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Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver

Nicole G. Ashino[a,1], Karen N. Saito[a,1], Flavia D. Souza[a], Fernanda S. Nakutz[a], Erika A. Roman[b], Licio A. Velloso[c], Adriana S. Torsoni[d], Marcio A. Torsoni[a,*]

[a]Universidade Braz Cubas, CEP 08.773-380, Mogi das Cruzes, São Paulo, Brazil
[b]Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas-UNICAMP, Campinas, São Paulo, Brazil
[c]Departamento de Medicina Interna, Faculdade de Ciências Médicas, Universidade Estadual de Campinas-UNICAMP, Campinas, São Paulo, Brazil
[d]Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas-UNICAMP, Campinas, São Paulo, Brazil

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Abstract

The exposure to an increased supply of nutrients before birth may contribute to offspring obesity. Offspring from obese dams that chronically consume a high-fat diet present clinical features of metabolic syndrome, liver lipid accumulation and activation of c-Jun N-terminal kinases (JNK) consistent with the development of nonalcoholic fatty liver disease (NAFLD). However, in spite of the importance of the resistance to insulin for the development of NAFLD, the molecular alterations in the liver of adult offspring of obese dams are yet to be investigated. In this study, we tested the hypothesis that the consumption of excessive saturated fats during pregnancy and lactation contributes to adult hepatic metabolic dysfunction in offspring. Adult male offspring of dams fed a high-fat diet (HN) during pregnancy and lactation exhibited increased fat depot weight; increased serum insulin, tumor necrosis factor α and interleukin 1β; and reduced serum triglycerides. Liver showed increased JNK and I kappa B kinase phosphorylation and PEPCK expression in the adult. In addition, liver triglyceride content in the offspring 1 week after weaning and in the adult was increased. Moreover, basal ACC phosphorylation and insulin signaling were reduced in the liver from the HN group as compared to offspring of dams fed a standard laboratory chow (NN). Hormone-sensitive lipase phosphorylation (Ser565) was reduced in epididymal adipose tissue from the HN group as compared to the NN group. It is interesting that all changes observed were independent of postweaning diet in 14-week-old offspring. Therefore, these data further reinforce the importance of maternal nutrition to adult offspring health.

Keywords: Offspring; High-fat diet; Fatty liver; Insulin resistance

1. Introduction

The prevalence of childhood obesity has increased dramatically around the world, and studies have shown that exposure to an increased supply of nutrients before birth may contribute to overweight or obesity later in life [1,2]. Several studies in animal models support the role of altered nutritional condition during pregnancy and in early postnatal period in predisposing offspring to the development of metabolic disorders [3–6]. In the model of diet-induced obesity, high fat consumption during pregnancy induced features of metabolic syndrome in adult offspring, independent of adult environmental factors [7]. Thus, the fetus responds to nutritional stress by 'programming' its own growth in a way that it can lead to an increased risk of future metabolic disorders, such as insulin resistance, increased body fat mass and reduced hypophagic effect of central insulin later in life [8].

The consumption of a high-fat diet (HFD) during pregnancy may alter the development of peptide systems in the uterus, producing neuronal changes in the offspring that persist postnatally in the absence of the diet and that have long-term consequences. Interestingly, the offspring of dams on an HFD showed increased expression of orexigenic peptides, galanin, enkephalin and dynorphin in the paraventricular nucleus and orexin, and melanin-concentrating hormone in the perifornical lateral hypothalamus [9]. In an elegant study performed with Japanese macaques, McCurdy and colleagues [6] showed that fetal offspring from both lean and obese mothers who chronically consumed an HFD had a threefold increase in liver triglycerides and exhibited elevated hepatic expression of gluconeogenic enzymes. In addition, fetal offspring from HFD-fed mothers showed increased evidence of hepatic oxidative stress early in the third trimester, lipid accumulation and activation of the c-Jun N-terminal kinases (JNK) in the fetal liver consistent with the development of nonalcoholic fatty liver disease (NAFLD).

According to the American Association for the Study of Liver Diseases, NAFLD is defined as fat accumulation in the liver exceeding

* Corresponding author. Universidade Braz Cubas-Área da Saúde-Campus I, Av. Francisco Rodrigues Filho, 1233, Mogilar, Mogi das Cruzes-São Paulo, Brazil, CEP 08773-380. Tel.: +55 19 35218592; fax: +55 19 37888950.
E-mail address: torsoni@yahoo.com (M.A. Torsoni).
[1] Contributed equally for this study.

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5% to 10% by weight. Nonalcoholic fatty liver disease is perhaps the most common liver disease in the Western hemisphere. Insulin resistance is central to the pathogenesis of the metabolic syndrome, and recent data indicate that NAFLD should be considered as the hepatic manifestation of the metabolic syndrome [10].

Obesity and type 2 diabetes are associated with a state of abnormal inflammatory response. Challier and colleagues [11] showed that obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. This inflammatory environment in which the fetus develops may have critical consequences for the short- and long-term programming of obesity. Several downstream mediators and signaling pathways that provide the crosstalk between inflammatory and metabolic signaling have been identified (for review, see Refs. [12,13]). Cytokines and free fatty acids (FFAs) are potent JNK and I kappa B kinase (IKK) activators and are key components in the pathogenesis of insulin resistance and the metabolic syndrome [14].

Studies performed with distinct forms of insulin resistance in humans suggest that partial postreceptor hepatic insulin resistance is a key element in the development of metabolic dyslipidemia and hepatic steatosis [15]. In the present study, we investigated the effects of *in utero* and lactation HFD exposure on the development of insulin resistance and hepatic steatosis in mice. We identified reduced insulin signaling, lipid accumulation and activation of the JNK and IKKbeta in the liver of adult offspring.

2. Material and methods

2.1. Animals and diets

The experiment was carried out according to the COBEA (Brazilian College of Animal Experimentation) guidelines, which are adopted by the Universidade Braz Cubas, Mogi das Cruzes, São Paulo, Brazil. Sixteen virgin female Swiss mice (70 days old) were taken from the university's central breeding colony. Before mating, the females were randomly divided in fed *ad libitum* either with HFD or standard laboratory chow (control diet; CD) for 1 week for adaptation. Mating was performed by housing females with adult males overnight, and pregnancy was confirmed by examining vaginal smears for the presence of sperm. Pregnant and virgin females were separated and maintained in individual polypropylene cages in a room at 24°C±1°C with lights on from 6:00 a.m. to 6:00 p.m. They were fed diets and water *ad libitum* during the pregnancy and lactation periods. During the experimental period, the animals received two types of diets: eight females were fed CD, and eight females were fed HFD (Table 1). The diet was prepared in according to the AIN-93G modified for high fat (35%) content. The offspring were divided into two groups according to maternal feeding: offspring from female mice fed HFD (group HN) and offspring from female mice fed CD (group NN). On day (d) 1 after birth, the litters of both groups (HN and NN) were assigned to mixed-gender groups of five animals of similar body weight. The pups were weaned on d21 and separated according to sex. All male offspring were fed standard chow after weaning.

2.2. Sample collection and analysis

At the end of the experimental period (82 days) and after overnight fasting, all mice were sacrificed, blood samples were collected and centrifuged, and serum aliquots were used to measure serum glucose by enzymatic colorimetry (glucose oxidase method). Serum aliquots were stored at −80°C for hormone measurements. Serum insulin, leptin, interleukin (IL) 1β and tumor necrosis factor (TNF) α were determined by enzyme-linked immunosorbent assay using Kits Crystal Chem. Inc., USA, and SABiosciences, USA. Serum triglyceride was determined using a specific kit. The epididymal fat pad was removed and measured to determine the fresh weight (g/g of body weight).

Table 1
Nutritional composition of experimental diet and standard chow used during gestation and lactation

Nutrients	Standard chow g%	High-fat diet g%
Protein	20	20
Carbohydrate	66	43
Saturated fat	4	23
Choline	0.25	0.25
Fiber	5	5
Vitamin	1	1
Mineral	3.5	3.5
Cystine	0.3	0.3
Energy (KJ/100 g)	1591	1939
Calories from lipids (%/100 g of diet)	9	45

2.3. Evaluation of liver histology and triglyceride content

Frozen tissues (200 mg) from HN and NN specimens were homogenized in 1.5 ml of phosphate-buffered saline. The protein concentration of homogenate was determined, and an aliquot of 300 μl was extracted with 5 ml of chloroform/methanol (2:1) and 0.5 ml of 0.1% sulfuric acid. An aliquot of organic phase was collected, dried under nitrogen and resuspended in 2% Triton X-100 [16,17]. Triglyceride content was determined using a commercially available kit.

Fragments of liver from NN and HN animals were fixed in 10% formalin and embedded in paraffin. Serial sections were stained with hematoxylin–eosin (HE) and observed in light microscopy.

2.4. Immunoprecipitation and immunoblotting

Briefly, mice were treated according to the protocols described in the preceding section. They were then anesthetized and subjected to tissue extraction. Tissues were obtained and homogenized in freshly prepared ice-cold buffer (1% Triton X-100, 100 mM Tris, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF and 0.01 mg aprotinin/ml). Insoluble material was removed by centrifugation (10,000*g*) for 25 min at 4°C. Aliquots of the resulting supernatants containing 2.0 mg of total protein were used for immunoprecipitation with antibodies against IR, IRS1, and IRS2 at 4°C overnight, followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transfer to nitrocellulose membranes and blotting with antiphosphotyrosine (anti-Py). For direct immunoblot analyses, 0.2 mg protein from the extracts was separated by SDS-PAGE, transferred to nitrocellulose membranes and blotted with specific antibodies against AKT, GAPDH, FAS, PEPCK, p-HSL_{565} and p-JNK. The proteins were detected by enhanced chemiluminescence. The results were visualized by autoradiography with preflashed Kodak XAR film. Band intensities were quantified by optical densitometry of developed autoradiographs (Scion Image software, ScionCorp).

2.5. Data presentation and statistical analysis

All numerical results are expressed as means±S.D. of the indicated number of experiments. Blot results are presented as direct band comparisons in autoradiographs and quantified by densitometry using the Scion Image software (ScionCorp). Student's *t* tests of unpaired samples and analysis of variance analysis for multiple comparisons were used as appropriate. Post hoc test (Tukey) was employed when required. The level of significance was set at $P<.05$.

3. Results

3.1. Body weight, food intake and adiposity

The HFD dams were 11% heavier than controls during the gestational period (30.6±1.2 g vs. 34.1±1.1 g, respectively) ($P\leq.05$), and the body mass difference was maintained throughout lactation (until d20 postpartum).

The pups were weighed on d2 and weekly thereafter. The body weight of HN animals was higher than that of NN from d2 (Fig. 1A) to d82 (Fig. 1B). In d28 and d82, the epididymal fat pad was weighed; HN animals showed a fat mass larger than that of NN (0.3±0.03 g in HN offspring vs. 0.09±0.025 g in NN to d28; 0.77±0.01 g in HN offspring vs. 0.4±0.01 g in NN to d82) (Fig. 1C). No differences in food intake were observed between the NN and HN offspring (data not shown).

3.2. Liver lipid content and histological analysis

One week after weaning, the HN group already presented higher hepatic triglycerides levels than NN animals (25.1±3.0 g vs. 37.2±4.1 g, respectively) (Fig. 2A). In adult animals, the liver was yellowish in appearance (data not shown), and the quantitative determination revealed that the liver triglyceride content of hepatic triglycerides was about 1.4-fold higher in HN mice than in the NN group (Fig. 2B).

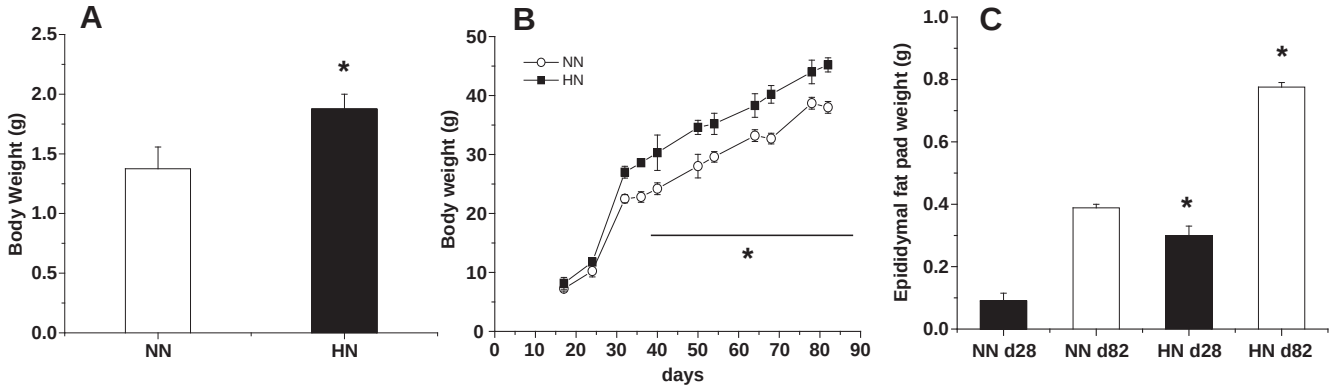

Fig. 1. Body parameters of NN and HN animals. (A) Body weight at d2, (B) body mass evolution from d17 to d82 and (C) epididymal fat pad weight at d28 (black bars) and d82 (white bars). Data are means±S.D., n=8–10. *HN vs. NN.

In addition, the analysis of HE-stained liver sections showed that the HN group presented large lipid vacuoles within hepatocytes, while the NN group had normal liver structure, without lipid vacuoles (Fig. 2C).

3.3. Plasma analyses

Serum leptin and insulin concentrations were not significantly different across pregnancy and lactation in either group (control dams and HFD dams). However, serum leptin and insulin levels in pregnant (d14) and lactating (d14) dams were affected by an HFD (Table 2). Inflammatory cytokines also were quantified in the serum of dams during pregnancy (d14) and lactation (d14). Serum TNFα and IL1β levels were significantly greater in HFD dams than in control dams (Table 2).

There were no significant differences in lipid profiles or plasma glucose between HN and NN offspring. The levels of fasting insulin and TNFα were higher in HN than in NN animals (Table 3).

3.4. Expression and activation of proteins of insulin signaling in the liver

Initially, we examined whether the offspring of dams fed an HFD during gestation and lactation presented changes in the protein of the insulin-signaling pathway in liver tissue. The expression of total IR, IRS1 and AKT was not different between the groups at d82 (Fig. 3). However, the HN group presented reduced phosphorylation of the insulin signaling components as compared to the NN group (Fig. 4). As expected, the caval administration of insulin induced tyrosine phosphorylation of the insulin receptor (2.4-fold) (Fig. 4A), as well as IRS-1 (2.1-fold) (Fig. 4B) and IRS-2 (2.6-fold) (Fig. 4C), when compared to saline-treated controls. Moreover, serine phosphorylation of PKB/AKT also increased (6.7-fold) after insulin administration (Fig. 4D) when compared with the vehicle. However, in the HN group,

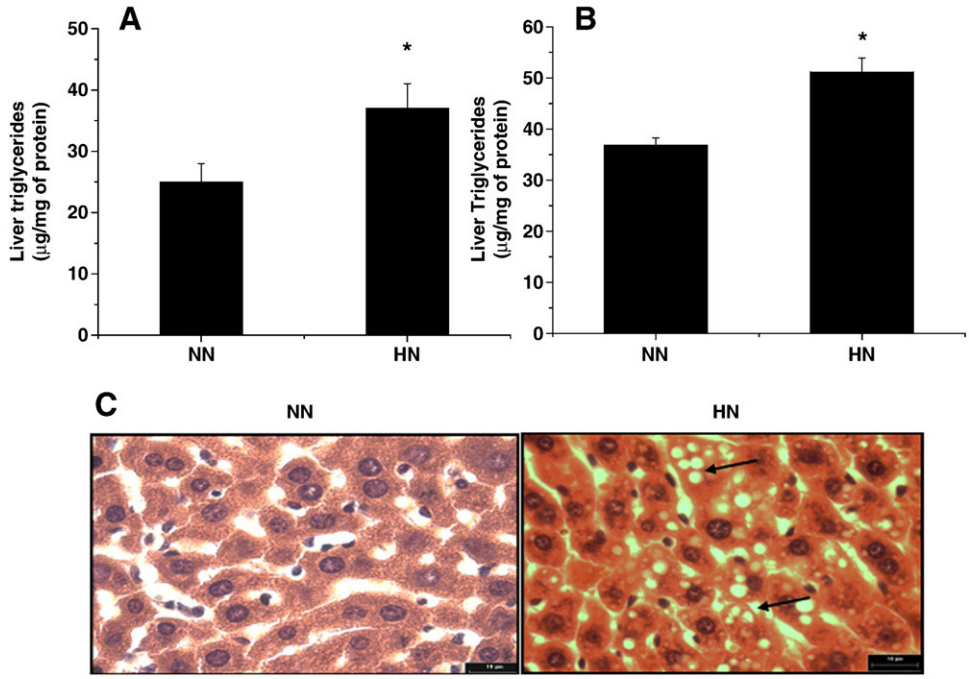

Fig. 2. Liver triglyceride content in NN and HN animals at d28 (A) and d82 (B) and photomicrographs of liver stained with HE from NN and HN animals at d82 (C). Data are means±S.D., n=5 (from different litters). To A and B $P \leq .05$, *HN vs. NN.

Table 2
Maternal serum biochemical parameters of control dams (C) and fed dams (HFD) during pregnancy (d14) and lactation (d14)

	Groups			
	C		HFD	
	Pregnancy d14	Lactation d14	Pregnancy d14	Lactation d14
Serum insulin (ng ml^{-1})	a 1.6±0.17	b 1.0±0.23	ab 2.7±0.15	b 1.9±0.20
Serum leptin (ng ml^{-1})	ab 25.2±4.5	cd 26.9±8.4	ac 68.6±9.1	bd 75.0±12.0
Serum IL1β (pg ml^{-1})	ab 61±7.0	c 67±9.0	a 98±7.0	bc 102±11.0
Serum TNFα (pg ml^{-1})	ab 102±12.4	c 110±9.0	a 146±9.4	bc 155±13.0

Data are means±S.E.M., n=6 per group. $P \leq .05$ to means with the same letters.

the effect of insulin was significantly reduced. The phosphorylation of IR, IRS1, IRS2 and AKT was increased by 1.6-fold, 1.5-fold, 1.8-fold and 3.1-fold, respectively, when compared to the saline-treated control.

3.5. Liver p-ACC, FAS and PEPCK expression

Total FAS expression in the liver of the NN and HN animals was evaluated. The expression of FAS was higher in the NN group than the HN group (1.4-fold) (Fig. 5A). The basal p-ACC level was reduced (3.3-fold) in HN when compared to NN (Fig. 5B). On the other hand, PEPCK expression increased in the group HN by about 35% when compared to the NN group (Fig. 5C).

3.6. JNK, IKK and hormone-sensitive lipase phosphorylation

Basal JNK and IKK phosphorylation in liver tissue of the NN and HN groups was evaluated. Compared to saline-treated controls, basal phosphorylation of JNK and IKK in the liver from HN mice increased (3.2-fold and 2.3-fold, respectively) as compared to NN mice (Fig. 6A and B). Basal hormone-sensitive lipase (HSL) phosphorylation (SER_{565}) was evaluated in the epididymal fat pad of NN and HN animals. The basal phosphorylation of HSL of HN animals was reduced 1.8-fold as compared to the NN group (Fig. 6C).

4. Discussion

It is well established that maternal diet during gestation and lactation influences fetal and postnatal development. Prenatal and suckling exposure to a diet rich in animal fat leads to excessive body weight, insulin resistance, pancreatic β-cell dysfunction and the development of a metabolic-syndrome-like phenotype in adult life [18–20].

Insulin resistance is central to the pathogenesis of the metabolic syndrome, and recent data indicate that NAFLD should be considered the hepatic manifestation of the metabolic syndrome [10]. In our study model, the adult offspring did not develop glucose intolerance,

Table 3
Postnatal serum biochemical parameters at d82 in offspring of HN and NN groups

Parameters	Groups	
	NN	HN
Fasting serum glucose (mg dl^{-1})	137±23	161±30
Fasting serum insulin (ng ml^{-1})	0.8±0.07	1.4±0.1 *
Serum TNFα (pg ml^{-1})	85±9.2	147±18.4 *
Serum IL1β (pg ml^{-1})	45±7.0	85±12.1 *
Serum triglycerides (mg dl^{-1})	170±30	102±25

Data are means±S.E.M., n=5–7 per group.
* $P \leq .05$ to NH vs. NN.

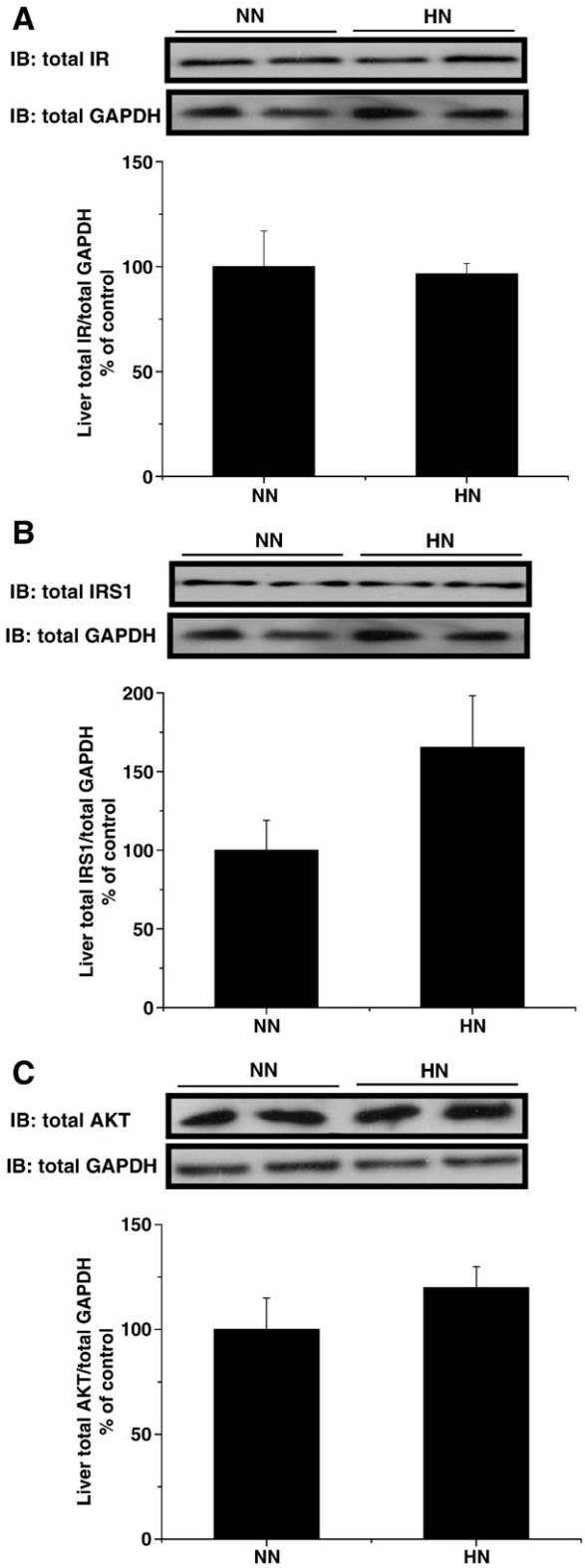

Fig. 3. Expression of IR, IRS-1 and AKT protein in liver of NN and HN animals at d82. (A) Immunoblotting (IB) with anti-IR; (B), IB with anti-IRS-1 and (C) IB with anti-AKT. Bars show quantification of total IR, IRS-1 and AKT normalized by total GAPDH in tissue. Data are means±S.D., n=5 (from different litters). $P \leq .05$.

as evaluated by glucose tolerance test (GTT) (data not shown). In addition, fasting glucose was not different between HN and NN mice. However, the body weight at birth and the epididymal fat pad (d28

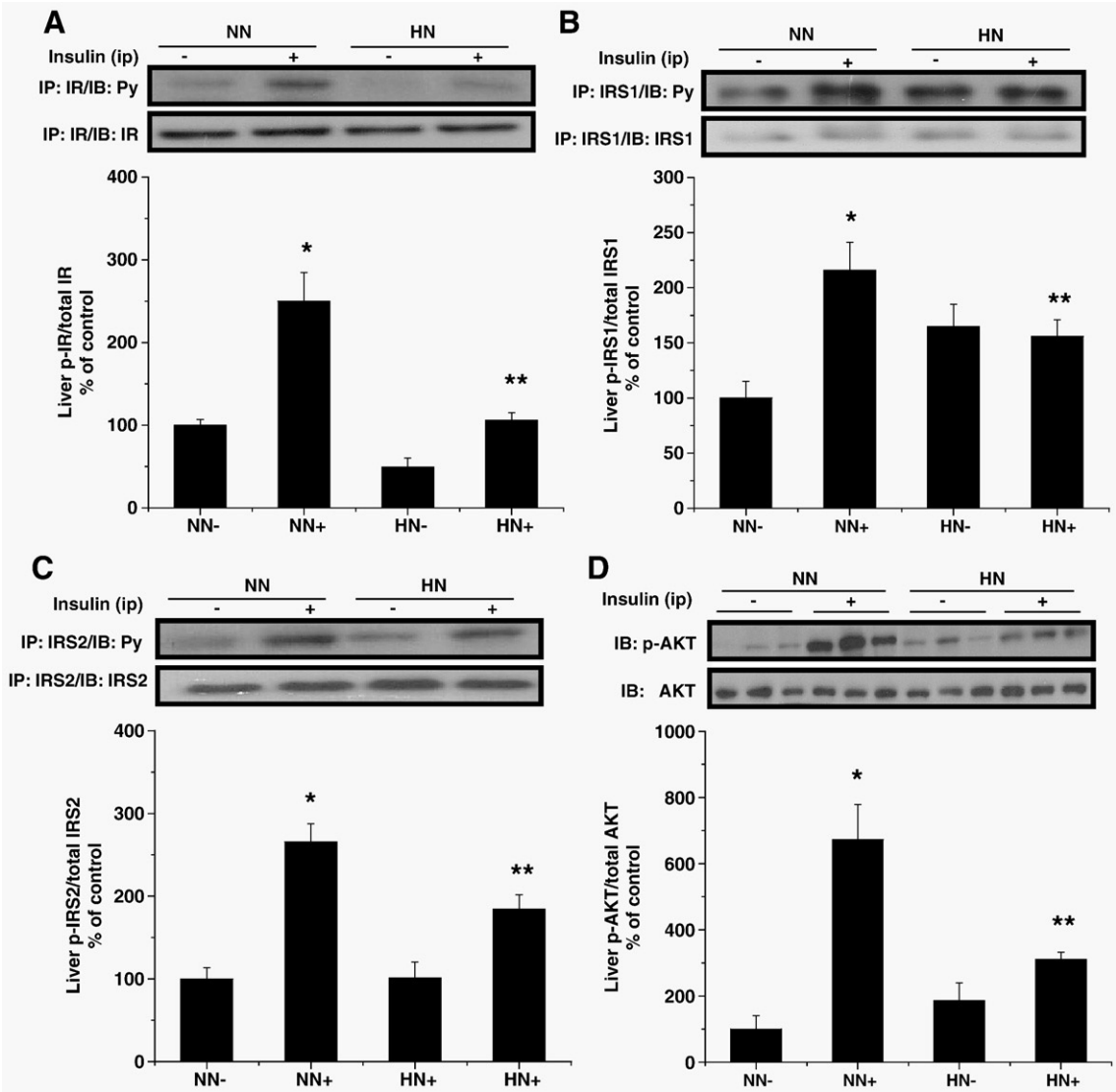

Fig. 4. Insulin signaling in liver of NN and HN animals at d82. (A) Immunoprecipitation (IP) with anti-IR and immunoblotting (IB) with anti-Py antibodies; (B) IP with anti-IRS-1 and IB with anti-pY; (C) IP with anti-IRS-2 and IB with anti-pY; (D) IB with anti-p-AKT. Mice subjected to an overnight fast received either saline (−) or insulin (+) (3 pmol) through the cava vein before liver excision. Bars show quantification of phosphorylated protein normalized by total protein in tissue. Data are means±S.D., $n=5$ (from different litters). $P \leq .05$, *NN+ vs. NN, **HN+ vs. NN+.

and d82) were higher in HN than NN animals, and there was increased body weight gain up to d82. This phenotype may be a result of the inflammatory milieu in which the fetus developed. The maternal body weight was higher in HFD dams than in control dams. Besides, when compared to control dams, HFD dams were hyperleptinaemic and hyperinsulinaemic during pregnancy and lactation. Seeber and colleagues [21] suggest that the increase in the serum leptin during gestation in control dams is associated to leptin resistance in the pregnancy, a physiological adaptation. More recently, Howie and colleagues [22] demonstrated that rats fed with HFD during pregnancy and lactation were hyperleptinaemic and hyperinsulinaemic at the end of the nursing period (d22) when compared to control dams. Then, the maternal consumption of HFD during pregnancy could be affecting the hormonal signaling and leading to maternal leptin and insulin resistance and gestational diabetes.

Obesity during pregnancy stimulates macrophage accumulation and inflammation in the placenta [11], and although in the present study we did not evaluate the macrophage accumulation in the placenta of the HFD dams, an increased serum inflammatory cytokine level (TNFα and IL1β) was observed. Tumor necrosis factor α activated pathways have been described as potential links between increased placenta inflammation and altered maternal metabolic homeostasis [11,23]. These findings indicate potential programming effects of an altered intrauterine environment induced by the maternal consumption of HFD.

The hypothalamus plays a central role in energy homeostasis by regulating both appetite and energy expenditure. In the present study, we did not evaluate the fetal hypothalamic alterations in HN mice. However, Gupta and colleagues [24] showed that fetuses of female rats fed an HFD had significantly increased expression of both leptin and insulin receptor, and serum leptin and insulin levels. Furthermore, young adults exposed early (intrauterine/perinatal) to hydrogenated fat rich in trans-fatty acids showed loss of insulin-induced hypophagia [8], indicating that the mismatch between early and late nutritional environments was relevant. Therefore, the maternal and fetal environments during pregnancy and obesity in rats fed an HFD may contribute to metabolic-syndrome-like phenotype in adult life.

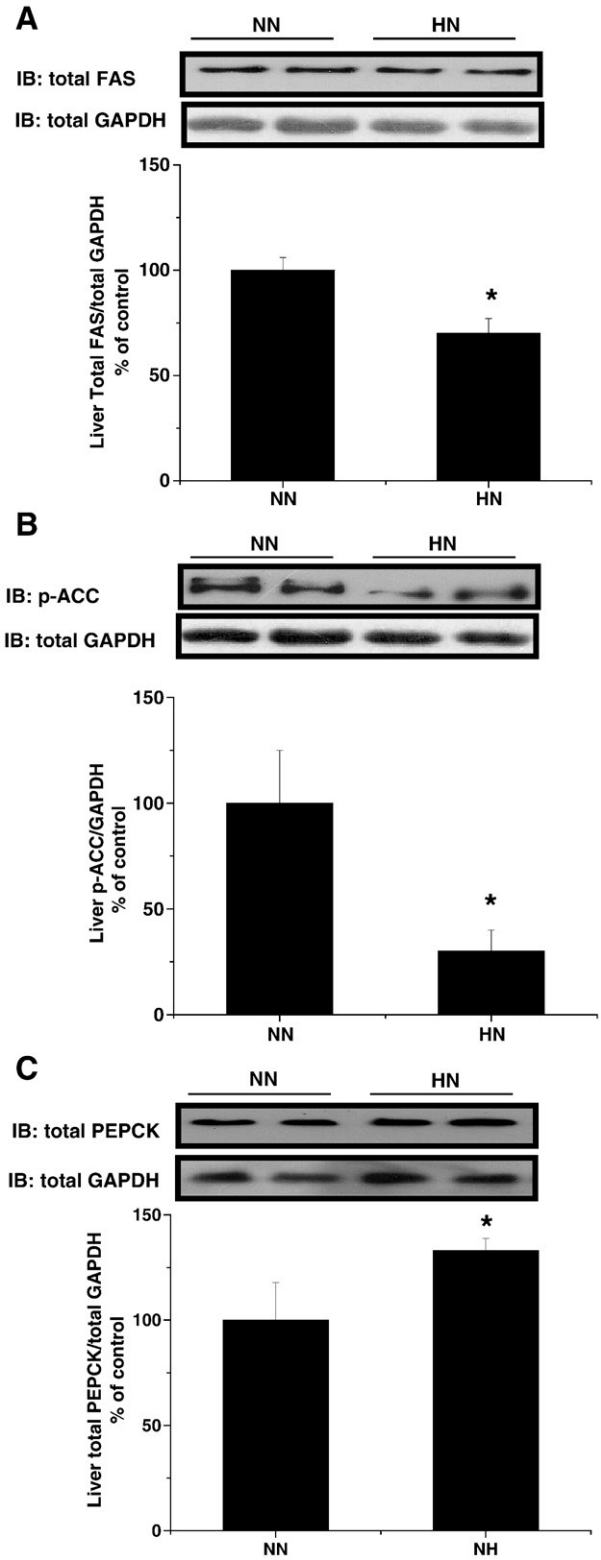

Fig. 5. Basal level of FAS (A), p-ACC (B) and PEPCK (C) in liver from NN and HN animals. (A) Immunoblotting (IB) with anti-FAS antibodies; (B) IB with anti-p-ACC and (C) IB with anti-PEPCK. Bars show quantification of total protein normalized by total GAPDH in tissue. Data are means±S.D., $n=5$ (from different litters), $P\leq.05$, *HN vs. NN.

Interestingly, although insulin resistance was not observed through GTT in HN animals, the animals of the HN group were hyperinsulinaemic and euglycaemic when compared to the NN group. Therefore, we believe that the high insulin level could be compensating peripheral insulin resistance. A similar result was found by White and colleagues [25] who demonstrated that offspring of obese

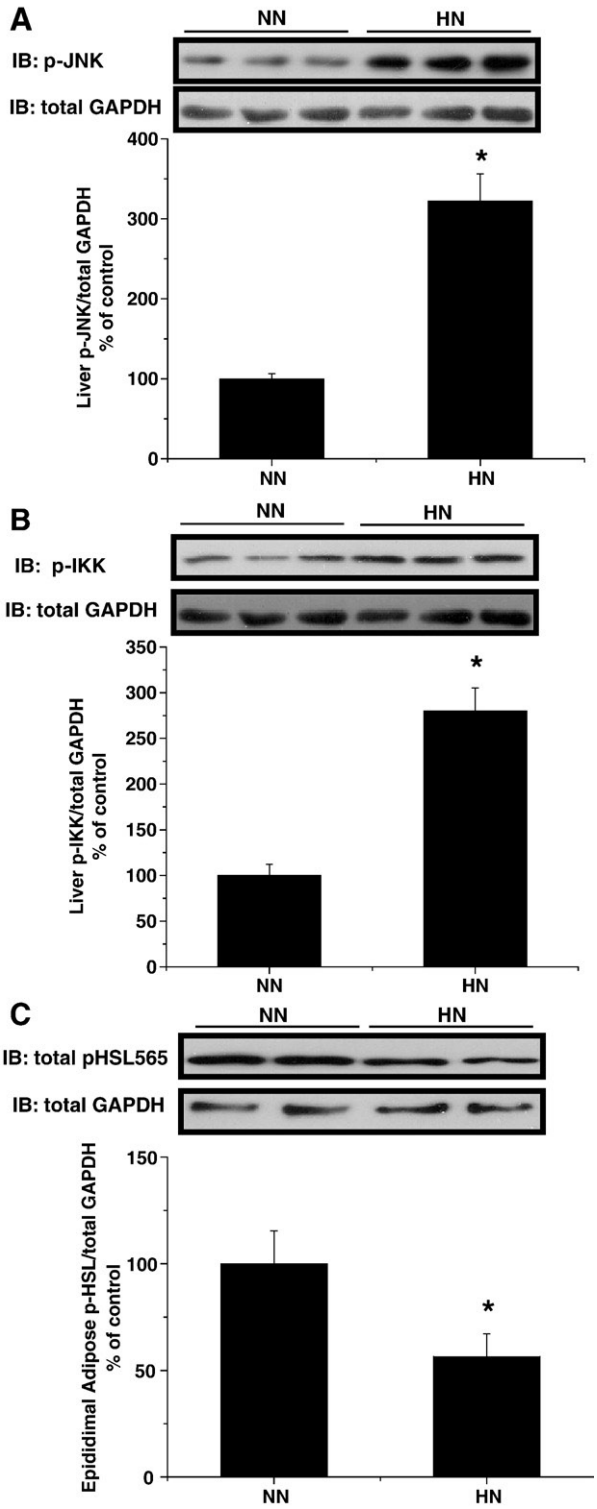

Fig. 6. Liver basal phosphorylation of JNK (A), IKK (B) and HSL_{565} (C) in NN and HN groups. (A) Immunoblotting (IB) with anti-p-JNK antibodies; (B) IB with anti-p-IKK and (C) IB with anti-p-HSL_{565}. Bars show quantification of total protein normalized by total GAPDH in tissue. Data are means±S.D., $n=5$ (from different litters). $P\leq.05$, *HN vs. NN.

and lean dams had similar sensitivity to insulin at 8 weeks of age, although they had been less sensitive at weaning.

The hepatic insulin resistance seems to be an essential requirement for the development of NAFLD. It contributes to NAFLD by altering TG synthesis and transport, increasing the lipolysis rate in the adipose tissue and the transport of FFAs to the liver [26,27]. *De novo* lipogenesis contributes significantly to hepatic accumulation of TG in NAFLD [28], and in this condition, the amounts of enzymes of the FA biosynthesis pathway may be increased. Here, we showed that HN mice presented lipid vacuoles in liver cells, indicating an imbalance in triglyceride formation and turnover, a characteristic of NAFLD. Moreover, serum triglycerides were not increased in the HN group, suggesting an inadequate liver TG export, which might be related to reduced liver's ability to secrete large very low density lipoprotein [27]. Besides, 1 week after weaning, the offspring of obese dams also showed hepatic accumulation of TG and increased epididymal fat pad when compared to NN animals, indicating that the liver phenotype is a direct outcome of maternal fat feeding. Interestingly, at 82 days of age, the HN animals presented reduced expression of FAS and smaller phosphorylation of ACC than animals from the NN group, corroborating the data that showed an increase in the lipid content in the liver of HN mice. ACC is a multifunctional enzyme that catalyses the conversion of acetyl-CoA to malonyl-CoA, a precursor of lipid synthesis pathway when activated (dephosphorylated form). Additionally, malonyl-CoA is a potent inhibitor of carnitine palmitoyl transferase-1, a rate-limiting step for the entry of long-chain FAs into the mitochondria for oxidation [29]. Therefore, the inhibition of oxidation, rather than the stimulation of synthesis, may have contributed to lipid accumulation in the liver.

In spite of several studies that describe a broad range of metabolic abnormalities presented by offspring of HFD-fed dams during gestation and lactation [5–7,9,18,30], the presence of triglycerides in the liver of adult offspring of HFD-fed dams was demonstrated by some studies [18,19,31]. Recently, in an elegant study with nonhuman primates, McCurdy and colleagues suggested that the increased level of liver triglycerides in offspring of HFD-fed dams resulted from maternal lipid transfer because there was no change in either mRNA or protein expression of any of the lipogenic enzymes. Although maternal lipid transfer may have contributed to fetal NAFLD, this possibility was discarded in our study, as the offspring were 12 weeks old. However, maternal lipid transfer may be associated with permanent metabolic changes in fetal liver that increase the risk of steatosis during fetal development, such as reduced TG export and decreased FA oxidation. It is possible that the liver from adult offspring presents diminished FA oxidation, as indicated by reduced ACC phosphorylation in the HN group. As discussed above, dephosphorylated ACC is active and produces malonyl-CoA, an inhibitor of FA oxidation. We acknowledge limitations in the evaluation of the p-ACC level; however, these animals also presented reduced phosphorylation of HSL_{ser565} in the adipose tissue and a higher level of serum FFAs when compared to the NN group, indicating that the flux of FAs to the liver increased. Altogether, these results may at least partially explain liver lipid accumulation and the ensuing NAFLD and insulin resistance.

To investigate insulin resistance, we evaluated insulin-induced phosphorylation of liver IR, IRS and AKT proteins. Although insulin resistance was not observed through the insulin or glucose tolerance tests, the animals of the HN group presented lesser insulin-induced phosphorylation of liver IR, IRS and AKT as compared to the NN animals. Thus, the liver of the offspring of dams fed an HFD in pregnancy presents molecular resistance to insulin, which may contribute to hepatic steatosis. Insulin resistance is an important event that may precede triglyceride accumulation in the liver [10,26]. The conventional explanation for hepatic triglyceride accumulation is that visceral obesity and insulin resistance, mostly mediated by adipokines, such as TNF-α, result in increased FFA release from adipocytes with the consequent enhanced delivery of lipids to the liver. Additionally, the reduced capacity of insulin to inhibit the production of hepatic glucose aggravates the peripheral insulin resistance and contributes to hepatic lipogenesis [26].

The pathogenesis of insulin resistance has the activation of serine kinases as a key event. The offspring of HFD-fed dams (HN group) showed increased basal phosphorylation of liver IKK and JNK and higher levels of serum IL1β and TNFα when compared to the offspring of dams fed chow (group NN). The concentration of serum FFAs also increased in the HN when compared to the NN group. In an animal model of diet-induced fatty liver disease, the increase in the expression of inflammatory cytokines contributed to worsening of insulin signaling and activation of gluconeogenic and lipidogenic pathways [32]. Cytokines are potent JNK and IKK activators, two serine kinases that may contribute to the deregulation of the insulin-signaling pathway [33–35]. Thus, we believe that two mechanisms may contribute to lipid accumulation in the liver. Initially, it is likely that the exposure to a dysmetabolic condition in the uterine environment induced by HFD consumption alters the leptin and insulin signaling in the late fetal period. Gupta and colleagues [24] have demonstrated potential programming effects of an altered intrauterine environment on appetite-regulating neuropeptides and leptin and insulin signaling in the late fetal period. This effect is an important aspect to be investigated and can explain the increased adiposity and excessive body weight in adult life in the offspring of fat-fed dams (HN group at d82). However, the food intake was similar between both groups studied, suggesting that a reduction in energy expenditure may be present. One possibility would be the reduction in the expression of UCP2, as shown in the brain, white adipose tissue and muscle of diet-induced obesity compared to normal rats [36].

The contribution of the uterine environment to obesity and metabolic syndrome in adult mice is not yet completely known. In rodent models, maternal HF feeding has been reported to have variable effects on metabolic changes in offspring. These discrepancies are likely due to differences in FA composition of fat-enriched diets across studies and varying levels of maternal intake of saturated fat [22,37,38]. Caluwaerts and colleagues [30] showed that overweight, adipocyte hypertrophy and increased TNFα gene expression in white adipose tissue were observed from early life to d35, but not in postpubertal rats. On the other hand, offspring of fat-fed dams presented impaired glucose homeostasis and mitochondrial abnormalities in 3–9-month-old and 1-year-old mice [20] and hyperinsulinaemia and hyperleptinaemia [22].

Our study showed that the offspring presented liver lipid deposition at d82, as well as molecular insulin resistance and excessive body weight. In addition, serum insulin, TNFα and IL1β, and liver p-JNK and p-IKK were higher in HN animals. Taken together, these results reinforce the importance of maternal nutrition during these critical windows of development and suggest that consumption of an HFD by the mother predisposes the offspring to develop a metabolic-syndrome-like phenotype in adult life independent of postnatal nutrition.

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