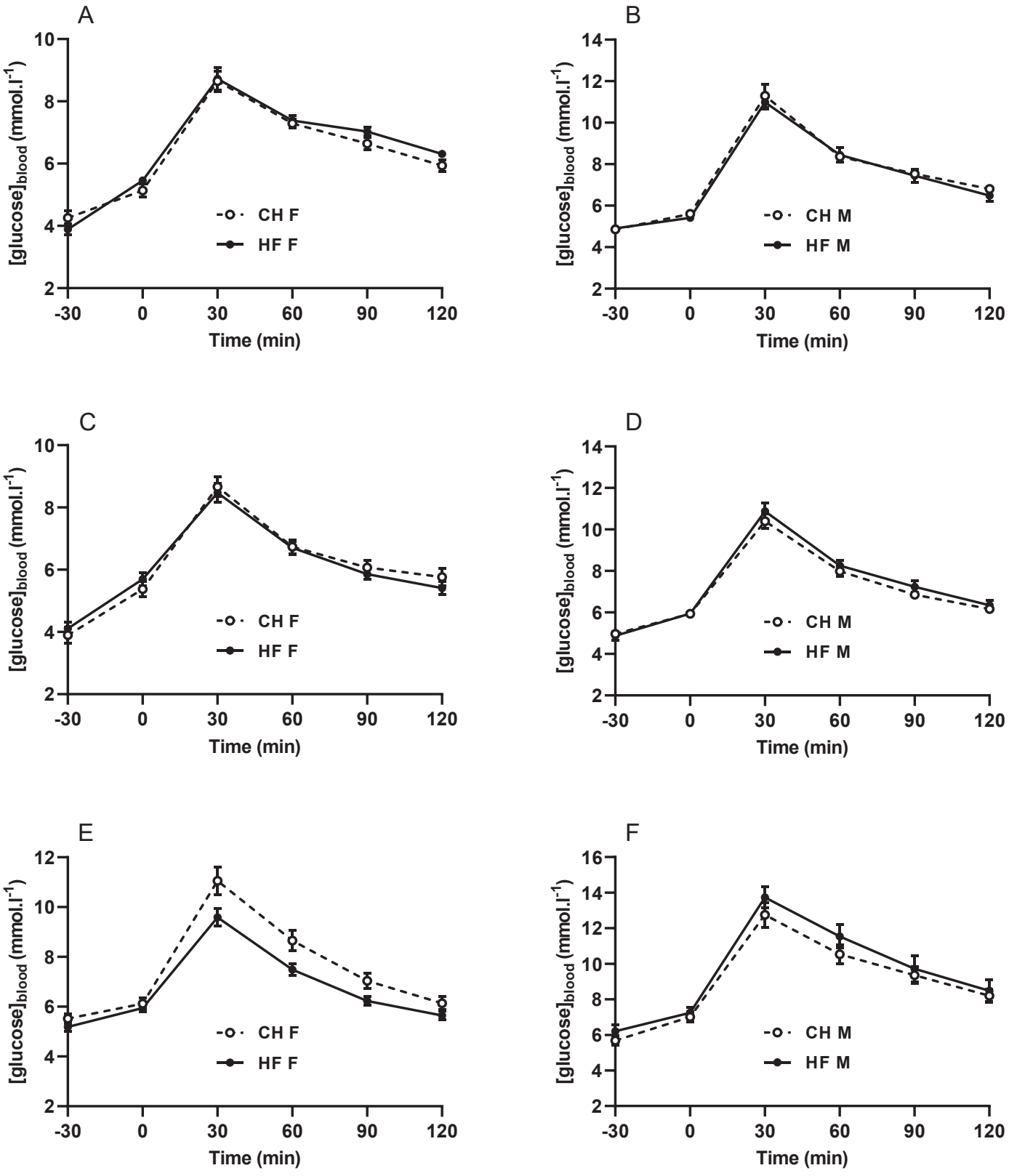


***Figure 10.*** *Blood glucose levels in an oral glucose tolerance test in male and female offspring at age 6 weeks (A females, B males), 12 weeks (C females, D males) and 6 months (E females, F males) from mothers fed either a standard chow (CH) or high fat (HF) diet, (n = 15 per group). *P<0.05, **P<0.01. Values are expressed as means ± S.E.M.*

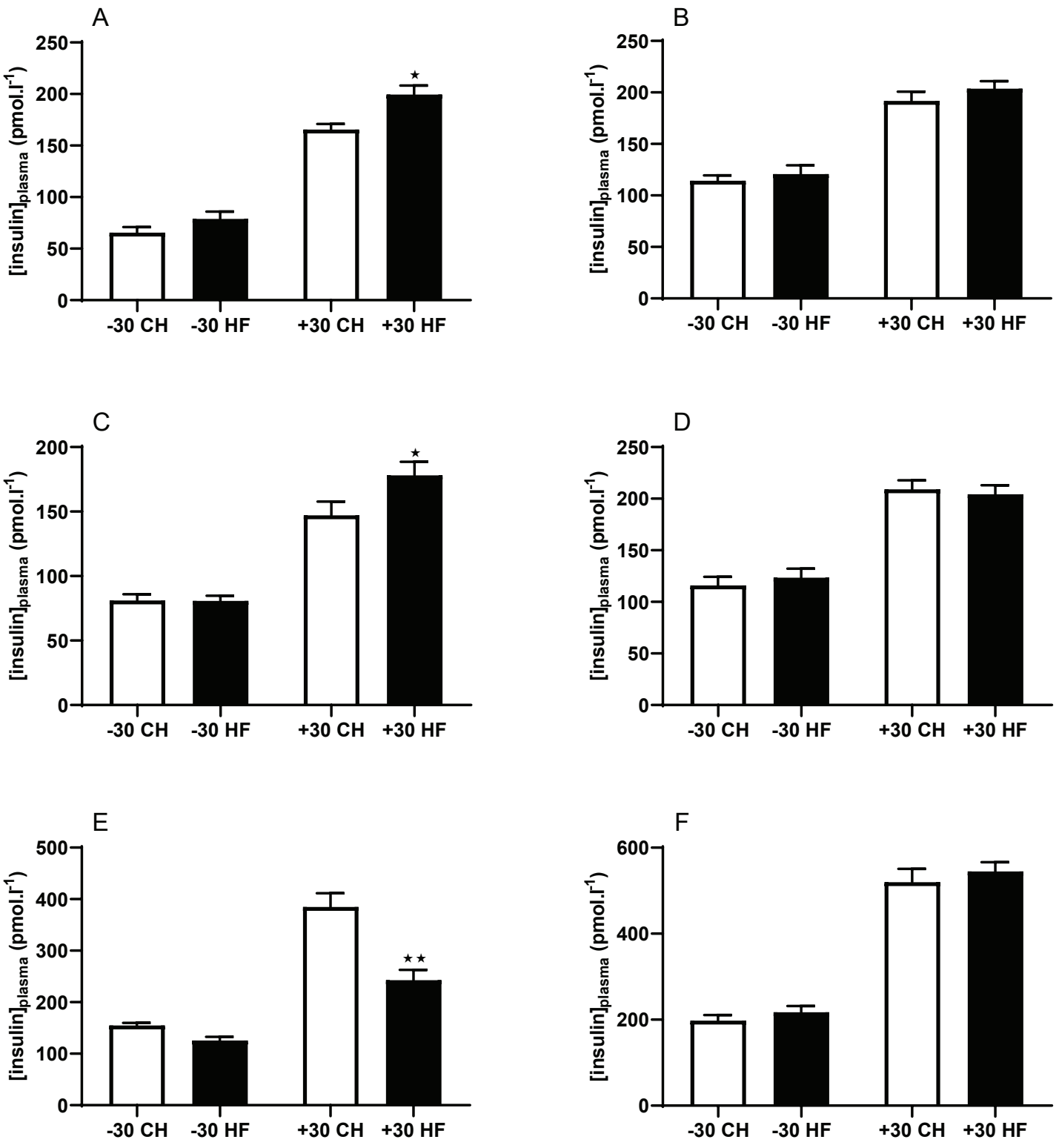


***Figure 11.*** *Plasma insulin levels 30 minutes before (-30) and after (+30) an oral glucose load in male and female offspring at age 6 weeks (A female, B male), 12 weeks (C female, D male) and 6 months (E female, F male) from mothers fed either a standard chow (CH) or high fat (HF) diet, (n = 15 per group). *P<0.05, **P<0.01. Values are expressed as means ± S.E.M.*

0.05, Figure 2A). However, the absolute fat mass was not different between these mice (F CH: 2.8 ± 0.2 g vs. F HF: 3.2 ± 0.2 g, P = 0.1, figure 2A).

*At 6 weeks of age male offspring from mothers fed a high fat diet had a shorter body length but did not contain a higher proportion of fat than those from mothers fed standard chow*

Similar to the female offspring, the body length of male mice from mothers fed on a high fat diet was significantly less than that of mice from mothers fed on chow (M CH: 9.05 ± 0.08 cm vs. M HF: 8.75 ± 0.09 cm, P < 0.05, figure 3A). Similarly, tibia length of male mice from mothers fed high fat diet was significantly less than that of mice from mothers on chow (M CH: 2.37 ± 0.01 cm vs. M HF: 2.31 ± 0.01 cm, P < 0.001, figure 3B). The bodyweight of 6-week-old male offspring of dams fed high fat diet was not less than from mothers fed standard chow (M CH: 20.4 ± 0.4 g vs. M HF: 19.2 ± 0.5 g, P = 0.08, figure 2B). By contrast with the female offspring, the lean mass of male mice from mothers fed high fat diet was significantly less than that of mice from mothers on chow, (M CH: 18.8 ± 0.5 g vs. M HF: 17.5 ± 0.5 g, P = 0.04, figure 2B).

There was no difference in fat mass between six-week-old male mice from mothers fed high fat diet and mice from mothers on chow (M CH: 1.61 ± 0.05 g vs. M HF: 1.72 ± 0.08 g, P = 0.78, figure 2B). Unlike the female offspring, the proportion of fat in these mice was also no different (M CH: 8.0 ± 0.3 % vs. M HF: 9.1 ± 0.6 %, P = 0.45, figure 2B).

*Female but not male offspring had decreased energy intake at 6 weeks of age*

At 6 weeks of age the female offspring of high fat fed dams consumed significantly less energy than their control groups (F CH: 3.0 ± 0.1 g.animal-1.day-1 vs. F HF: 2.7 ± 0.1 g.animal$^{-1}$.day$^{-1}$, P < 0.05, figure 4A). Maternal diet had no effect on energy consumption in male offspring (M CH: 3.7 ± 0.1 g.animal$^{-1}$.day$^{-1}$ vs. M HF: 3.7 ± 0.1 g.animal$^{-1}$.day$^{-1}$, P = 0.98). There was no difference in the energy expenditure between offspring of either sex from mothers fed high fat or chow (figures 5A and 5B).

*Fasting plasma leptin and adiponectin concentrations were higher in female, but not male, offspring from dams fed on a high fat diet*

Consistent with the percentage fat, 6-week-old female but not male offspring of high fat-fed dams had increased fasting plasma leptin concentrations compared to offspring of chow-fed dams (F CH: 105 ± 19 pmol.l$^{-1}$ vs. F HF: 232 ± 31 pmol.l$^{-1}$, P < 0.0001, figure 6A). They had also significantly elevated fasting adiponectin levels (F CH: 29 ± 2 µg.ml$^{-1}$ vs. F HF: 41 ± 3 µg.ml$^{-1}$, P < 0.01, figure 6B). Plasma leptin and adiponectin were not altered in the male offspring. Fasting plasma resistin was not affected by maternal diet in either sex at 6 weeks of age (figure 6C).

*The gonadal fat pads were larger in 6-week-old female offspring of dams fed high-fat diet and contained larger adipocytes, whereas male offspring had proportionally more smaller adipocytes*

Consistent with the percentage fat, 6-week-old female but not male offspring of high fat-fed dams had heavier average gonadal fat depot weights than females from chow-fed dams (F CH: 102 ± 5 mg vs. F HF: 163 ± 9 mg, P < 0.0001 (Figure 7A). There was no effect of maternal diet on gonadal fat pad weight in 6-week-old male offspring.

Six-week-old female offspring had significantly fewer adipocytes smaller than 1000 µm2 and significantly more adipocytes larger than 1000 µm$^2$ in offspring from mothers on high fat diet than in offspring from mothers on standard chow (Figure 8A). 6-week-old male mice from mothers on high fat diet had significantly more adipocytes smaller than 500 µm$^2$ but no difference in numbers of adipocytes larger than this (Figure 8B).

Consistent with the percentage fat, 6-week-old female but not male offspring of high fat-fed dams had increased gonadal adipose gene expression of sterol regulatory element-binding protein (SREBP, P < 0.0001, Figure 9A) and acetyl CoA carboxylase 1 (ACC1, P < 0.001 Figure 9B), whereas expression of carnitine palmitoyl transferase was significantly decreased (P < 0.0001, Figure 9C). Hexokinase 2 gene expression was significantly increased in female offspring of dams fed high-fat diet (P < 0.0001, Figure 9D). The expression of these genes was also significantly different to that in the male gonadal adipose tissue (SREBP, P < 0.0001, Figure 9A; acetyl CoA carboxylase 1, P < 0.01 Figure 9B; carnitine palmitoyl transferase, P < 0.0001, Figure 9C; hexokinase 2, P < 0.0001, Figure 9D).

*Maternal high fat diet had no effect on glucose tolerance in 6-week-old mice, but insulin levels were raised in females*

Maternal diet had no effect on oral glucose tolerance or fasting plasma insulin concentrations in either male or female offspring (Figures 10A,10B,11A,11 B). Female offspring did, however, have significantly higher plasma insulin levels 30 minutes after the glucose load (F CH: 165 ± 5 pmol.l$^{-1}$ vs. F HF: 200 ± 9 pmol.l$^{-1}$, P < 0.05, Figure 11A) but males did not (M CH: 192 ± 9 pmol.l$^{-1}$ vs. M HF: 204 ± 7 pmol.l$^{-1}$, P = 0.3, Figure 11B).

## Parameters in 10- to 12-week-old offspring

By 12 weeks of age, the effect of maternal diet on female and male offspring body weight and composition (Figures 2C,2D) and fasting plasma leptin, adiponectin and resistin (Figures 6D,E,F) had disappeared. The differences in food consumption (Figure 4B) and glucose-induced insulin secretion (Figure 11C) in female offspring remained. There was no difference in any of these measured parameters in male offspring (Figures 4B,6D,6E,6F,11D). Neither was there a difference in offspring of either sex at this age in energy expenditure (Figures 5C,5D) or glucose tolerance (Figures 10C,10D).

## Parameters in 6-month-old offspring after 3 months on high fat diet

*After 3 months on the high fat diet female offspring from mothers fed a high fat diet were leaner than those from mothers fed standard chow*

Female offspring of 6-month-old dams, which had been fed on a high fat diet for three months, gained less weight than offspring from dams on chow (F CH: 32.3 ± 0.6 g vs. F HF: 28.6 g ± 1.0 g, P < 0.05, Figure 2E). They also had a lower fat mass (F CH: 12.0 ± 0.7 g vs. F HF: 8.5 g ± 0.7 g, P < 0.01, Figure 2E). There was no difference in lean mass .

Maternal diet had no effect on body weight, lean or fat mass in the male offspring fed high fat diet between the age of 3 and 6 months (Figure 2F).

*After 3 months on high fat diet, female, but not male, offspring of high fat-fed dams had decreased energy intake*

Consistent with their lower weight gain, female mice from high fat fed-dams consumed significantly less high fat diet at age 6 months than the female offspring from mothers on standard chow diet (F CH: 3.3 ± 0.2 g.animal$^{-1}$.day$^{-1}$ vs. F HF: 2.7 ± 0.1 g.animal $^{1}$.day$^{-1}$, P < 0.05, Figure 4C). Maternal diet had no effect on energy consumption in male offspring. There was no difference in the energy expenditure between offspring of either sex from mothers fed high fat or chow.

### Morphology of gonadal fat depot

Consistent with their lower body fat content, female offspring of high fat-fed dams had lighter gonadal fat depots than females from chow-fed dams (F CH: 1.22 ± 0.07 g vs. F HF: 0.78 ± 0.02 g, $P < 0.0001$, Figure 7B). There was no effect of maternal diet on gonadal fat pad weight in male offspring.

There were significantly more adipocytes smaller than 2000 $\mu m^2$ in female offspring from mothers on high fat diet than in female offspring from mothers on standard chow (Figure 8C). There was no effect of maternal diet on adipocyte size in the male offspring (Figure 8D).

Consistent with the percentage fat, 6-month-old female but not male offspring of high fat-fed dams had decreased gonadal adipose gene expression of sterol regulatory element-binding protein (SREBP, $P < 0.01$, Figure 9E) and acetyl CoA carboxylase 1 ($P < 0.001$, Figure 9F), whereas expression of carnitine palmitoyl transferase was significantly increased ($P < 0.0001$, Figure 9G). Hexokinase 2 gene expression was significantly increased in female offspring of dams fed high-fat diet ($P < 0.0001$, Figure 9H). The expression of these genes was also significantly different to that in the male gonadal adipose tissue (SREBP, $P < 0.05$, Figure 9E; acetyl CoA carboxylase 1, $P < 0.001$ Figure 9F; carnitine palmitoyl transferase, $P < 0.0001$, Figure 9G; hexokinase 2, $P < 0.0001$, Figure 9H).

*High fat diet-induced glucose intolerance and elevated plasma insulin concentration were improved in female, but not male, offspring from dams fed high fat diet*

At six months of age, after 3 months on high fat diet, female offspring from dams fed on the high fat diet had better glucose tolerance than female offspring of dams fed on the chow diet (Figure 10E, $P < 0.05$ by 2-way ANOVA). Female offspring also had significantly lower plasma insulin levels 30 minutes after glucose load (F CH: 385 ± 27 $pmol.l^{-1}$ vs. F HF: 243 ± 20 $pmol.l^{-1}$, $P < 0.01$, Figure 11E). Maternal diet had no effect on either blood glucose or plasma insulin levels before or after glucose load in a tolerance test in male offspring (Figures 10F,11F).

*After 3 months on high-fat diet fasting plasma leptin and adiponectin concentrations were higher, and resistin levels lower, in female, but not male, offspring from dams fed on the high fat diet*

By contrast with their lower body fat content, female offspring from high fat-fed dams had higher fasting plasma leptin concentrations than offspring of chow-fed dams (F CH: 1330 ± 43 $pmol.l^{-1}$ vs. F HF: 2077 ± 115 $pmol.l^{-1}$, $P < 0.0001$, Figure 6G). They also had significantly elevated fasting adiponectin levels (F CH: 23 ± 1 $\mu g.ml^{-1}$ vs. F HF: 32 ± 2 $\mu g.ml^{-1}$, $P < 0.01$, Figure 6H). Fasting plasma resistin was significantly reduced in female offspring from mothers on a high fat diet (F CH: 72 ± 5 $ng.ml^{-1}$ vs. F HF: 52 ± 2 $ng.ml^{-1}$, $P < 0.01$, Figure 6I). Plasma leptin, adiponectin and resistin levels were not altered by maternal diet in the male offspring (Figures 6G,6H,6I, respectively).

## Discussion

To try to model the effect of obesity in pregnant women on susceptibility to obesity and diabetes in their offspring, we have fed pregnant mice on chow or, from ten weeks prior to mating, high fat diets. Their offspring were fed on a chow diet between weaning and 12 weeks of age and then on a high fat diet to 6 months. There were striking differences between the effects of the diet of the dams on the female offspring at six weeks and six months of age. The diet of the dams had less effect on the male offspring, and there was little difference in its effects on the males at six weeks and six months of age. We acknowledge that for logistical and ethical reasons, including the restriction of our UK Home Office licence, we could not conduct all measurements at the ideal time. Nevertheless, we believe that our results have value. Other studies have the same limitations. We also point out that our study was designed to investigate differences between offspring of dams fed on a chow or high fat diet and, within those categories, differences between male and female offspring. It did not allow us to compare offspring of the same age fed on a high fat versus a chow diet.

Focusing in more detail first on the female offspring, at six weeks of age the offspring of the high fat-fed dams ate less and their body length was less than the offspring of the chow-fed dams. However, they had a higher percentage body fat, higher plasma leptin and adiponectin, and heavier gonadal fat pads with larger adipocytes. Consistent with these findings, they appeared to achieve a normal glucose tolerance by secreting more insulin from enlarged pancreatic insulin stores. By contrast, at six months of age, which was after all the offspring had been fed for three months on a high fat diet, they were eating less and had gained less weight than the offspring of the chow-fed dams. Reduced weight gain could be accounted for by decreased fat accumulation, there being no effect on lean mass, indicating an increased high fat diet feed efficiency in the female offspring from dams themselves fed high fat diet [34]. Their body fat content and gonadal fat pad weights were now lower, and they had more small adipocytes than the offspring of the chow-fed dams. Consistent with these findings, they had lower resistin levels, better glucose tolerance and lower plasma insulin. It was surprising that, despite being leaner, their plasma leptin and adiponectin levels remained raised. We speculate below that that the female offspring of mothers fed on the high-fat diet were predisposed to hyperleptinemia, with the consequence that they were resistant to exposure to high-fat diet later in adult life.

The six-week-old male offspring of the high fat-fed dams were similar to the female offspring in having a lower body and tibia length than the offspring of the chow-fed dams. However, their food intake was not lower and neither did they display the paradoxical increases in percentage body fat and plasma leptin. They had higher numbers of small adipocytes, contrasting with the higher numbers of large adipocytes in the females. Consistent with the absence of differences in percentage body fat and plasma leptin, glucose tolerance and plasma insulin levels were not affected by the diet of the dams, though pancreatic insulin content was increased. At six months of age, there were no detectable effects in the male offspring of feeding their mothers on a high fat diet. So, the relatively mild effects in the six-week-old males had disappeared at six months of age, whereas females had flipped from being adversely affected by their mothers' high fat diet to appearing to have benefitted.

Other workers have reported diverse effects of feeding high fat diets to rat or mouse dams during pregnancy. Reductions, increases and no effect on birth weight have been described. These differences have been ascribed to differences in the proportion of fat in the diet, the timing of the intervention, whether rats or mice were studied and their age [19,20]. Studies on these offspring have also produced conflicting results [23]. Similar to the present study, Chang et al. [36] observed a small reduction in bodyweight in male offspring from mothers fed a high fat diet was transiently significant (at 4, 6 and 9 weeks of age, but not overall during the first 12 weeks of life on a standard chow diet). When these workers fed the offspring on a high fat diet from 13 weeks of age, the males gained more weight than the females and it was only the males in which the diet of the dams exacerbated obesity and glucose tolerance. They did not find the female offspring of high fat-fed

dams had less body fat and improved glucose tolerance as we did, but nevertheless, the female offspring appeared metabolically healthier than the males, as in the present study.

Findings that conflict with the general view that offspring of obese or high fat-fed mothers are more susceptible to obesity and impaired glucose homeostasis have been reported not only in rodents but also in humans [Current refs 27 (Howie) and 28 (Akyol)]. It may also be relevant that, in a cross-sectional study, a higher BMI in the mother was associated with a decreased risk of overweight or obesity in their female adolescent children [Shafaghi K 2014 Asia Pac J Clin Nutr 23(2):225-31], reminiscent of our results for female offspring of dams fed on a high fat diet. BMI was not measured before pregnancy in the study in humans, and the explanation for the surprising result may lie in societal factors. However, if so, such societal factors must affect the BMI of fathers and mothers differently, because a higher BMI in fathers was associated with an increased risk of overweight or obesity in their female adolescent children. Thus, our findings add to those that disagree with the general view of how the diet and obesity in the dam affect her offspring and may be relevant to similar findings in humans. Our model offers a means to investigate the mechanisms involved.

We suggest that at least two opposing primary forces are at play in studies such as ours. Firstly, the feeding dams on the high fat diet alters the growth and the partitioning of energy between fat and lean body mass in the foetus in a sexually dimorphic manner. Although we did not measure foetal growth, some other studies [18,19,27] have found that feeding the dams on a high fat diet reduced birth weight. Secondly, feeding the offspring on the high fat diet also affects the males and the females differently. Males are known to be more susceptible to the obesogenic effect of high fat diets [35], added to which there may be a different interaction with the effects of the dams' diets, such that inor variations between protocols may tip outcomes in opposite directions.

The mechanisms whereby feeding dams on the high fat diet alters the growth and the partitioning of energy between fat and lean body mass in the foetus are likely to be complex. We have previously reported evidence that suggests that leptin might play a role [29]. Leptin might also be a crucial part of the mechanism whereby the female offspring of the high fat-fed mice, when fed on a high fat diet, eat less, have a lower fat content, with decreased gonadal fat expression of acetyl CoA carboxylase 1, and increased expression of carnitine palmitoyl transferase 1 compared to the female offspring of the chow-fed mice. Thus, an especially intriguing finding was that at six months of age the female offspring of the high fat-fed dams had higher leptin levels than the female offspring of the chow-fed dams, despite having less fat. The mechanism behind these high leptin levels requires further investigation. Assuming that leptin secretion rather than degradation is key to the explanation, it is first necessary to ascertain whether the leptin levels are inconsistent with the numbers of adipocytes of various sizes in the whole animal. If this is the case, hormonal and molecular mechanisms than regulate leptin secretion [36] must be compared between female offspring of chow- and high fat-fed mice. Unfortunately, leptin levels were not measured in the rodent [37].

In conclusion, feeding dams on a high fat diet differentially affects the susceptibility of their male and female offspring to obesity and impaired glucose homeostasis. Surprisingly, when the female offspring were fed on a high fat diet, they were partially protected from obesity and impaired glucose homeostasis. This study is the first to show the opposing molecular adaptations to low- and high-fat diet in the adipose tissue of female offspring of obese mothers, with a lack of adaption in the male offspring. This may represent an important factor in females being more resistant to the metabolic consequences of a high fat diet. Further investigation of the mechanisms underlying these findings may help to explain similar unusual findings in rodents and humans.

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