Resveratrol Intake During Pregnancy and Lactation Modulates the Early Metabolic Effects of Maternal Nutrition Differently in Male and Female Offspring

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Poor maternal nutrition can have detrimental long-term consequences on energy homeostasis in the offspring. Resveratrol exerts antioxidant and antiobesity actions, but its impact during development remains largely unknown. We hypothesized that resveratrol intake during pregnancy and lactation could improve the effects of poor maternal nutrition on offspring metabolism. Wistar rats received a low-fat diet (LFD; 10.2% kcal from fat) or high-fat diet (HFD; 61.6% kcal from fat), with half of each group receiving resveratrol in their drinking water (50 mg/L) during pregnancy and lactation. Body weight (BW) of dams was measured at treatment onset and weaning [postnatal day (PND) 21] and of pups at birth and PND21, at which time dams and pups were euthanized. Although HFD dams consumed more energy, their BW at the end of lactation was unaffected. Mean litter size was not modified by maternal diet or resveratrol. At birth, male offspring from HFD and resveratrol (HFD + R) dams weighed less than those from LFD and resveratrol (LFD + R) dams. On PND21, pups of both sexes from HFD dams weighed more, had more visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT), and had higher serum leptin levels than those from LFD dams. Resveratrol reduced BW, leptin, VAT, and SCAT, with females being more affected, but increased glycemia. Neuropeptide levels were unaffected by resveratrol. In conclusion, resveratrol intake during pregnancy and lactation decreased BW and adipose tissue content in offspring of dams on an HFD but did not affect offspring from LFD-fed dams, suggesting that the potential protective effects of resveratrol during gestation/lactation are diet dependent. (***Endocrinology*** **159: 810–825, 2018)**

Poor maternal nutrition during pregnancy and lactation can have adverse short- and long-term consequences on energy homeostasis in the offspring. Indeed, numerous studies indicate that adequate nutrition during both gestation and lactation is critical in the establishment of the future endocrine and metabolic status of the offspring and their risk of various diseases in later life (1–4). Although the complex relationship between poor maternal nutrition and the offspring's metabolic outcome is not fully understood, developmental programming of metabolic

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Abbreviations: AgRP, Agouti-related protein; ANOVA, analysis of variance; BW, body weight; FASN, fatty acid synthase; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; HFD + R, high-fat diet and resveratrol; HOMA-IR, homeostatic model assessment for insulin resistance; HSL, hormone-sensitive lipase; InsR, insulin receptor; LDL-C, low-density lipoprotein cholesterol; LFD, low-fat diet; LFD + R, low-fat diet and resveratrol; LPL, lipoprotein lipase; mRNA, messenger RNA; NEFA, nonesterified fatty acid; NPY, neuropeptide Y; ObR, leptin receptor; PCR, polymerase chain reaction; PND, postnatal day; POMC, proopiomelanocortin; PPARγ, peroxisome proliferator–activated receptor γ; SCAT, subcutaneous adipose tissue; SIRT1, sirtuin 1; TG, triglyceride; UCP2, uncoupling protein 2; VAT, visceral adipose tissue.

neuronal circuits is most likely involved (5), with insulin and leptin playing a central role in this phenomenon (6, 7). In rodents, there is a neonatal leptin surge that promotes neuronal (6) and astroglial (8) growth and development, with the long-term metabolic effects of poor nutrition during development possibly being mediated through both of these cell types. In the postnatal animal, the hypothalamus is an important target for the anorectic actions of leptin and insulin, as well as for other metabolic signals, and these signaling pathways could possibly be altered by inadequate early nutrition. An adverse perinatal environment can also affect the development of other tissues, such as adipose tissue, also potentially affecting metabolic function later in life (9, 10). Indeed, enhanced adipogenesis and lipogenesis, low-grade inflammation, and modifications in hormonal tissue sensitivity after maternal nutritional manipulation have been reported (11).

Although numerous studies have analyzed the impact of a poor maternal diet on long-term metabolism in the offspring, less is known regarding protective effects of dietary substances. Resveratrol (3,4′,5-tridydroxystilbene) is a natural phytoalexin found in several plants species, including grapes, peanuts, berries, and pine nuts, that has been investigated since the 1990s for its cardiovascular, antioxidant, and antiobesity actions (12–15). This natural antioxidant exerts its effects, at least in part, via activation of sirtuin 1 (SIRT1) (16). The consumption of resveratrol, which crosses the placenta (17), is reported to be safe during pregnancy (18). However, it is unknown whether resveratrol can improve the impact of poor maternal nutrition on the offspring.

We hypothesized that resveratrol intake during pregnancy and lactation would improve the early metabolic changes observed in the offspring of mothers on a high-fat diet (HFD). Moreover, as males and females can respond differently to nutritional and metabolic signals during development (19–22), we determined whether male and female offspring were differentially affected.

Materials and Methods

Animals

The study was designed according to the European Community Council Directive (86/609/EEC; 2010/63/UE) and National Institutes of Health guidelines for animal care and complied with the Royal Decree 53/2013 pertaining to the protection of experimental animals. The studies and use of animals were approved by the Commission of Investigation of the Hospital Universitario Puerta de Hierro-Majadahonda and Ethical Committee of Animal Experimentation of the Comunidad Autónoma of Madrid. The rats were always treated respectfully, and the least possible number of animals was used in all experiments.

Adult male and female Wistar rats (8 weeks of age) were purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain) and allowed to acclimate for 2 weeks before mating. All rats were maintained at a constant temperature (21 ± 1°C) and humidity (50% ± 1%) in a 12-hour light-dark cycle, with lights on at 7:30 AM. Virgin females were mated and given free access to rat chow (A04-10/15022; Panlab, Barcelona, Spain) and tap water during mating. After mating, they were randomly divided into two dietary groups, one receiving a low-fat diet (LFD; 3.8 kcal/g, 10.2% fat; 18.0% protein, 71.8% carbohydrate; LabDiet, Sodispan Research SL, Madrid, Spain) and the other an HFD (5.1 kcal/g, 61.6% fat; 18.1% protein, 20.3% carbohydrate; LabDiet) during pregnancy and lactation. Half of each dietary group received resveratrol (R; Tokyo Chemical Industries Co. Ltd., Tokyo, Japan) in their drinking water (50 mg/L *trans*-resveratrol). Although a wide range of doses of resveratrol has been used in previously reported studies, we chose this route of administration and dose in attempt to more closely simulate what might be consumed normally (2.25 mg/kg/d) (15, 23). During pregnancy and lactation, the dams were individually housed.

Resveratrol treatment

Due to its low solubility in water, resveratrol was first dissolved in ethanol as previously described such that the final concentration of ethanol was 0.5% (23). The same concentration of ethanol was used as vehicle in the water of the nonresveratrol-treated dams. This resulted in the following four groups of dams: LFD, LFD and resveratrol (LFD + R), HFD and HFD and resveratrol (HFD + R).

All drinking water was changed twice per week to avoid resveratrol oxidation, and drinking bottles were protected from light. The mean dose of resveratrol ingested was 2.0 to 2.5 mg/kg/d/dam. Dams were weighed the first day after being allowed to mate and at the end of lactation. Daily food intake of the dams was measured once a week throughout the experiment. To account for spillage, food was retrieved from the bedding before weighing.

Litter organization

Only litters from mothers that gave birth to between 6 and 14 pups were used for cross-fostering and for data analysis (exclusion of three small litters and one large litter, with no difference between maternal diet in the number of litters excluded). On the day of birth, pups were weighed and sexed and the litters adjusted to eight pups per mother with an equal number of males and females. Cross-fostering was performed by randomly mixing pups from dams of the same maternal experimental group (*e.g.*, pups from HFD mothers were only fostered to pups from HFD mothers). All litters contained fostered pups. Rats were raised in these litters from birth to postnatal day (PND) 21 when they were euthanized.

Euthanization and tissue collection

Pups were allowed to nurse undisturbed until euthanized to avoid stress. Dams were fasted for 12 hours before euthanization. Both the dams and pups were weighed and euthanized by rapid decapitation between 9:00 and 11:00 AM. Glycemia was determined by using a glucometer (Optium Xceed; Abbott Diabetes Care, Inc., Alameda, CA). Brains were removed and weighed. The hypothalamus, defined rostrally by the optic chiasm and caudally by the anterior margin of the mammillary

bodies, was dissected out, frozen on dry ice, and stored at −80°C until processed. Subcutaneous (inguinal) and visceral (perigonadal) adipose depots were removed, weighed, frozen on dry ice, and stored at −80°C until processed. The amount of each adipose tissue depot is expressed as mg/g of body weight (BW). Blood was collected from the trunk, allowed to clot on ice, and centrifuged at 3000 rpm during 10 minutes at 4°C, and the serum was removed and kept at −80°C until processed. A total of 26 litters were used to obtain the final adjusted litters for this study, with part of these pups being euthanized at PND21 and the remaining pups being allowed to mature for future studies. In the data reported here, there are eight pups/group. Only dams that nursed pups were used for data analysis.

Biochemical and hormonal measurements

Circulating leptin, insulin, and adiponectin levels were determined by enzyme-linked immunofluorescent assay following the manufacturer's instructions (Millipore, Billerica, MA). Absorbance in each well was measured by using a Tecan Infinite M2000 (Grödig, Austria) and the concentrations calculated from the standard curve. The sensitivity of the method was 0.08 ng/mL for leptin, 0.1 ng/mL for insulin, and 0.4 ng/mL for adiponectin. The inter- and intra-assay variations were 3.4% and 2.2% for leptin, 7.6% and 1.9% for insulin, and 1.8% and 7.3% for adiponectin, respectively. All samples (eight per experimental group chosen at random) were run in duplicate.

Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as insulin (mU/L) × glucose (mg/dL).

Lipid profile

Serum cholesterol, triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and phospholipid levels were measured by using commercial kits purchased from SpinReact (Sant Esteve de Bas, Gerona, Spain). Serum glycerol and nonesterified fatty acid (NEFA) levels were determined by using kits purchased from Sigma-Aldrich (St Louis, MO) and Wako Bioproducts (Richmond, VA), respectively. Samples were measured in duplicate and the assays were performed following the manufacturer's instructions.

Western blotting

Hypothalamic tissue was homogenized in radioimmunoprecipitation assay buffer containing an EDTA-free protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Protein concentration was estimated by using the Bradford protein assay (Bio-Rad, Hercules, CA). Depending on the protein to be detected, either 20 or 40 μg of protein was resolved under denaturing conditions on an 8% or 12% sodium dodecyl sulfate–acrylamide gel. Proteins were then transferred to a polyvinylidine difluoride membrane (Bio-Rad) and transfer efficiency determined by Ponceau red dyeing. Membranes were then blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% (w/v) nonfat milk or bovine serum albumin (phosphorylated proteins) and incubated with the appropriate primary antibody and concentration overnight at 4°C under agitation. The antibodies and the dilutions used are shown in Supplemental Table 1. The following day after washing, membranes were incubated with the secondary antibody conjugated with peroxidase (Pierce Biotechnology, 1:1000 or 1:2000). Peroxidase activity was visualized by using chemiluminescence and quantified by densitometry using an Image-Quant LAS4000 mini TL Software (GE Health care Europe GmbH, Barcelona, Spain). All blots were rehybridized with the nonphosphorylated form of the protein or actin to adjust for loading and then normalized to control values on each gel.

RNA preparation and quantitative real-time polymerase chain reaction

Relative levels of messenger RNA (mRNA) of adipokines and neuropeptides were determined in the hypothalamus and/or adipose tissue by real-time polymerase chain reaction (PCR). Total RNA was extracted from hypothalami by using Trizol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's recommendations. Total RNA from adipose tissue were extracted by using an RNeasy lipid tissue Mini kit (Qiagen, Hilden, Germany). The concentration and purity of total RNA were quantified using a Nanodrop (Thermo Scientific, Washington, DE). Complementary DNA was synthesized from 1 μg total RNA by using a high-capacity complementary DNA RT kit (Applied Biosystems, Foster City, CA). Quantitative reverse transcription PCR was performed by using TaqMan Universal PCR Master Mix (Applied Biosystems). TaqMan Gene Expression assay-on-demand kits (Supplemental Table 2) were used to analyze neuropeptides and receptors involved in metabolic control, including neuropeptide Y (NPY), Agouti-related protein (AgRP), orexin, proopiomelanocortin (POMC), the leptin receptor (ObR), the insulin receptor (InsR), and SIRT1. In perigonadal and subcutaneous adipose tissue, the relative mRNA levels of leptin, fatty acid synthetase (*Fas*), hormone-sensitive lipase (HSL), peroxisome proliferator–activated receptor γ (PPARγ), lipoprotein lipase (LPL), uncoupling protein 2 (UCP2), and SIRT1 were measured. All samples were run in duplicate. Various housekeeping genes were tested, and those that did not vary between experimental groups were chosen to normalize the data [phosphoglycerate kinase (Pgk1) in hypothalamic samples and 18S and peptidylprolyl isomerase A (or cyclophilin A) for adipose tissue]. The ΔΔCT method was used to determine relative expression levels and for statistical analysis. All data are expressed as % control group (LFD for dams and male LFD in the pups at PND21).

Statistical analysis

The program SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for data analysis. In dams, a two-way analysis of variance (ANOVA) was performed (factors: diet and resveratrol), followed by one-way ANOVA or two-tailed Student *t* test if appropriate. In pups, in each sex, two-way ANOVAs were used to determine the effect and interaction of the maternal diet and maternal resveratrol treatment. When appropriate, this was followed by one-way ANOVAs or two-tailed Student *t* test. Scheffé *F* test was used as a *post hoc* test to determine whether specific differences existed between the experimental groups. All data are presented as mean ± standard error of the mean. The results were considered statistically significant at $P < 0.05$. The *P* values in the figures represent the results of the one-way ANOVA or *t* test. Two-way ANOVA results are represented by letters (a: diet, c: resveratrol effect) on the figures when no statistically significant effect was found in the *post hoc* analyses.

Results

Mothers/dams

During lactation, the mean total energy intake of HFD mothers was higher than LFD mothers ($F_{(1, 26)}$ = 33.4, $P < 0.0001$), with resveratrol having no effect on this parameter (Fig. 1). There was no difference during pregnancy. Although energy intake was greater, no difference in BW (LFD: 258.7 ± 9.4 g, LFD + R: 241.3 ± 4.8 g, HFD: 250.3 ± 10.1 g, and HFD + R: 238.8 ± 4.5 g), weight gain (LFD: 43.7 ± 4.1 g, LFD + R: 30.6 ± 4.7 g, HFD: 36.4 ± 4.5 g, and HFD + R: 33.7 ± 4.0 g), or visceral adipose tissue (VAT) was found at the end of the lactation period. There were no differences in glycemia or serum leptin levels (data not shown), but maternal insulin levels were affected by diet [$F_{(1, 25)}$ = 10.2, $P < 0.01$], with LFD mothers having higher insulin levels than HFD mothers regardless of resveratrol ($P < 0.05$; Table 1). HOMA-IR was also affected by diet [$F_{(1, 23)}$ = 7.6, $P < 0.02$], with LFD dams having higher levels than HFD dams regardless of resveratrol treatment ($P < 0.05$; Table 1).

There was an interaction between diet and resveratrol on total cholesterol [$F_{(1, 26)}$ = 5.8, $P < 0.05$], HDL-C [$F_{(1, 24)}$ = 5.4, $P < 0.05$], and LDL-C [$F_{(1, 25)}$ = 15.5, $P < 0.001$] levels. Resveratrol increased total cholesterol ($P < 0.05$), LDL-C ($P < 0.001$), and HDL-C ($P < 0.05$) levels in the HFD + R group compared with LFD + R but decreased LDL-C levels in LFD dams (P = 0.05). Triglyceride levels were influenced by diet [$F_{(1, 26)}$ = 15.2, $P < 0.001$] and resveratrol [$F_{(1, 26)}$ = 6.9, $P < 0.05$], being increased by HFD and decreased by resveratrol regardless of diet. Glycerol levels were only affected by diet [$F_{(1, 23)}$ = 13.1, $P < 0.01$], with HFD mothers having higher levels than LFD mothers. There was no effect of maternal diet or resveratrol on serum levels of phospholipids or NEFA (Table 1).

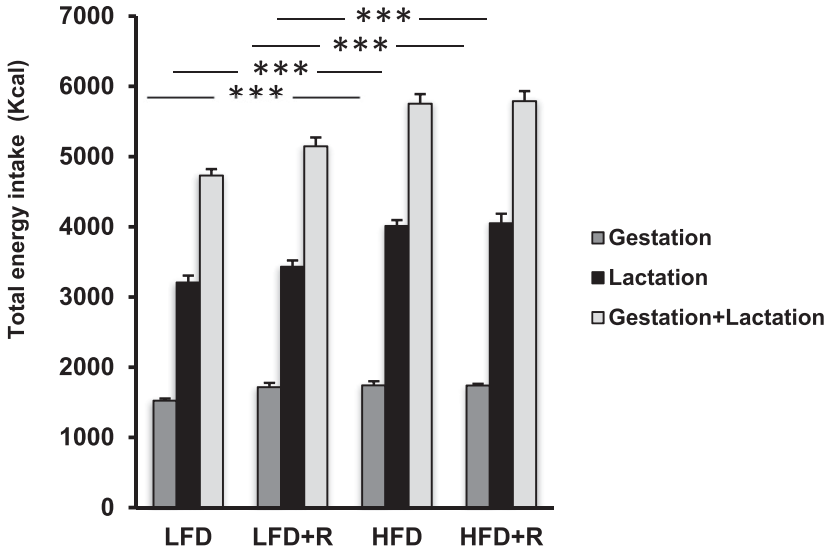

Figure 1. Energy intake of dams during gestation, lactation, and the total energy intake (n = 4 to 6). Data are shown as mean ± standard error of the mean. ***ANOVA: $P < 0.0001$.

PND0 (litter size and birth weight)

Neither maternal diet nor resveratrol affected mean litter size (LFD: 9.7 ± 2.9, LFD + R: 9.4 ± 0.8, HFD: 10.8 ± 1.1, and HFD + R: 11.2 ± 0.9 pups/litter). In males, there was an effect of diet [$F_{(1, 30)}$ = 8.9; $P < 0.003$], with an interaction between diet and resveratrol [$F_{(1, 30)}$ = 7.3; $P < 0.01$]. Male offspring from LFD + R dams weighed more than pups from all other groups ($P < 0.0001$; Fig. 2A). There was no effect of maternal diet or resveratrol intake on the birth weight of female offspring (Fig. 2B).

Pups at PND21

BW

At weaning (PND21), both males and females from HFD mothers weighed more than those from LFD mothers [diet effect: males: $F_{(1, 45)}$ = 52.0, $P < 0.0001$; females: $F_{(1, 43)}$ = 30.4, $P < 0.0001$; Fig. 2C and 2D] with an interaction between diet and resveratrol [males: $F_{(1, 45)}$ = 9.4, $P < 0.01$; females: $F_{(1, 43)}$ = 24.0, $P < 0.0001$]. When split by diet, resveratrol increased the BW in LFD offspring in both sexes, but this only reached statistical significance in females ($P < 0.05$). There was also a resveratrol effect decreasing BW in those pups from HFD mothers (males: $P < 0.05$; females: $P < 0.0001$).

Visceral and subcutaneous adipose tissue

The relative amount of VAT (Fig. 2E and 2F) was influenced by diet in both sexes [males: $F_{(1, 45)}$ = 109.3, $P < 0.0001$; females: $F_{(1, 45)}$ = 91.5, $P < 0.0001$] with higher VAT depots in HFD than LFD offspring. Resveratrol had an effect only in female offspring [$F_{(1, 45)}$ = 9.7, $P < 0.01$], with an interaction between diet and resveratrol [$F_{(1, 45)}$ = 12.4, $P < 0.001$]. When split by diet, resveratrol was found to reduce the amount of VAT in females from HFD mothers ($P < 0.0001$), with no effect in LFD offspring (Fig. 2F).

The percentage of subcutaneous adipose tissue (SCAT) was also influenced by diet [males: $F_{(1, 45)}$ = 31, $P < 0.0001$; females: $F_{(1, 44)}$ = 52.1, $P < 0.0001$; Fig. 2G and 2H], with offspring from HFD mothers having higher SCAT than LFD offspring. There was an interaction between diet and resveratrol [males: $F_{(1, 45)}$ = 6, $P < 0.05$; females: $F_{(1, 44)}$ = 7, $P < 0.05$], and when split by maternal diet, resveratrol was

Table 1. Metabolic Profile of Dams

Characteristic	LFD	LFD + R	HFD	HFD + R	ANOVA *P* Value
Insulin (ng/mL)	1.9 ± 0.6	2.3 ± 0.4	0.7 ± 0.1[a]	1.2 ± 0.2[b]	<0.05
Glycemia (mg/dL)	71.0 ± 3.6	75.0 ± 3.5	79.5 ± 4.9	75.3 ± 4.9	NS
HOMA-IR	9.7 ± 3.1	10 ± 1.5	4.2 ± 0.8	6.4 ± 1.1	<0.05
Total cholesterol (mg/dL)	82.6 ± 10.2	62.3 ± 5.6	76.2 ± 9.1	95.0 ± 7.2[b]	<0.05
LDL-C (mg/dL)	37.1 ± 0.5	30.6 ± 2.0[a]	36.6 ± 2.0	45.1 ± 2.7[b]	<0.001
HDL-C (mg/dL)	36.7 ± 6	21.9 ± 3.1	43.7 ± 6.4	39.1 ± 6.3[b]	<0.05
TG (mg/dL)	96.7 ± 7.6	74.6 ± 7.0	129 ± 8.5	107 ± 8.0[b]	<0.05
NEFA (mg/dL)	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	NS
Phospholipids (mg/dL)	1371 ± 168.2	1273.4 ± 68.3	1293.6 ± 43.0	1447.7 ± 99.7	NS
Glycerol (mg/dL)	33.4 ± 11.2	60.8 ± 20.9	142.4 ± 17.2	119.4 ± 30.3	NS

Data are shown as mean ± standard error of the mean.

Abbreviation: NS, not significant.

[a]Different from LFD.

[b]Different from LFD + R.

found to decrease SCAT in both males ($P < 0.0001$) and females ($P < 0.01$) from HFD dams.

Serum metabolic parameters

Glucose levels were affected by resveratrol treatment in both sexes [males: $F_{(1,\ 45)} = 8.5$, $P < 0.01$; females: $F_{(1,\ 45)} = 5.3$, $P < 0.05$; Fig. 3A and 3B]. Maternal resveratrol intake increased glucose levels in pups from both LFD and HFD mothers. Maternal diet had an effect on insulin levels [males: $F_{(1,\ 30)} = 9.8$, $P < 0.01$; females: $F_{(1,\ 32)} = 5.2$, $P < 0.05$; Fig. 3C and 3D] and HOMA-IR [males: $F_{(1,\ 30)} = 8.92$, $P < 0.01$; females: $F_{(1,\ 32)} = 4.6$, $P < 0.05$; Fig. 3E and 3F]. The offspring from HFD mothers had higher insulin levels and HOMA-IR than those from LFD mothers regardless of resveratrol treatment.

Circulating leptin levels were affected by diet [males: $F_{(1,\ 31)} = 78.5$, $P < 0.0001$; females: $F_{(1,\ 32)} = 66.2$, $P < 0.0001$; Fig. 3G and 3H]. Maternal HFD increased serum leptin in offspring of both sexes, with resveratrol having no effect (males and females: $P < 0.0001$).

In male offspring, circulating adiponectin levels were not different between the experimental groups (Fig. 3I). In female offspring, diet had an effect on serum adiponectin levels [$F_{(1,\ 29)} = 21.4$, $P < 0.0001$], being increased by HFD ($P < 0.001$; Fig. 3J).

Maternal diet determined serum levels of total cholesterol [males: $F_{(1,\ 44)} = 48.3$, $P < 0.0001$; females: $F_{(1,\ 45)} = 12.1$, $P < 0.001$; Fig. 4A and 4B], LDL-C [males: $F_{(1,\ 45)} = 47.4$, $P < 0.0001$; females: $F_{(1,\ 45)} = 14$, $P < 0.001$; Fig. 4C and 4D], HDL-C [males: $F_{(1,\ 44)} = 63.2$, $P < 0.0001$; females: $F_{(1,\ 44)} = 23.3$, $P < 0.0001$; Fig. 4E and 4F], NEFA [males: $F_{(1,\ 45)} = 24.8$, $P < 0.0001$; females: $F_{(1,\ 44)} = 16.6$, $P < 0.0001$; Fig. 4G and 4H], phospholipids [males: $F_{(1,\ 45)} = 43.1$, $P < 0.0001$; females: $F_{(1,\ 44)} = 25.4$, $P < 0.0001$; Fig. 4I and 4J], and glycerol [males: $F_{(1,\ 44)} = 36.5$, $P < 0.0001$; females: $F_{(1,\ 45)} = 7.6$, $P < 0.01$; Fig. 4K and 4L] in offspring of both sexes. Males and females from HFD dams had higher levels of these lipids compared with offspring from LFD dams. However, resveratrol supplementation in the maternal diet only affected serum NEFA [$F_{(1,\ 43)} = 4.1$, $P < 0.05$; Fig. 4G] and TG [$F_{(1,\ 44)} = 9$, $P < 0.01$; Fig. 4M] levels in males offspring, causing an overall increase. In females, TG levels were not affected by resveratrol or diet (Fig. 4N).

Analysis of adipose tissue of offspring on PND21

Because the relative amount of both adipose tissue depots was influenced by maternal diet and resveratrol at PND21, we analyzed adipokine expression and factors involved in adipogenesis, lipogenesis, and lipolysis to better understand these changes (24–26).

VAT

Maternal HFD increased leptin mRNA levels in VAT from male [$F_{(1,\ 24)} = 95.4$, $P < 0.0001$; Fig. 5A] and female [$F_{(1,\ 24)} = 39.5$, $P < 0.0001$; Fig. 5B] offspring, similar to the changes observed in circulating leptin levels. HFD offspring had higher leptin mRNA levels than those from LFD dams regardless of resveratrol treatment (males and females: $P < 0.0001$).

There was an overall effect of diet on adiponectin expression in VAT in males [$F_{(1,\ 23)} = 6.0$, $P < 0.05$; Fig. 5C], with no significant differences in the *post hoc* analysis. In females, adiponectin mRNA levels were influenced by diet [$F_{(1,\ 24)} = 9.7$, $P < 0.01$] with an interaction between diet and resveratrol [$F_{(1,\ 24)} = 7.6$, $P < 0.05$; Fig. 5D]. When split by diet, resveratrol was found to decrease adiponectin expression in females from HFD mothers (ANOVA, $P < 0.05$), with no effect in pups from LFD mothers.

The mRNA levels of LPL, the rate-limiting enzyme in fatty acid uptake and lipogenesis were dependent on

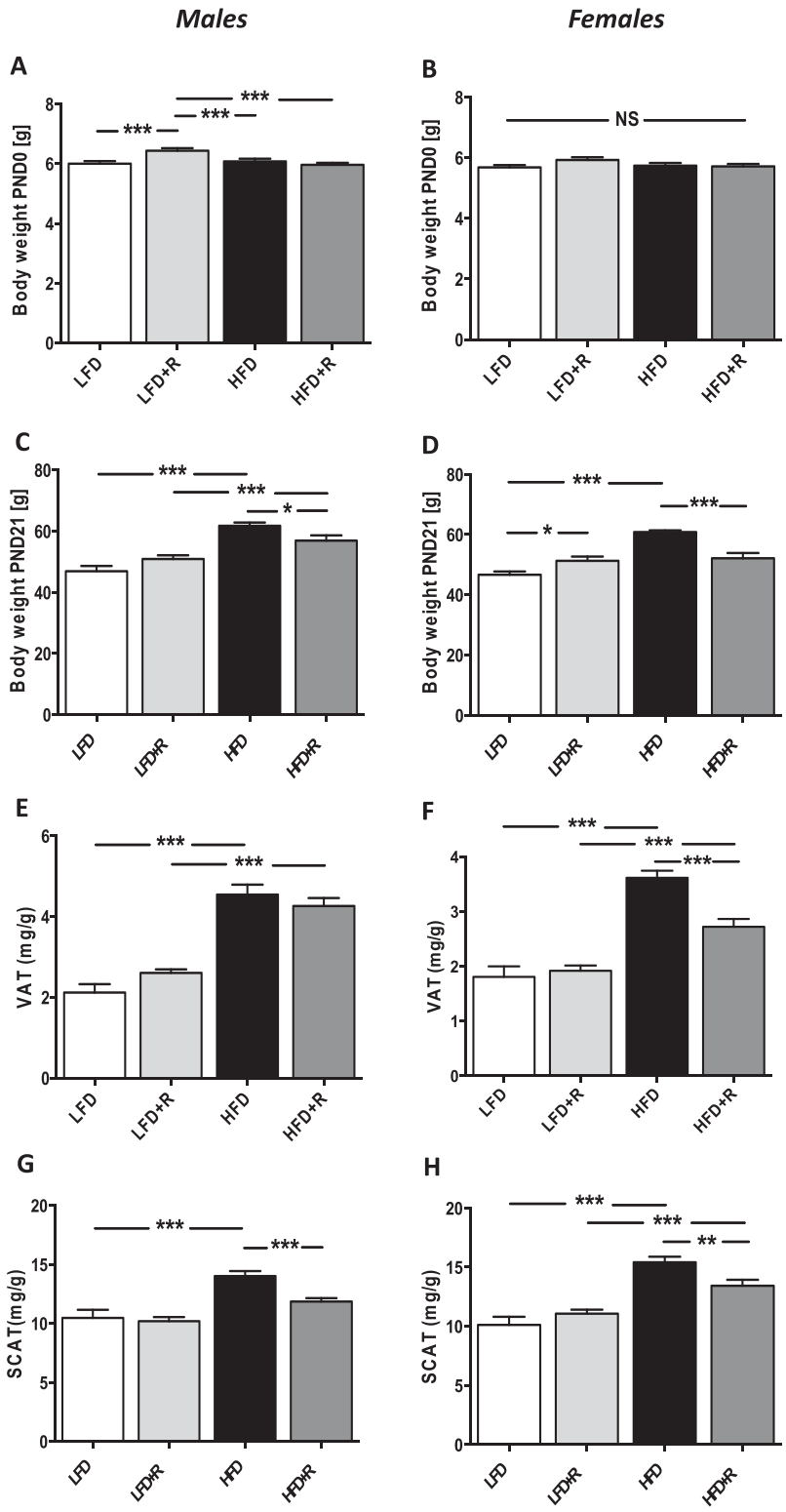

Figure 2. (A, B) BW on the day of birth and (C, D) BW, (E, F) VAT, and (G, H) SCAT mass at PND21. The left panel represents males and the right panel females. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. *ANOVA: $P < 0.05$; **ANOVA: $P < 0.001$; ***ANOVA: $P < 0.0001$. NS, not significant.

maternal diet in both sexes [males: $F_{(1,\ 24)} = 30$, $P < 0.0001$; females: $F_{(1,\ 24)} = 45.1$, $P < 0.0001$; Fig. 5E and 5F]. Offspring of both sexes from HFD mothers had higher LPL expression than those from LFD mothers regardless of resveratrol treatment (both sexes $P < 0.0001$).

PPARγ is an adipogenesis-inducing factor, controlling the expression of LPL and regulating fatty acid storage by fat cells, as well as glucose metabolism (24). PPARγ mRNA levels in VAT were affected by maternal diet in males [$F_{(1,\ 24)} = 5.01$, $P < 0.05$; Fig. 5G], with an overall effect of maternal HFD to increase PPARγ levels. There was no difference between groups in females (Fig. 5H).

Fatty acid synthase is a rate-limiting enzyme in the synthesis of fatty acids (25), and its expression in VAT was affected by resveratrol, but only in females [$F_{(1,\ 24)} = 5.4$, $P < 0.05$; Fig. 5J]. Resveratrol induced an overall decrease in fatty acid synthase (FASN) mRNA in females from both LFD and HFD dams. Although this tendency was found in males (Fig. 5I), it was not significant.

HSL, also known as triglyceride lipase, is activated when the body mobilizes energy stores (26). Maternal intake of an HFD resulted in lower HSL expression levels in VAT of male offspring [maternal diet: $F_{(1,\ 23)} = 8.1$, $P < 0.01$; Fig. 5K]. There was no effect in females (Fig. 5L).

Mitochondrial uncoupling proteins (UCPs) mediate heat production in response to environmental temperature or diet and consequently can be important regulators of BW. The mRNA levels of UCP2 were modified by maternal diet in both males [$F_{(1,\ 24)} = 4.5$, $P < 0.05$; Fig. 5M] and females [$F_{(1,\ 23)} = 37.7$, $P < 0.0001$; Fig. 5N]. UCP2 expression was higher in offspring from HFD dams than those from LFD mothers regardless of resveratrol treatment, although this reached statistical significance in the *post hoc* analysis only in females ($P < 0.0001$).

Resveratrol is reported to mediate many of its effects through SIRT1; however, the expression levels of this sirtuin were unaffected (Fig. 5O and 5P).

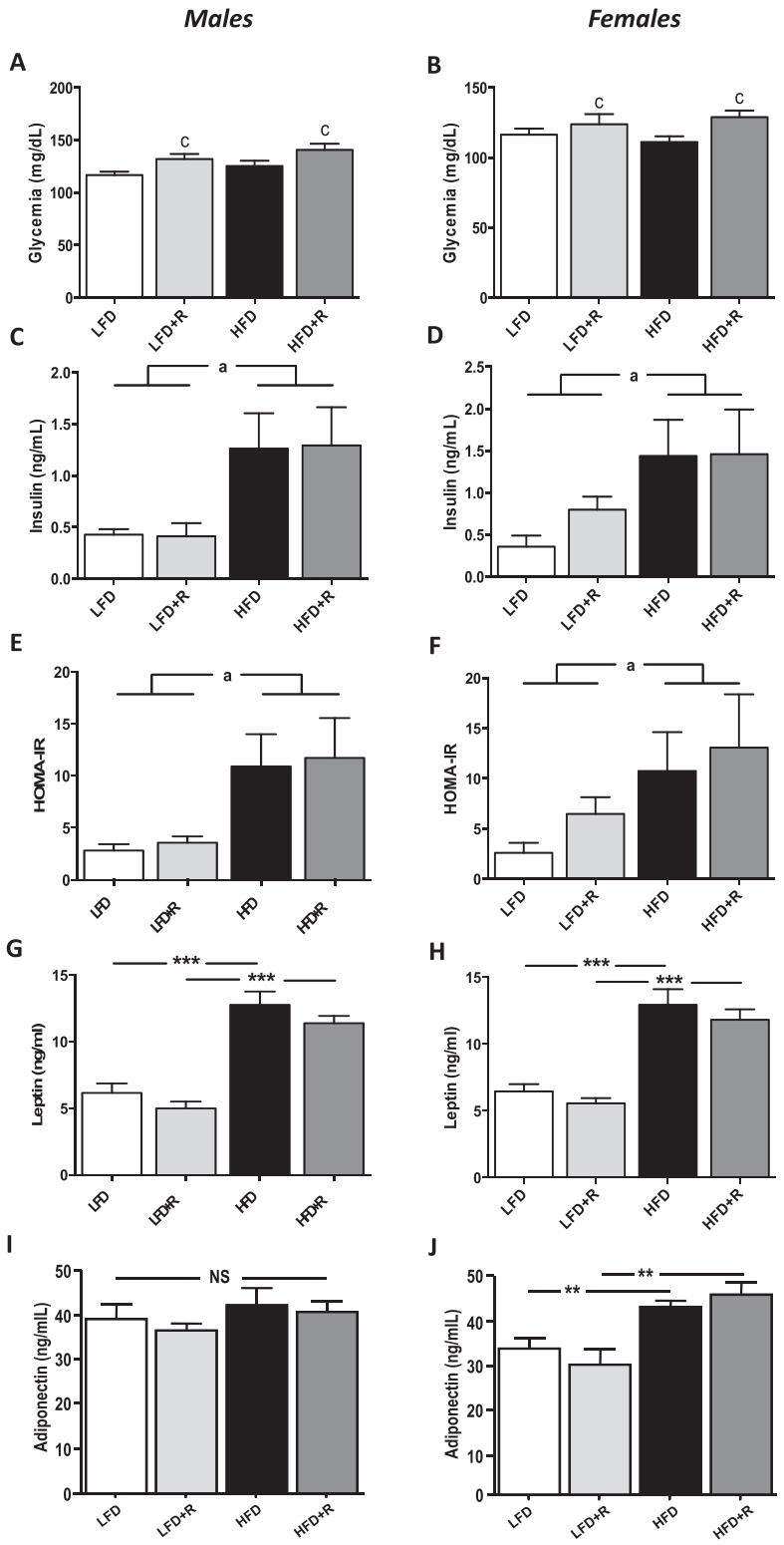

Figure 3. Circulating (A, B) glucose, (C, D) insulin, (E, F) HOMA-IR, and (G, H) leptin levels at PND21. The left panel represents males and the right panel females. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. **ANOVA: $P < 0.001$; ***ANOVA: $P < 0.0001$. a, overall effect of diet; c, overall effect of resveratrol; NS, not significant.

SCAT

Leptin mRNA levels in SCAT were modified by maternal diet in males [$F_{(1,\ 22)} = 16.0$, $P = 0.001$; Fig. 6A], with this being significant in offspring of mothers that did not receive resveratrol ($P < 0.01$). There was an overall effect of maternal HFD to induce leptin expression levels in female offspring [$F_{(1,\ 24)} = 9.8$, $P < 0.01$; Fig. 6B], although no statistically significant differences were found in the *post hoc* analysis.

Adiponectin mRNA levels in SCAT were not influenced by diet or resveratrol supplement in either sex (Fig. 6C and 6D).

The expression levels of LPL were affected by maternal diet in both males [$F_{(1,\ 22)} = 14.0$, $P < 0.001$] and females [$F_{(1,\ 24)} = 14.0$, $P < 0.001$], with pups from HFD mothers having overall higher levels of LPL mRNA compared with those from LFD mothers, reaching statistical significance in males from mothers not receiving resveratrol ($P < 0.01$; Fig. 6E) and in females from dams receiving resveratrol ($P < 0.01$; Fig. 6F).

There was no significant effect of maternal diet or resveratrol on PPARγ (Fig. 6G and 6H), FASN (Fig. 6I and 6J), HSL (Fig. 6K and 6L), or UCP2 (Fig. 6M and 6N) expression in SCAT.

Effects of maternal diet and resveratrol intake on the hypothalamus

Neuropeptides: POMC, AgRP, NPY, and orexin. The mRNA levels of the anorexigenic neuropeptide POMC (Fig. 7A and 7B) were affected by diet in females only [$F_{(1,\ 22)} = 4.6$, $P < 0.05$], with offspring from HFD dams having overall higher expression. In contrast, maternal HFD reduced AgRP mRNA levels in males [$F_{(1,\ 22)} = 5.1$, $P < 0.05$; Fig. 7C], with no effect in females (Fig. 7D).

There was no effect of maternal diet or resveratrol intake on NPY (Fig. 7E and 7F) or orexin expression levels in either sex (Fig. 7G and 7H).

ObR and InsR. Maternal HFD tended to decrease hypothalamic ObR mRNA levels in male offspring (Fig. 7K), but this was not significant [diet effect:

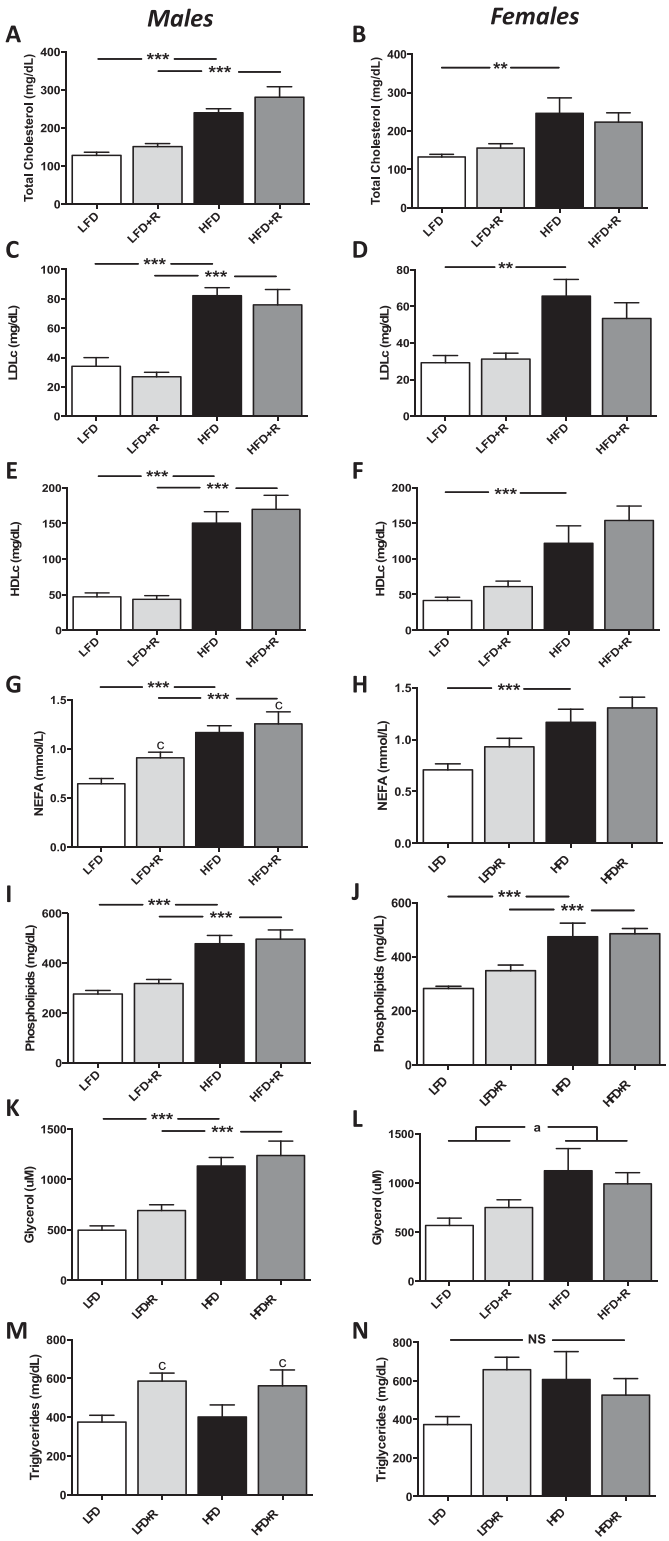

Figure 4. Circulating levels of (A, B) total cholesterol, (C, D) LDL-C, (E, F) HDL-C, (G, H) NEFA, (I, J) phospholipids, (K, L) glycerol, and (M, N) TGs in male and female pups on PND21 that were exposed to different maternal diets. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. **ANOVA: $P < 0.001$; ***ANOVA: $P < 0.001$. a, overall effect of diet; c, overall effect of resveratrol; NS, not significant.

$F_{(1,\ 22)} = 4.0$, $P = 0.06$]. Resveratrol increased ObR mRNA levels in females regardless of maternal diet [resveratrol effect: $F_{(1,\ 21)} = 4.2$, $P = 0.05$; Fig. 7J].

Resveratrol induced an overall increase in hypothalamic InsR expression in male offspring [$F_{(1,\ 21)} = 8.5$, $P < 0.01$; Fig. 7K] regardless of maternal diet. There was no effect in females (Fig. 7L).

SIRT1 expression. There was an effect of maternal diet on SIRT1 mRNA levels (Fig. 7M and 7N) in female offspring [$F_{(1,\ 22)} = 5.4$, $P = 0.05$], with pups from LFD mothers having overall higher levels than those from HFD mothers. There was no significant effect in males.

Hypothalamic protein levels

Markers of cell turnover, astrocytes, and microglia. Hypothalamic glial fibrillary acidic protein, an astrocyte structural protein, and Iba1 levels, a marker of microglia, were not affected. Protein levels of proliferating cell nuclear antigen, used to evaluate cell proliferation, were also unchanged (Table 2).

Oxidative stress. Resveratrol is reported to have antioxidant effects; thus, we analyzed the protein levels of oxidative stress markers in the hypothalamus of the offspring. Heat shock protein 70 was not affected in males (Supplemental Fig. 1A), but resveratrol slightly, but significantly, decreased Hsp70 levels [$F_{(1,\ 20)}$: 6.7, $P < 0.05$] in females (Supplemental Fig. 1B).

In contrast, supraoxide desmutase levels were modified by maternal diet in males [$F_{(1,\ 21)}$: = 15.4, $P < 0.001$; Supplemental Fig. 1C]. Maternal HFD increased SOD levels, with a significant difference between LFD + R and HFD + R offspring ($P < 0.01$). There was no effect on SOD levels in females (Supplemental Fig. 1D).

Leptin signaling. To determine whether basal levels of leptin signaling in the hypothalamus were affected by the hyperleptinemia observed in maternal HFD offspring, key proteins of the leptin signaling pathway were analyzed. Hypothalamic pSTAT3^{Tyr705}, pSTAT3^{Ser727}, and SOCS3

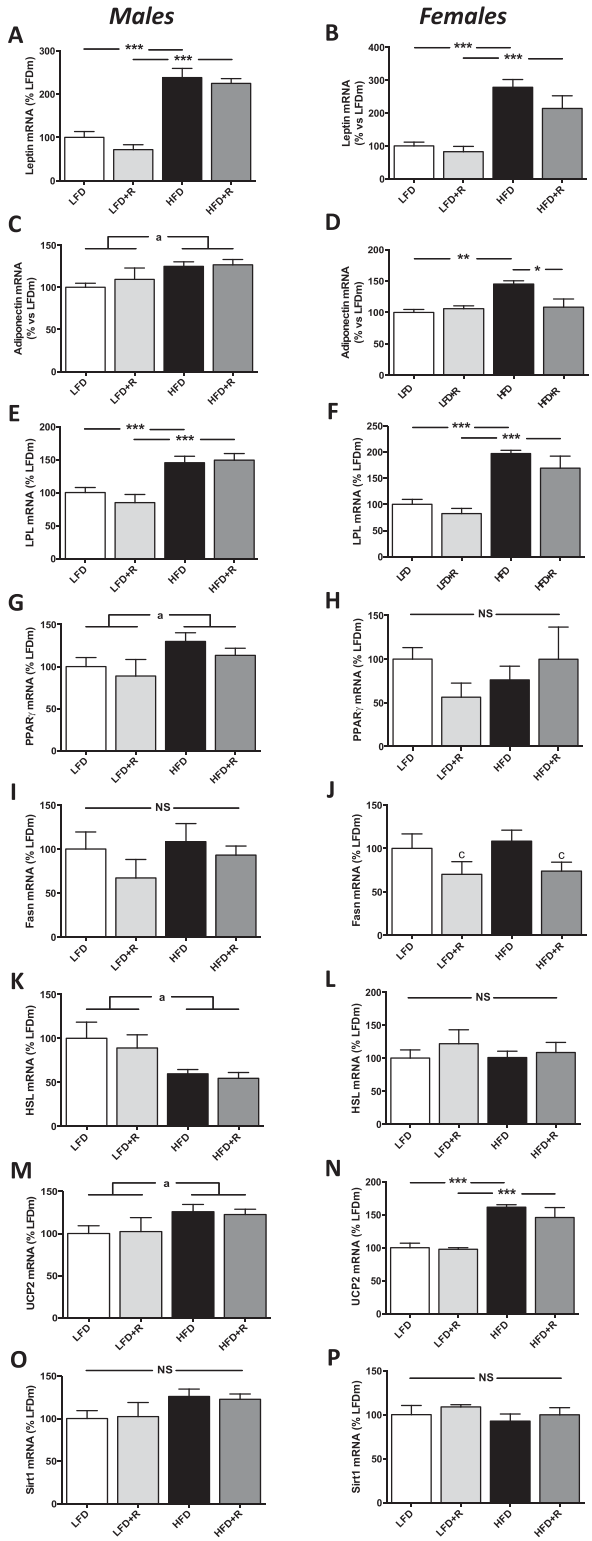

Figure 5. Mean mRNA levels of (A, B) leptin, (C, D) adiponectin, (E, F) LPL, (G, H) PPARγ, (I, J) FASN, (K, L) HSL, and (M, N) UCP2 in VAT at weaning (PND21). The left panel represents males and the right panel females. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. *ANOVA: $P < 0.05$; ***ANOVA: $P < 0.001$. a, overall effect of diet; c, overall effect of resveratrol; NS, not significant.

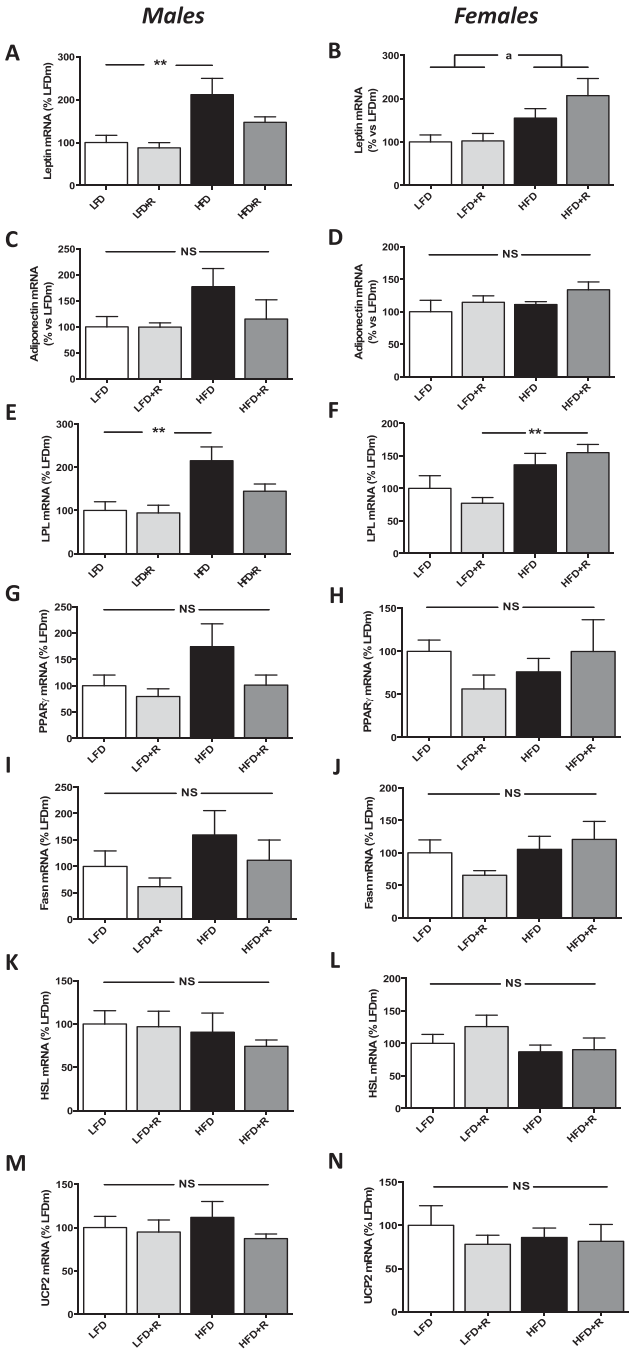

Figure 6. Mean mRNA levels of (A, B) leptin, (C, D) adiponectin, (E, F) LPL, (G, H) PPARγ, (I, J) FASN, (K, L) HSL, and (M, N) UCP2 in SCAT. The left panel represents males and the right panel females. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. **ANOVA: $P < 0.001$. a, overall effect of diet; NS, not significant.

levels were unaffected in males (Fig. 8A, 8C, and 8E, respectively). However, in females, there was an effect of maternal diet on $pSTAT3^{Tyr705}$ [$F_{(1,\ 21)} = 8.3$, $P = 0.01$; Fig. 8B] and $pSTAT3^{Ser727}$ [$F_{(1,\ 21)} = 6.9$, $P < 0.05$; Fig. 8D], with no effect on SOCS3 levels (Fig. 8F). Maternal HFD increased $pSTAT3^{Tyr705}$ levels in females [$F_{(3,\ 20)} = 3.9$, $P < 0.05$] compared with LFD offspring. There was an overall decrease in pSTAT3 Ser727 in HFD female offspring.

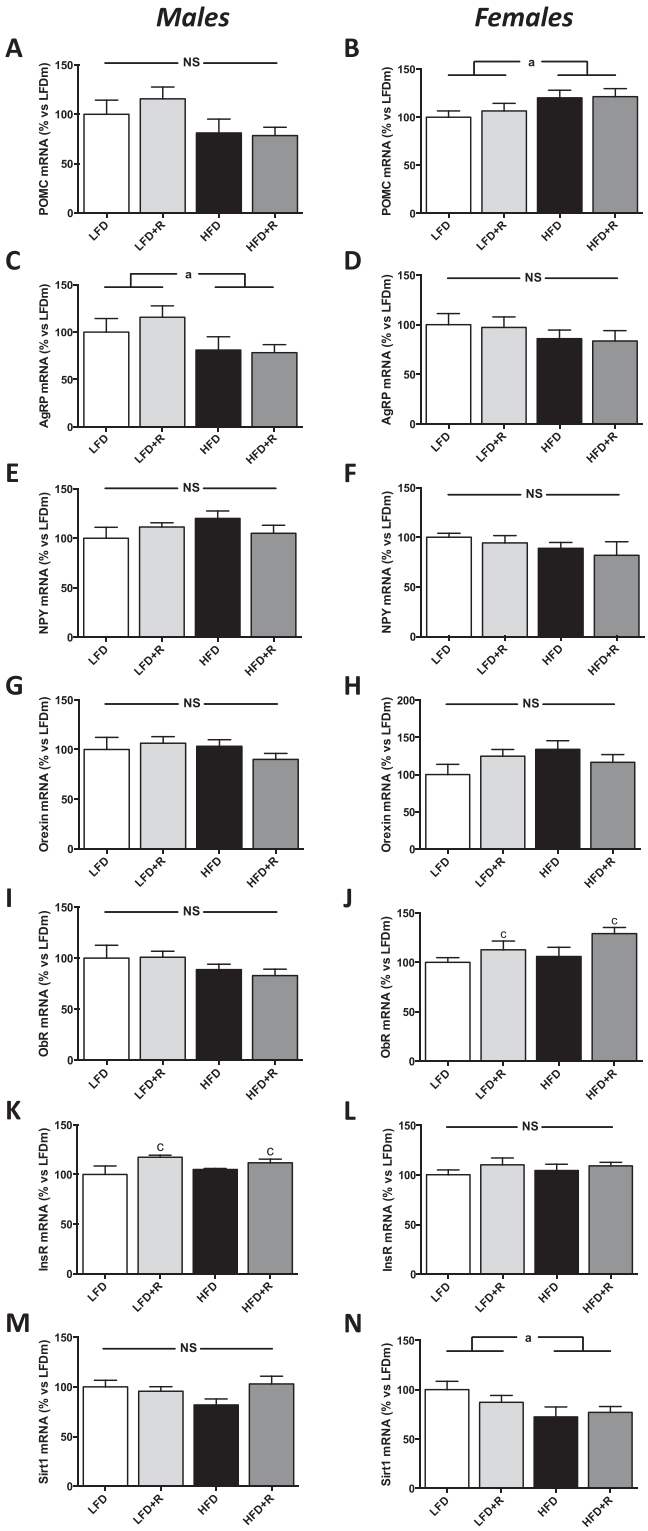

Figure 7. Mean mRNA levels of hypothalamic neuropeptides and InsR and ObR on PND21: (A, B) POMC, (C, D) AgRP, (E, F) NPY, (G, H) orexin, (I, J) ObR, (K, L) InsR, and (M, N) SIRT1. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. a, overall effect of diet; c, overall effect of resveratrol; NS, not significant.

Discussion

Resveratrol is a naturally occurring antioxidant that is reported to protect against poor dietary habits (12–14, 27–29). Not only is it possible that resveratrol improves maternal metabolic status, which would indirectly affect the offspring, but as it is known to pass the placenta, it could also have direct effects on the fetus (17). The composition of maternal breast milk partially reflects the maternal diet (30), but little is known regarding the effects of resveratrol during lactation, the excretion of this polyphenol into breast milk, or its safety and efficacy in nursing mothers and infants (http://toxnet.nlm.nih.gov; last updated in February 2017). Here we show that even though resveratrol did not affect BW, visceral fat mass, or serum leptin levels in pregnant/lactating dams fed with either an LFD or an HFD, it modified the offspring's BW, fat mass, glycemia, lipid profile, and serum leptin levels at weaning. Moreover, some of these effects were sex and maternal diet specific.

It is well established that nutritional perturbations of the maternal diet during pregnancy and nursing can have long-term effects on the offspring's nutritional status and their susceptibility to metabolic diseases in adulthood (31, 32), with both undernutrition and overnutrition having been shown to increase the propensity of the offspring to become overweight in later life (33–35). Not only the excess intake of kilocalories but the type of diet is also important (36). Fatty acids cross the placenta during pregnancy and are also present in breast milk, with excessive or insufficient fatty acid intake during perinatal stages being associated with metabolic and endocrine disruptions (37, 38). In mice, the exposure to HFD only during lactation is sufficient to predispose the offspring to later metabolic disorders, and this is at least in part due to alterations in the innervation of the arcuate nucleus (39). Imbalances in fatty acid intake during fetal development can also cause functional and structural changes in metabolic circuits (37, 38), including modifications in the response of hypothalamic POMC neurons to dopamine (40). Even a normocaloric diet during pregnancy and lactation can cause deleterious effects in the offspring if the composition of the diet is not appropriate (41). Dietary substances can also be helpful; for example, consuming *n*-6/*n*-3 polyunsaturated fatty acids in a ratio of approximately 1–2:1, before conception and throughout lactation, is reported to be beneficial in brain development through increasing PPARγ in mice (42).

Mothers on the HFD had a greater mean energy intake compared with LFD mothers, but there were no differences in BW, VAT depot, or serum leptin levels at the end of the lactation period. The lack of effect on BW is in accordance with most studies of rodents fed an HFD

Table 2. Hypothalamic Protein Levels

	GFAP		Iba1		PCNA	
Characteristic	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
LFD	100.0 ± 8.3	100.0 ± 14.3	100.0 ± 19.2	100.0 ± 3.9	100.0 ± 10.4	100.0 ± 27.1
LFD + R	129.8 ± 29.4	119.3 ± 20.7	94.4 ± 7.7	109.1 ± 13.2	105.6 ± 8.7	104.4 ± 9.6
HFD	122.7 ± 23.1	126.4 ± 15.5	93.0 ± 10.7	94.4 ± 13.3	115.0 ± 8.8	109.4 ± 12.2
HFD + R	126.6 ± 27.7	118.5 ± 19.1	78.3 ± 5.9	85.7 ± 12.7	118.0 ± 7.8	98.3 ± 14.5

There were no significant differences between groups.

Abbreviations: GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adapter molecule; PCNA, proliferating cell nuclear antigen.

during gestation and/or lactation (41, 43, 44) and suggests a mechanism of compensation for this increased energy intake. Although some authors report that maternal HFD consumption increases abdominal fat and plasma leptin levels in dams at the end of lactation (45), others report a decrease (44) or no change (36). These discrepancies might be attributed to differences in various aspects of the experimental design—namely, type of diet (cafeteria *vs* HFD), strain of rat (Wistar *vs* Sprague Dawley rats), and experimental period length (pregestational 10 weeks *vs* pregnancy and lactation).

The observation that LFD dams had significantly higher insulin levels and HOMA-IR could be due to the difference in carbohydrate concentration in the diets, as the energy derived from carbohydrates was 67.4% in the LFD and 25.9% in the HFD. Some authors report no change in plasma glucose, insulin, or HOMA-IR index in dams fed an HFD but a higher level of insulin during glucose tolerance testing (44), whereas others report a slight elevation of serum insulin concentrations in HFD mothers compared with those on normal chow (39). Again, these discrepancies are most likely due to differences in experimental design but together further emphasize the importance of diet on maternal metabolism.

Because fatty acids cross the placenta, as well as into breast milk, maternal diet can directly influence fetal/newborn programming independent of maternal obesity (36, 38). At the end of lactation, circulating TG levels were significantly increased by the HFD in the dams, which is in agreement with some authors (43). Females on an HFD are reported to have higher TG and cholesterol levels during pregnancy compared with the end of lactation, in both animal models (36) and human pregnancy (46); however, unfortunately, we did not measure lipid levels at the end of pregnancy. The increase in maternal fatty acid intake and circulating lipid levels are reflected in the higher levels of lipids in their offspring, except for TGs. TG levels were unaffected by diet but increased in response to maternal resveratrol intake, as were NEFA levels. Interestingly, in male offspring, this effect of resveratrol was independent of diet, whereas in females, it was only observed in the offspring from LFD dams. In female offspring from LFD dams, phospholipid levels were increased by resveratrol, with glycerol levels being increased by resveratrol in male offspring from LFD dams. The availability of specific dietary lipids, lipid-soluble vitamins, and some trace elements affects lipid/phospholipid metabolism; thus, the dam's diet determines the lipid availability to the offspring and their lipid metabolism. Resveratrol had little effect on the lipid profile in the dams, suggesting that the effect of resveratrol on the lipid profile in the offspring could be due to a direct effect of this factor on their development. Indeed, dietary fatty acid composition influences lipid-signaling molecules in the brain, with effects on neural function, eating behavior, and obesity-related problems (37, 47). However, the role of fatty acids in metabolic programming during development remains to be elucidated.

At birth, BW was greater in pups from LFD + R compared with all other groups, reaching significance in males. This effect of resveratrol to increase BW in the offspring of LFD dams continued at weaning in both sexes, here reaching statistical significance only in females. However, at this stage of development, the effect of resveratrol had an opposite effect in pups from HFD mothers, causing a reduction in BW. The maternal HFD-induced increase in BW, VAT, and SCAT in the pups at weaning, which is in accordance with previous studies (39, 41, 43), most likely reflects the increased maternal energy intake, but the mechanism of action of resveratrol remains to be determined. The effect of resveratrol has been reported to depend on the cellular redox status and may be effective only under context-specific metabolic stress (15), but to our knowledge, this is the first time that different resveratrol effects have been shown in offspring depending on the maternal diet.

Males and females can respond differently to early nutritional and hormonal manipulations (21, 22, 48, 49). Here BW at weaning was similarly affected by maternal HFD in both sexes, but VAT accumulation was greater in males. This is similar to what we previously observed in neonatally overnourished rats, where males had more

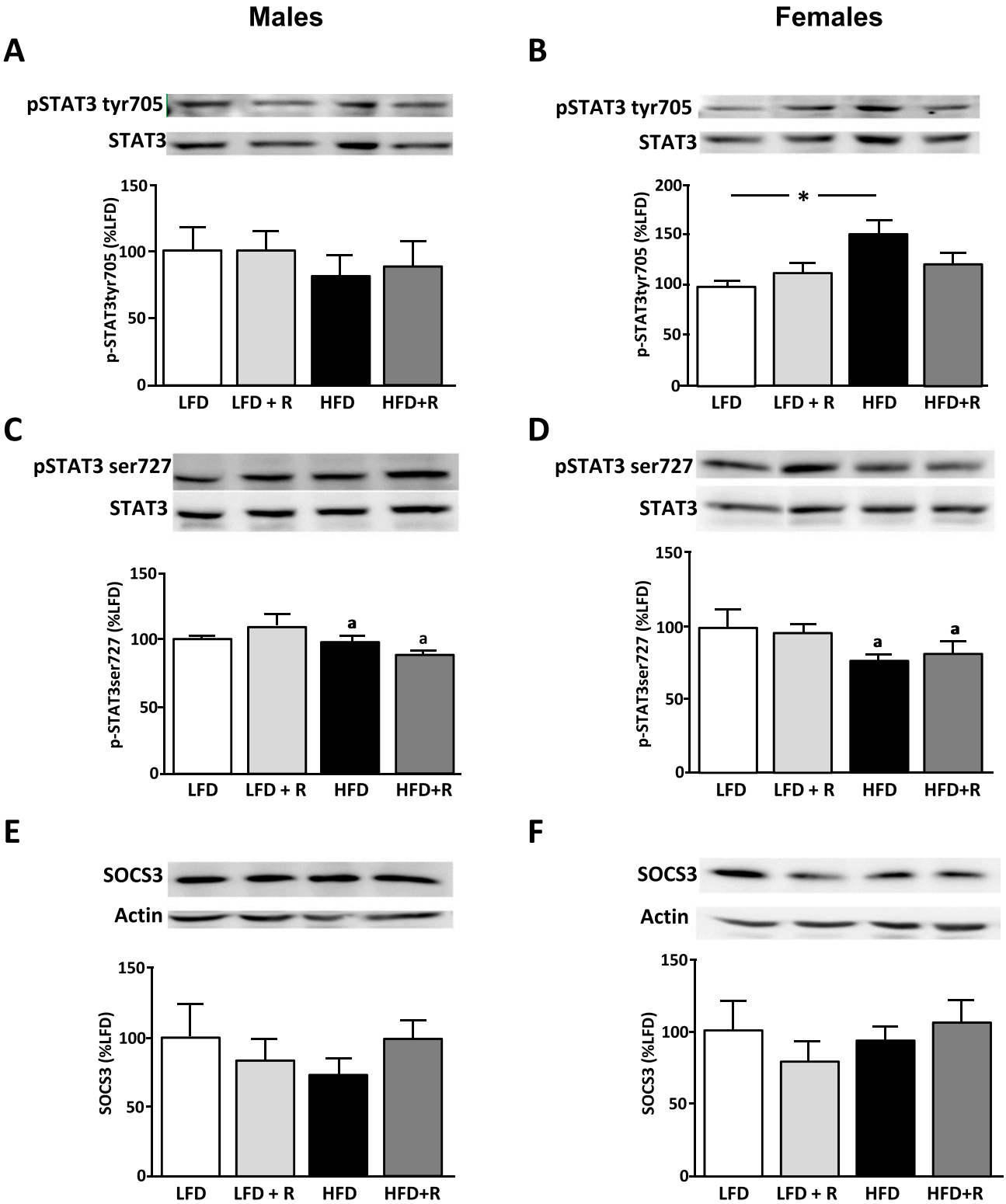

Figure 8. Relative hypothalamic protein levels of pSTAT3-Tyr705, pSTAT3-Ser727, and SOCS3, key protein in leptin signaling, in (A) males and (B) females. Maternal diet during pregnancy and lactation: LFD, LFD + R, HFD, and HFD + R. Data are shown as mean ± standard error of the mean. *ANOVA: $P < 0.05$. a, overall effect of diet.

VAT at PND21 than females even though there was no difference in BW (22). In addition, although resveratrol decreased SCAT in both sexes from HFD mothers, it only decreased VAT in females. In both sexes, the increase in adiposity was associated with higher serum leptin levels and increased leptin expression in both VAT and SCAT. Thus, the increased serum levels are at least in part due to the endogenous production by the offspring as opposed

to ingestion via maternal milk. The maternal HFD-induced increment in VAT and SCAT was associated with higher LPL expression in these depots from male and female offspring, but PPARγ mRNA levels, also involved in fatty acid uptake and lipogenesis, were only increased in VAT from males. Although one drawback of this study is that protein levels of these factors involved in adipose tissue turnover were not measured, the observed changes in mRNA levels suggest that there is an increase in lipogenesis as a result of maternal HFD, and this might differ depending on the fat depot involved, as well as between the sexes.

Perinatal hyperinsulinemia in the offspring of malnourished or diabetic mothers has been implicated in the long-term metabolic effects of these conditions in human epidemiological and animal studies (50, 51). Thus, it is conceivable that the hyperinsulinemia observed here in the offspring of HFD mothers could negatively influence their metabolic programming. In addition, there could also be an effect of dietary fat and/or metabolic signals from the mother (*e.g.*, the increase in TG levels) on the metabolism of the offspring.

In contrast to the effects of poor maternal nutrition, there is relatively little information regarding the beneficial effects of specific nutritional additives or substances on the fetus or nursing child. The antioxidant resveratrol is reported to protect against poor dietary habits in adult animals (14, 15, 28, 29, 52) and could possibly improve maternal metabolic status that in turn affects the fetus, as well as a possible direct effect as it crosses the placenta (28). Resveratrol, at doses up to 750 mg/kg/d, was shown to be well tolerated in pregnant Sprague-Dawley rats and with no effect on litter size or fetal or placental weights (18). Similarly, we found no effect on litter size. Resveratrol induced a 30% maternal weight loss, improved glucose tolerance, and decreased placental inflammation and liver TG deposition in pregnant nonhuman primates on a Western-style diet (28). However, we found no effect on maternal weight, possibly due to differences in the experimental model. In the study by Roberts and colleagues (28), there was concern regarding an unexplained alteration in fetal pancreatic development. We unfortunately did not analyze the pancreas, but we observed an increase in glycemia in all offspring of mothers ingesting resveratrol. Although insulin levels were not modulated by resveratrol at this early age, it remains to be determined if pancreatic function is affected and if glycemic control worsens with age.

Maternal resveratrol intake increased BW in male offspring but only from mothers on an LFD, pointing out the differential effect of resveratrol depending on the diet. This natural phenol was previously shown to increase fetal weight on gestational day 18.5 in a diabetic mouse model (53), but the sex of these mice was not reported. Again, at the end of lactation, the effect of resveratrol on weight was dependent on the type of diet, with an increase in weight in LFD offspring but a decrease in pups from HFD mothers. Moreover, this decrease in BW and fat mass induced by maternal resveratrol intake in HFD offspring was greater in female offspring. This is in accordance with the hypothesis that resveratrol protects against poor maternal dietary habits. In contrast, resveratrol tended to increase BW at PND21 in the offspring from LFD, with this being statistically significant in females, but with no significant change in fat mass. Thus, some of the effects of maternal resveratrol intake on the offspring are diet dependent, which is in accordance with studies indicating that this antioxidant is effective only under context-specific metabolic stress and depends on the cellular redox status (15, 54).

Resveratrol decreased circulating leptin levels in the offspring regardless of the maternal diet. This antioxidant has been reported to reverse the hyperleptinemia effects of early weaning or maternal HFD in male rats when treated with this antioxidant from PND150 to PND180 (50). The decrease in circulating leptin levels could be due to the lower fat mass, at least in the HFD + R offspring, and the fact that resveratrol tended to decrease leptin mRNA levels in both VAT and SCAT but differently in males and females. The concentration of leptin is relatively stable throughout lactation and is positively associated with maternal body mass index (55, 56). As maternal weight was unaffected here, the differences between the offspring in serum leptin levels are most likely not due to differences in the ingestion of this hormone.

Maternal resveratrol intake increased glycemia in the offspring, with no effect on insulin. In contrast, in adult obese animals, resveratrol is reported to improve glycemic control (28, 29). Likewise, although studies indicate no change in lipid profiles in response to resveratrol in adult animals or humans (57), we found an increase in NEFA levels in the offspring from mothers ingesting resveratrol as well as an increase in TG levels, but only in pups from LFD mothers. These observations raise the question as to whether the effects on glycemia and lipid parameters are direct effects of the resveratrol in the offspring or are the result of some metabolic change in the mother.

Resveratrol has antiadipogenic effects by reducing the expression of PPARγ, the main adipogenesis-inducing factor and regulator of glucose metabolism and fatty acid storage by fat cells, both *in vitro* and in adult animals (24, 25, 58, 59). Resveratrol decreased the amount of adipose tissue in HFD offspring, indicating that this antiadipogenic mechanism may be implicated when energy is in abundance, but no significant effect of resveratrol

on PPARγ mRNA levels was observed. The mRNA levels of LPL, an enzyme controlled by PPARγ and involved in fatty acid uptake and lipogenesis, were increased with HFD but not affected by resveratrol. Maternal HFD has been shown to increase LPL expression in VAT in offspring when they become adults, and this is suggested to contribute to the adipocyte hyperplasia and hypertrophy that occurs during extrauterine white adipose tissue development (60). The expression of FASN, which catalyzes the *de novo* formation of long-chain fatty acids, was decreased by resveratrol in VAT, reaching significance in females, supporting an antiadipogenic effect of this antioxidant. Different responses were found in the two adipose depots studied, and this could be due to differences in the metabolic activity between VAT and SCAT.

Although numerous studies have indicated that the metabolic effects of resveratrol are due to SIRT1 activation, other reports have suggested that it could elicit its effects through other sirtuins or targets unrelated to sirtuins (23, 61, 62). Here, the expression levels of SIRT1 were not affected by diet, resveratrol, or sex in VAT.

Central effects

Maternal HFD consumption can program hypothalamic circuits involved in the regulation of energy intake, stimulating orexigenic pathways in the offspring (63). Perinatal maternal HFD induces obesity, hyperleptinemia, and changes in central leptin signaling in the hypothalamus of adult male rodents, with resveratrol supplementation from PND150 to PND180, ameliorating the modifications in central sensitivity to leptin but with no change in hypothalamic ObR expression (50). Here the expression of the orexigenic neuropeptide AgRP was decreased in male HFD offspring, whereas POMC mRNA levels were increased by maternal HFD in females. There were no changes in the other neuropeptides analyzed, nor was there an effect of resveratrol. However, although mRNA levels are not affected, it is possible that protein levels, neuron numbers, and/or synaptic connectivity could be altered. Indeed, Vogt and coworkers (39) demonstrated that whereas the number and neuropeptide expression of POMC and AgRP/NPY neurons in the arcuate nucleus were unaffected at 3 weeks of age in response to maternal HFD during lactation, the formation of POMC and AgRP projections to hypothalamic target sites was severely impaired. This deserves further investigation.

The hyperleptinemia observed in the maternal HFD offspring did not affect basal hypothalamic leptin signaling in PND21 males, which could indicate decreased sensitivity to this hormone. In contrast, in females from HFD mothers that did not receive resveratrol, there was an increase in $pSTAT3^{Tyr705}$, as this phytoalexin tended to decrease $pSTAT3^{Tyr705}$ levels. Franco *et al.* (52) reported that in adult male offspring from HFD mothers, leptin signaling was improved by resveratrol; thus, it is possible that this effect of resveratrol might be observed at later stages of development. Interestingly, there was a sexually dimorphic effect of resveratrol on ObR and InsR mRNA levels in the hypothalamus. ObR mRNA levels were increased in females and InsR mRNA levels were increased in males from mothers receiving resveratrol, and this was regardless of maternal diet. These changes in ObR and InsR expression could modify the trophic effects of these two hormones on metabolic circuits (6), resulting in sexually dimorphic outcomes. However, it remains to be determined if these alterations persist into adulthood and are cell specific and if the protein levels of these receptors also change in a sex-specific manner and are involved in sex differences in long-term metabolic effects induced by maternal resveratrol intake.

In summary, our findings indicate that the outcome of resveratrol supplementation to pregnant dams depends on both the maternal dietary intake and the sex of the offspring. Resveratrol could potentially have beneficial effects in protecting against poor maternal dietary habits, although further studies are necessary to understand the mechanisms involved and whether there are potential adverse effects. Indeed, a more widespread analysis of the long-term changes induced by maternal resveratrol intake and the mechanisms involved is necessary. Moreover, the observation that resveratrol has differential effects depending on diet indicates that this variable must be taken into consideration when analyzing or performing studies with this antioxidant.

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