



# Long-chain *n*-3 PUFA given before and throughout gestation and lactation in rats prevent high-fat diet-induced insulin resistance in male offspring in a tissue-specific manner

Nathalie Guriec<sup>1</sup>, Christelle Le Foll<sup>1†</sup> and Jacques Delarue<sup>1,2\*</sup>

<sup>1</sup>Department of Nutritional Sciences, University Hospital/Faculty of Medicine/University of Brest, Brest, France

<sup>2</sup>ER 7479 SPURBO, University Hospital/Faculty of Medicine/University of Brest, Brest, France

(Submitted 11 September 2022 – Final revision received 9 January 2023 – Accepted 16 January 2023 – First published online 23 January 2023)

## Abstract

This study investigated whether long-chain *n*-3 PUFA (LC *n*-3 PUFA) given to pregnant rats fed a high-fat (HF) diet may prevent fetal programming in male offspring at adulthood. Six weeks before mating, and throughout gestation and lactation, female nulliparous Sprague–Dawley rats were given a chow (C) diet, HF (60.6 % fat from maize, rapeseed oils and lard) or HF in which one-third of fat was replaced by fish oil (HF *n*-3). At weaning, the three offspring groups were randomly separated in two groups fed C diet, or HF without LC *n*-3 PUFA, for 7 weeks until adulthood. Glucose tolerance and insulin sensitivity were assessed by an oral glucose tolerance test both at weaning and at adulthood. Insulin signalling was determined in liver, muscle and adipose tissue by quantification of the phosphorylation of Akt on Ser 473 at adulthood. At weaning, as at adulthood, offspring from HF-fed dams were obese and displayed glucose intolerance (GI) and insulin resistance (IR), but not those from HF *n*-3 fed dams. Following the post-weaning C diet, phosphorylation of Akt was strongly reduced in all tissues of offspring from HF dams, but to a lesser extent in liver and muscle of offspring from HF *n*-3 dams. However, it was abolished in all tissues of all offspring groups fed the HF post-weaning diet. Thus, LC *n*-3 PUFA introduced in a HF in dams partially prevented the transmission of GI and IR in adult offspring even though they were fed without LC *n*-3 PUFA from weaning.

**Key words:** EPA/DHA: Fetal programming: Nutrition: Insulin resistance: *n*-3 fatty acids: High-fat diet

The increase in obesity and diabetes prevalence is a worldwide major public health concern. According to WHO<sup>(1)</sup>, worldwide obesity has nearly tripled since 1975. In 2020, 764 million adults were obese<sup>(2)</sup>. According to the International Diabetes Federation<sup>(3)</sup>, 537 million adults are living with diabetes in 2021. Insulin resistance (IR) is a major factor that leads from obesity to type 2 diabetes<sup>(4)</sup>. Although overfeeding and lack of exercise during infancy and adulthood predispose to non-communicable diseases, many epidemiological studies strongly suggest that maternal undernutrition or obesity<sup>(5–8)</sup> could lead to obesity, IR, type 2 diabetes and CVD in the offspring during adulthood. This phenomenon has been called: Barker's hypothesis, fetal programming or Developmental Origins of Health and Disease. Growing bodies of evidence from rodent models demonstrate that maternal nutrition during pregnancy can increase the risk of developing metabolic alterations in offspring during early life and adulthood<sup>(8)</sup>.

The amount and type of dietary fatty acids (FA) also contribute to fetal programming<sup>(10,11)</sup>. In rodent models, a high-fat (HF) diet rich in SFA given during pregnancy induces several metabolic alterations in offspring during adulthood: liver steatosis, increased body and visceral fat mass, adipocyte hypertrophy, pro-inflammatory cytokines production, IR, pancreatic islet hypertrophy and oxidative stress<sup>(11,12)</sup>. Long-chain (LC) *n*-3 PUFA, mainly EPA and DHA, can prevent and even decrease IR in rodents fed a HF diet, which has also been shown in humans<sup>(13–18)</sup>. During pregnancy, the only source of PUFA for the developing fetus is via the placenta. Placental tissue expresses lipoprotein receptors and enzymes with lipase and phospholipase activities involved in the maternal FA transfer across the placenta. NEFA that originate from maternal metabolism and dietary FA can also cross the placenta<sup>(11,19)</sup>. Several studies have demonstrated selectivity in placental transfer of FA and a strong preference by the placental plasma membrane

**Abbreviations:** AT, adipose tissue; BAT, brown adipose tissue; FA, fatty acid; GI, glucose intolerance; HF, high fat; HOMA-IR, homeostasis model assessment for insulin resistance; IR, insulin resistance; LC *n*-3 PUFA, long-chain *n*-3 PUFA; OGTT, oral glucose tolerance test.

\* **Corresponding author:** Jacques Delarue, email [jacques.delarue@univ-brest.fr](mailto:jacques.delarue@univ-brest.fr)

† Present address: Institute of Veterinary Physiology, University of Zurich, CH-8057, Zurich, Switzerland.



binding sites for LC-PUFA, especially DHA<sup>(20–22)</sup>. Nishimura *et al.*<sup>(23)</sup> demonstrated that the maternal dietary EPA and DHA content during the third trimester of pregnancy is directly associated with the content of these FA in mature breast milk. Additionally, the same human study showed a direct relation between the dietary *n*-3 to *n*-6 ratio in the postpartum period and milk composition<sup>(23)</sup>. In gestational diabetes mellitus-exposed mothers, DHA transfer to the fetus (assessed by <sup>13</sup>C DHA) is reduced<sup>(24)</sup>. Several studies performed in animal models have assessed, in offspring at adulthood, the effects of LC *n*-3 PUFA<sup>(11,25)</sup> given during pregnancy and/or lactation on adiposity, liver steatosis, inflammation<sup>(26–30)</sup> and/or oxidant/anti-oxidant status, and DNA methylation. Other studies have assessed their effects on insulin sensitivity<sup>(28–33)</sup>, but with different models: glucocorticoids-induced IR or LC *n*-3 PUFA given only in the offspring diet and not to dams or use of fat-1 mice model, rendering somewhat difficult to draw conclusions about the specific role of diet given to dams.

A meta-analysis of eleven randomised controlled trials<sup>(34)</sup> and a recent review<sup>(35)</sup> concluded no beneficial effect of LC *n*-3 PUFA given during pregnancy on obesity in offspring. Another one concluded no benefit towards IR but to a significant decrease in C-reactive protein<sup>(36)</sup>. In the MIGHT randomised controlled trial, a supplementation during pregnancy of 800 mg/d of DHA *v.* 200 mg/d associated with dietary counselling in overweight or obese mothers did not reduce the risk of gestational diabetes mellitus, macrosomia or IR in neonates<sup>(37)</sup>. A Danish study compared the metabolic parameters of offspring aged 9–16 years from mothers with gestational diabetes mellitus or healthy mothers<sup>(38)</sup>; no association was found between LC *n*-3 PUFA and seafood intakes of mothers during pregnancy with offspring metabolic outcomes, but gestational diabetes mellitus-exposed women reporting no fish intake between week 12 and 30 *v.* intake > 2 times/week had an offspring with a higher BMI, waist circumference and homeostasis model assessment for insulin resistance (HOMA-IR). It is to note that following supplementation, the plasma concentrations of LC *n*-3 PUFA were less raised in obese pregnant women than in lean ones, which could have attenuated their beneficial effects<sup>(39,40)</sup>.

Because of the discrepancy in results and the small number of studies, the present study aimed to determine whether *n*-3 LC PUFA administered during pregnancy and lactation to female rats fed a HF diet could prevent IR in the adult male offspring, and examined the basic mechanisms potentially involved in their favourable effects.

## Experimental methods

### Animal care

The experimental procedure was approved by the Animal Ethical Committee (agreement R-2012-NG-01). The protocol was carried out in conformity with French laws and regulations and the ARRIVE Guidelines for Reporting Animal Research.

Twenty-one nulliparous 5-week-old female Sprague–Dawley rats (Elevage Janvier) were individually housed in a temperature-, humidity- and light-controlled room. After 1 week of acclimation, they were randomly divided into three groups (*n* 7 per group), fed

*ad libitum* with either a standard chow (C) diet (control group; *n*°210 SAFE), or a HF diet (HF group, 60.6 % of energy content as fat, *n*°230 SAFE), or a HF *n*-3 diet (HF*n*-3 group, 60.6 % of the energy content as fat; *n*°230 modified SAFE). Fat in HF diet was provided by maize oil, rapeseed oil and lard (Table 1). In HF*n*-3 diet (60.6 % of energy content as fat), one-third of fat was replaced by a marine-derived TAG oil rich in LC *n*-3 PUFA (Omegavie® 1050 TG Quality Silver, kindly provided by Polaris) (Table 1). The FA composition of the three diets is reported in Table 2.

After 6 weeks of diet, two dams of each group were mated for 48 h with one Sprague–Dawley male rat fed a C diet (eleven different males). Dams were further continued on their initial diet throughout gestation and lactation. At birth, all the litters were weighed and adjusted to eleven pups (male and female) per dam to ensure a standard litter size per dam, with four males per litter. At weaning (day 21 after birth), female pups were given for adoption at Brest University Animal Facility. For each dam's diet, male pups of each dam (seven dams per diet, four male pups per dam) were divided at weaning in two equal subgroups, one receiving the C diet (10–14 pups), the other one the HF diet without LC *n*-3 PUFA (HF diet, 11–14 pups) for 7 weeks before euthanasia (Fig. 1). The dams and pups were weighed twice a week all along the study.

At sacrifice (2 days after weaning for the dams and 7 weeks after weaning for the male offspring), dams and adult offspring were submitted to the same protocol. After 12 h fast in the dark phase, rats were randomised and were euthanised (decapitation after intra-peritoneal pentobarbital injection, 50 mg/kg). Half of each group of dams or of adult offspring received, 10 min before sacrifice, an intra-peritoneal injection of either 9% NaCl (vehicle) or 4 µg/kg of insulin (Umuline). Blood was immediately collected, and the plasma was separated by centrifugation and stored at –20°C until subsequent analysis. Liver, leg muscles (gastrocnemius) and peri-epididymal fat tissue were weighted and snap frozen into liquid N<sub>2</sub> and stored at –80°C.

### Oral glucose tolerance test

An oral glucose tolerance test (OGTT) (3 g/kg body weight) was performed in dams 3 d before mating, in pups at weaning (post-natal day 21) and 6 weeks after weaning (Fig. 1). After 12 h fasting, a local anaesthesia with lidocaine (5 % EMLA cream; AstraZeneca) was performed on the tail, and a vein blood sample was taken (*t* = 0). The glucose solution was then administered orally by gavage. Blood samples were collected at 15, 30, 45, 60, 90 and 120 min. For each sample, a fraction was immediately used for glucose determination with a GLUCOTOUGH Pro Glucometer (Lifescan), another fraction was collected in heparinised tubes (Microvette; Sarstedt) and plasma was separated by centrifugation and stored at –20°C.

*HOMA-IR score* was calculated using the formula: fasting insulin (µIU/ml) × (fasting blood glucose (mmol/L)/22.5).

### Milk and plasma glucose, insulin, leptin and NEFA plasma concentrations

Plasma insulin and leptin concentrations were determined using ELISA kits (Merck-Millipore). Plasma and milk NEFA

**Table 1.** Composition of the different diets

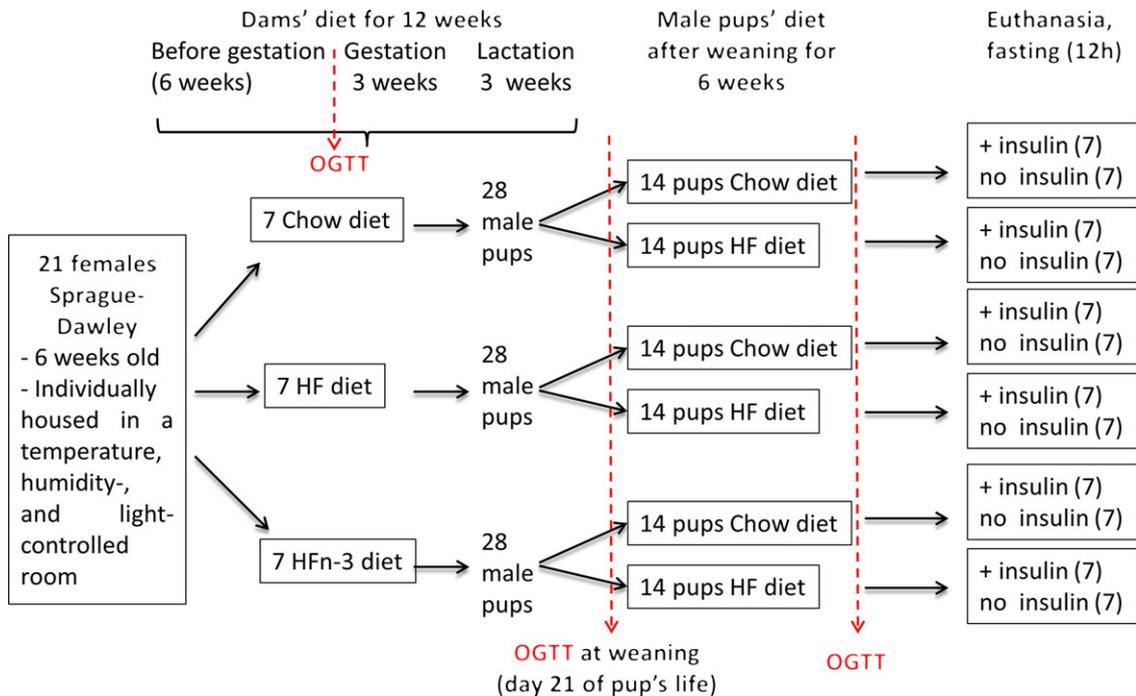
	Chow diet (C)		Hyperlipidic diet (HF)		Hyperlipidic with <i>n</i> -3 diet (HF <i>n</i> -3)	
	Weight (%)	Energy content (%)	Weight (%)	Energy content (%)	Weight (%)	Energy content (%)
Proteins	19.6	22.8	17.4	13.1	17.4	13.1
Carbohydrates	55.1	64.1	36.8	26.3	36.8	26.3
Lipids	5	13.1	35.8	60.6 (maize oil, rapeseed oil, lard)	35.8	60.6 (1/3 as fish oil, 2/3 as maize oil, rapeseed oil, lard)
Minerals	7	0	5.9	0	5.9	0
Vitamins	1	0	1	0	1	0
Fibres (cellulose)	6	0	0	0	0	0
Energy supply (kcal/100g)		343.8		531.7		531.7

Diet composition was provided by the diet manufacturer (SAFE).

**Table 2.** Fatty acid composition of the different diets in mg per 1000 g of diet

Fatty acid	Chow diet (C)	High-fat diet (HF)	High-fat diet with <i>n</i> -3 (HF <i>n</i> -3)
Palmitic acid	8744	86 278	57 683
Stearic acid	4299	47 256	31 594
Palmitoleic acid	940	10 622	7102
Oleic acid	20 050	143 916	96 218
Linoleic acid	10 165	33 473	22 379
$\alpha$ -Linolenic acid	1222	3598	2406
EPA			15 080
DHA			30 320
other <i>n</i> -3 (18:4, 20:4)			8120
Sum <i>n</i> -3	1222	3598	85 926
Sum <i>n</i> -6	10 165	33 473	22 379
Cholesterol	26		203

Fatty acid composition was determined by the diet manufacturer SAFE (Augy) and Polaris (Quimper).



**Fig. 1.** Schematic overview of the experimental protocol.



**Table 3.** Morphometric and biochemical characteristics of dams (Mean values with their standard error of the means)

	Chow diet (n 7)		HF diet (n 7)		HF $n$ -3 diet (n 7)							
	Mean	SEM	Mean	SEM	Mean	SEM						
<b>Initial body weight (g)</b>	201.6	2.6	202.5	2.3	198.1	2.3						
<b>After 6 weeks of diet and before mating</b>												
Body weight (g)	302.4	3.5	311.2	2.7	296	4.4						
Body weight gain (g)	93.63	8.5	103.00	7.7	110.73	6.89						
Food intake (kcal/week)	421.46	7.93 <sup>a</sup>	493.75	15.7 <sup>b</sup>	415.28	23.19 <sup>a</sup>						
Glucose at T0 (mg/dl)	78.44	5.61	76.64	1.63	78.44	4.49						
Insulin at T0 (ng/ml)	0.84	0.13	0.88	0.14	0.85	0.13						
HOMA-IR	4.73	0.50	4.96	0.53	4.66	0.47						
AUC glycaemia (mg/dl per 2 h)	11 801	339 <sup>a</sup>	13 618	243 <sup>b</sup>	12 348	313 <sup>a</sup>						
AUC insulinaemia (ng/dl per 2 h)	120.42	10.08 <sup>a</sup>	188.67	21.70 <sup>b</sup>	96.18	15.48 <sup>a</sup>						
NEFA acids (mM)	0.69	0.02 <sup>a</sup>	0.89	0.02 <sup>b</sup>	0.77	0.03						
Leptin (ng/ml)	2.63	0.37 <sup>a</sup>	6.44	0.25 <sup>b</sup>	3.97	0.34 <sup>c</sup>						
<b>At the end of the gestation and lactation period</b>												
Body weight at the end of gestation (g)	392.1	3.40 <sup>a</sup>	422.40	6.60 <sup>b</sup>	415.10	4.40 <sup>b</sup>						
Body weight gain at the end of gestation (g)	83.63	8.53 <sup>a</sup>	103.00	8.75 <sup>b</sup>	116.00	5.69 <sup>b</sup>						
Cumulative energy intake during gestation (kcal)	235.65	4.76 <sup>a</sup>	327.22	17.86 <sup>b</sup>	375.55	27.13 <sup>a</sup>						
Body weight at the end of lactation (g)	348.90	5.90 <sup>a</sup>	369.00	5.60 <sup>b</sup>	340.90	8.80 <sup>a</sup>						
Body weight gain at the end of lactation (g)	12.88	5.72 <sup>a</sup>	7.25	3.26 <sup>a</sup>	-19.75	7.50 <sup>b</sup>						
Cumulative energy intake during lactation (kcal)	411.54	6.9 <sup>a</sup>	494.67	72.39 <sup>b</sup>	375.55	27.13 <sup>a</sup>						
Number of pups in litters before normalisation	12.29	0.91	11.43	1.20	9.43	0.99						
Percentage of males in litters before normalisation	58.45	2.62	50.19	4.41	48.62	8.57						
<b>At sacrifice (48 h after weaning)</b>												
	Chow diet + vehicle (n 3)		Chow diet + insulin (n 4)		HF diet + vehicle (n 3)		HF diet + insulin (n 4)		HF $n$ -3 diet + vehicle (n 3)		HF $n$ -3 diet + insulin (n 4)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NEFA acids (mM)	0.90	3.5 <sup>a</sup>	0.46	0.03 <sup>a</sup>	1.13	0.05 <sup>b</sup>	0.93	0.03 <sup>b</sup>	0.89	0.04 <sup>a</sup>	0.59	0.03 <sup>a</sup>
Leptin (ng/ml)	2.09	0.13 <sup>a</sup>	2.26	0.16 <sup>a</sup>	5.95	0.42 <sup>b</sup>	6.16	0.62 <sup>b</sup>	3.84	0.74 <sup>c</sup>	4.87	0.50 <sup>b</sup>

The number of rats per group is indicated under each heading. OGTT was performed in a fasting state (12 h). The difference for body weight after lactation was calculated between the body weight at the end of lactation and the body weight after birthing. HOMA-IR (homeostasis model assessment of insulin resistance) score was calculated using the formula: fasting insulin ( $\mu$ U/ml)  $\times$  (fasting blood glucose (mmol/l)/22.5. Results for each parameter were analysed by one-way ANOVA or two-way ANOVA (data at sacrifice). For each row, data points with differing superscript letters (a,b) differ from each other by  $P < 0.05$  by post hoc test after intergroup differences were found by one-way ANOVA.

concentrations were determined by a colorimetric method using a NEFA C kit (Wako Chemical).

**Western blot**

Liver, leg muscles (gastrocnemius) and peri-epididymal fat tissue (1 g) were ground in liquid N<sub>2</sub> and tissue proteins were extracted in ice-cold buffer containing 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM ethylene glycol-bis( $\beta$ -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 1% triton, 0.5% NP40, 2 mM phenylmethylsulfonyl fluoride (PMSF), 10  $\mu$ g/mL leupeptin, 10  $\mu$ g/mL aprotinin, 100 mM Na fluoride, 10 mM Na pyrophosphate, 2 mM Na orthovanadate and pH 7.4. After 45 min on ice, homogenates were centrifuged 5 min at 10 000 g at 4°C. Protein concentrations in supernatants were determined by a DC protein assay (Bio-Rad). Lysates were separated by SDS-PAGE, and immunoblotting was performed using antibodies directed against Akt or Akt phosphorylated on Serine 473 or actin (secondary antibody Goat Anti-Rabbit HRP-linked at a concentration of 1/2500, ref #7074; primary antibody anti-Akt at a concentration of 1/1000, ref # 9272; primary antibody anti-pAkt Ser<sup>473</sup> Akt at a concentration of 1/1000, ref #9271; primary antibody anti-actin at a concentration of 1/4000, ref #4970; Cell Signaling Technology). The results were analysed using Image J software

to obtain the optical density (NIH). Actin was used as loading control.

**Statistics**

Data are presented as mean values with their standard error of the means. Statistical analyses were performed using ANOVA test and Bonferroni post-test with Prism statistics Software for MacOS system (Graphpad software Inc.). A  $P$  value  $< 0.05$  was considered as statistically significant.

**Results**

*Morphometric and biochemical characteristics of dams*

Body weights were not different among the three groups at the start of the study as well as after 6 weeks of diet (Table 3). At the end of gestation, body weight was higher in HF and HF $n$ -3 dams than in C dams ( $P = 0.031$  and  $0.039$ , respectively) with no difference between HF and HF $n$ -3 dams (Table 4). Litter size was not different among the three groups (Table 3). At the end of lactation, body weight of HF dams was higher than that of HF $n$ -3 ( $P = 0.031$ ) and C groups ( $P = 0.034$ ) (Table 3).

**Table 4.** Morphometric and biochemical characteristics of rat male offspring at weaning (day 21) (Mean values with their standard error of the means)

Dam's diet	Chow diet (n 23)		HF diet (n 28)		HF <i>n</i> -3 diet (n 21)	
	Mean	SEM	Mean	SEM	Mean	SEM
Body weight at birth (day 1, g)	8.31	0.32 <sup>a</sup>	10.24	0.41 <sup>b</sup>	8.02	0.31 <sup>a</sup>
Body weight at day 7 (g)	15.70	0.37 <sup>a</sup>	19.00	0.55 <sup>b</sup>	15.06	0.65 <sup>a</sup>
Body weight at day 14 (g)	26.40	0.48 <sup>a</sup>	33.31	0.73 <sup>b</sup>	24.75	0.99 <sup>a</sup>
Body weight at day 21 (g)	35.96	0.64 <sup>a</sup>	46.64	1.38 <sup>b</sup>	32.85	1.29 <sup>a</sup>
Body weight gain at day 21 (g)	27.65	0.56 <sup>a</sup>	36.40	1.15 <sup>b</sup>	24.83	1.20 <sup>a</sup>
NEFA in milk at day 21 (mM)	3.13	0.29 <sup>a</sup>	8.09	0.30 <sup>b</sup>	7.43	0.30 <sup>b</sup>
Plasma NEFA at day 21 (mM)	0.62	0.03 <sup>a</sup>	0.72	0.03 <sup>b</sup>	0.75	0.11 <sup>b</sup>
Plasma leptin at day 21 (ng/ml)	0.48	0.09 <sup>a</sup>	2.22	0.10 <sup>b</sup>	1.19	0.10 <sup>c</sup>
Glucose at T0 (mg/dl)	107.83	3.11 <sup>a</sup>	80.59	4.20 <sup>b</sup>	98.04	3.42 <sup>c</sup>
Insulin at T0 (ng/ml)	0.21	0.03	0.23	0.02	0.16	0.08
HOMA-IR	1.50	0.17	1.31	0.09	1.10	0.16
AUC glycaemia at day 21 (mg/dl per 2 h)	13 230	494 <sup>a</sup>	14 547	339 <sup>b</sup>	14 090	431 <sup>a,b</sup>
AUC insulinaemia at day 21 (ng/dl per 2 h)	36.37	6.35 <sup>a</sup>	52.98	5.08 <sup>b</sup>	28.96	3.36 <sup>a</sup>

HOMA-IR (homoeostasis model assessment of insulin resistance) score was calculated using the formula: fasting insulin ( $\mu$ U/ml)  $\times$  (fasting blood glucose (mmol/l)/22.5. The number of rats per group is indicated in the table heading. Results for each parameter were analysed by one-way ANOVA. For each row, data points with differing superscript letters (a, b) differ from each other by  $P < 0.05$  or less by post hoc test after intergroup differences were found by one-way ANOVA.

Before and during gestation as well as during lactation, energy intake of HF-fed rats was higher than that of HF*n*-3 and C rats ( $P < 0.05$ ) (Table 3); energy intake of HF*n*-3 was not different from that of C rats. The difference of food intake between groups occurred as soon as week 2 on the diet (data not shown).

After 6 weeks on their respective diets, NEFA concentrations were increased by 28% in HF dams compared with C dams (0.89 (SEM 0.02) mM *v.* 0.69 (SEM 0.02) mM,  $P = 0.021$ , Table 3) reflecting the higher percentage of fat in the diet. NEFA plasma levels in HF*n*-3 dams (0.77 (SEM 0.03) mM) were not different from those of HF and C dams (Table 3). Leptin plasma levels before gestation were different among the three groups with HF dams showing a 144% increase as compared with C dams, while HF*n*-3 leptin levels were decreased by 38% as compared with HF dams but were still higher than in C dams (Table 3).

At sacrifice, NEFA plasma levels were higher in HF dams than in C ( $P = 0.022$ ) and HF*n*-3 dams ( $P = 0.021$ ), but not different between HF*n*-3 and C dams (Table 3). Following insulin acute injection before sacrifice, NEFA plasma levels decreased significantly in C rats by 49% ( $P = 0.001$ ) and in HF*n*-3 rats by 30% ( $P = 0.002$ ) but not in HF-fed dams (Table 3). In addition, NEFA plasma levels of HF*n*-3 dams injected with insulin were statistically different and intermediate from that of C- and HF-insulin injected dams ( $P = 0.023$  and 0.002, respectively; Table 3). As we observed before gestation, leptin plasma levels were higher in HF than in C dams ( $P = 0.004$ ) and in HF*n*-3 ones ( $P = 0.031$ ), but no difference was observed between HF*n*-3 and C dams (Table 3).

#### Effect of diet on glycaemic and insulinaemic responses to oral glucose tolerance test and peripheral tissues insulin signalling in dams

At time 0, glycaemia and insulinaemia were not different among the three groups. AUC of glycaemia and insulinaemia during OGTT before gestation were higher in HF than in HF*n*-3 ( $P = 0.032$  and 0.015, respectively) and C rats ( $P = 0.025$  and

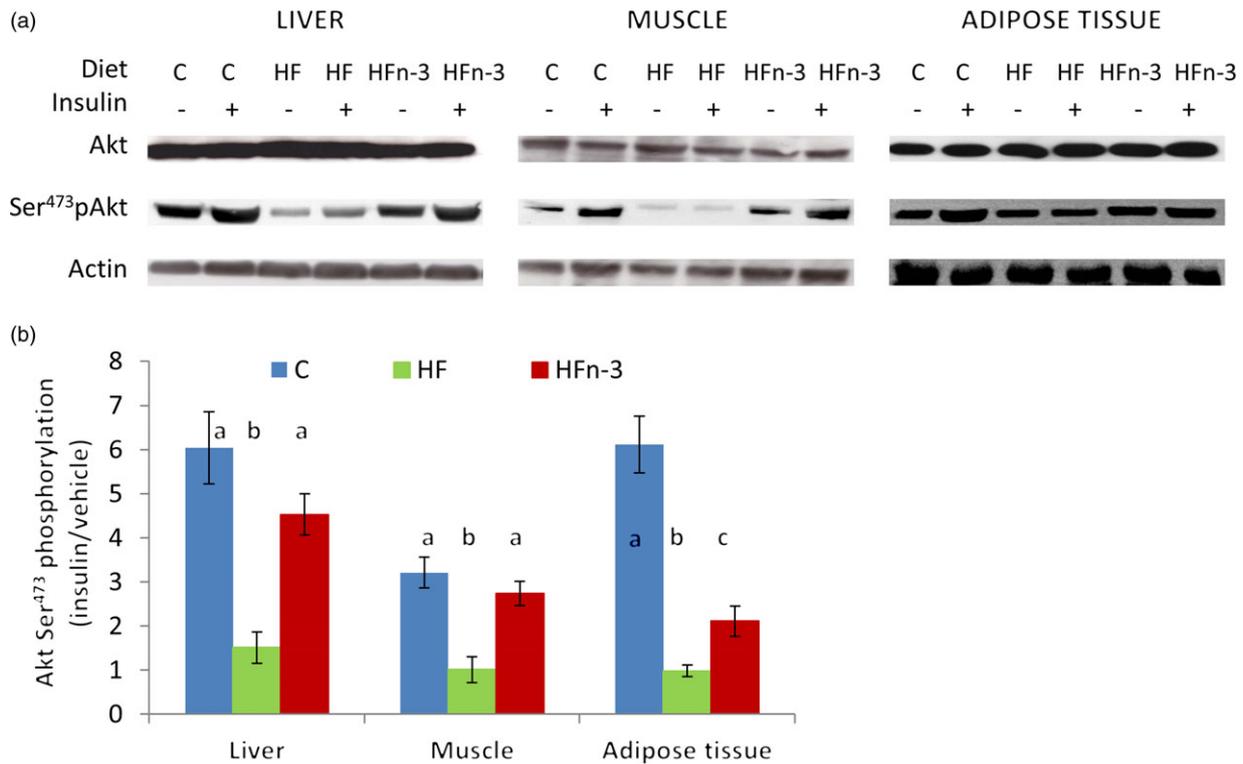
0.026, respectively; Table 3). No difference was observed between HF*n*-3 and C rats ( $P = 0.264$  and 0.244, respectively). HOMA-IR was not different between the three groups of dams (Table 3).

To determine if any of the diet altered insulin signalling, total Akt and Ser<sup>473</sup> Akt phosphorylation were determined in liver, muscle and adipose tissue (AT) of dams at sacrifice. The activation of Akt occurs downstream of phosphoinositide 3'-kinase. Phosphoinositide 3'-kinase generates phosphatidylinositol-3,4,5-triphosphate PIP(3), a lipid second messenger essential for the translocation of PKB/Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1. Akt is a key mediator of the distal steps in insulin signalling, such as modulation of glucose uptake by GLUT4, glycogen synthesis by glycogen synthase kinase (GSK3), protein and fat synthesis by mTOR and gene expression by forkhead family box O<sup>(4)</sup>.

Akt protein abundance remained unaffected by diets in any of the analysed tissues, as estimated by the ratio Akt/ $\beta$ -actin (Fig. 2(a)). Ser<sup>473</sup> Akt phosphorylation was almost completely abolished in the three tissues of HF dams (ratio insulin/vehicle Ser<sup>473</sup>Akt < 1; Fig. 2(b)) while it was activated by 4- and 3-fold, respectively, in liver and muscle of HF*n*-3 dams (Fig. 2(b)). However, it was partially reduced in AT of HF*n*-3 dams as compared with C dams ( $P = 0.033$ ) (Fig. 2(b)). This absence of impairment of the Ser<sup>473</sup> Akt phosphorylation in HF*n*-3 dams may have contributed to the improved glucose tolerance during the OGTT.

#### Characteristics of rat male offspring at birth and at weaning (postnatal day 21)

No difference in sex ratio and number of pups per dam according to the dam's diet was observed. At birth, the male offspring born to HF dams were heavier than those born to C dams (10.24 (SEM 0.41) g *v.* 8.31 (SEM 0.32) g,  $P = 0.007$ ) or to HF*n*-3 dams (8.02 (SEM 0.31) g,  $P = 0.006$ ) while no difference occurred between C and HF*n*-3 pups (Table 4). At weaning,



**Fig. 2.** Analysis of dams' response to insulin at sacrifice after 12 h fasting. Insulin was injected intra-peritoneally at a dose of 4 µg/kg 10 min before euthanasia. C means chow, H means high fat, HFn-3 means high-fat n-3 enriched diet. (a) Western blots of Akt and phosphorylated Akt on Ser<sup>473</sup>. To ensure equal protein loading and transfer, membranes were re-probed for β-actin; (b) quantification of Ser<sup>473</sup> Akt phosphorylation ratio insulin/vehicle. Data are mean values with their standard error of the means (n 7/group). For each tissue, data with differing superscripts differ from each other by  $P < 0.05$  or less when assessed by one-way ANOVA followed by post hoc test.

HF male offspring were heavier (46.64 (SEM 1.38) g) than C (35.96 (SEM 0.64) g,  $P = 0.001$ ) and HFn-3 pups (32.85 (SEM 1.29) g,  $P = 0.002$ ) while body weight and body weight gain were not different between C and HFn-3 pups (Table 4). The difference in body weight between HF pups and C or HFn-3 pups increased every week during lactation (Table 4) and at weaning, HFn-3 male offspring gained 31.8% less weight than HF male ones.

NEFA concentrations in the dam's milk at weaning were higher in HF and HFn-3 than in C dams (8.09 (SEM 0.30) mM and 7.43 (SEM 0.30) v. 3.13 (SEM 0.29) mM,  $P = 0.027$  and 0.024, respectively). They were not different between HF and HFn-3 dams (Table 4).

#### Glucose, insulin, leptin and NEFA concentrations of pups at weaning

During OGTT, glycaemia was increased from time 30 min to time 90 min in HF pups as compared with C pups ( $P < 0.05$ ) (Fig. 3(a)). Glycaemic response in HFn-3 pups was intermediate, but not different from that of HF and C offspring. AUC of glycaemia was higher in HF pups than in HFn-3 and C pups ( $P = 0.02$  and 0.03, respectively), and not different between HFn-3 and C pups (Table 4). Even though glucose levels were decreased in HF pups, insulinaemia at time 0 was not different between the three groups (Table 4). However, insulin levels during OGTT were increased at time 15, 60, 120 min in HF pups as compared with C and HFn-3 pups ( $P < 0.05$ ) (Fig. 3(b)). Insulin AUC was increased by 2-fold for HF pups as compared with HFn-3 pups ( $P = 0.006$ ), whereas it was not different

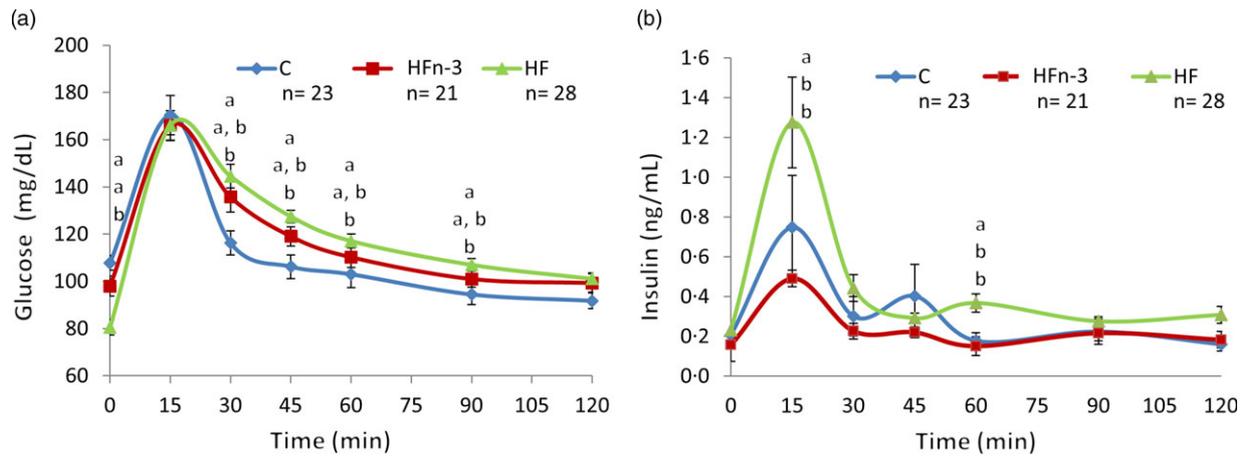
between HFn-3 and C ones. As a result, HF pups need to secrete more insulin than HFn-3 ones to maintain a glycaemia close to that of HFn-3 pups (Fig. 3, Table 4). HOMA-IR was not different between the three groups of pups (Table 4).

Basal plasma leptin levels were higher in HF (2.22 (SEM 0.10) ng/ml) than in C pups (0.48 (SEM 0.09) ng/ml,  $P = 0.003$ ) and HFn-3 ones (1.19 (SEM 0.10) ng/ml,  $P = 0.007$ ), and in HFn-3 than in C pups ( $P = 0.012$ ). Notably, they were higher in HFn-3 than in C rats ( $P = 0.004$ ; Table 4) despite displaying similar body weight. Basal NEFA plasma concentrations were higher in HF and HFn-3 diet pups as compared with chow-fed ones (Table 4).

#### Body weight and energy intake of 9-week-old male rat offspring fed a C or HF diet for 6 weeks

After weaning, male rat's offspring of each dam were randomly divided into two groups and fed either a chow diet (C-C, HF-C, HFn-3-C) or a HF diet (C-HF, HF-HF, HFn-3-HF) over 6 weeks (Fig. 1). HF-C rats presented a higher body weight than C-C ( $P = 0.036$ ) or HFn-3-C ones ( $P = 0.029$ ; Table 5). Nevertheless, body weight of C-C and HFn-3-C rats was not different (Table 5). Body weight of C-HF and HF-HF rats was similar, but body weight of HFn-3-HF rats was lower than that of C-HF and HF-HF pups ( $P = 0.039$  and 0.026, respectively; Table 5).

Cumulated energy intake over 6 weeks was higher for HF-C pups than for C-C ones ( $P = 0.037$ ) and HFn-3-C ( $P = 0.031$ ), and not different between C-C and HFn-3-C rats (Table 5). Energy



**Fig. 3.** Oral glucose tolerance test at weaning after 12 h fasting (day 21). This figure related to Table 3. C means chow, H means high fat, HF*n*-3 means high-fat *n*-3 enriched diet. (a) Blood glucose plasma levels; (b) insulin plasma levels. Data are mean values with their standard error of the means ( $n$  21–28/group). Data were analysed using a repeated-measures one-way ANOVA followed by post hoc test. For each time point, data with differing superscripts differ from each other by  $P < 0.05$  or less when assessed by one-way ANOVA followed by post hoc test.

intake was also higher in HF-HF pups than in C-HF and HF*n*-3-HF pups ( $P = 0.043$  and  $0.037$ , respectively) while it was lower in HF*n*-3-HF than in C-HF rats ( $P < 0.05$ ; Table 5). In male offspring fed a C diet after weaning, HF*n*-3 diet in gestation and lactation resulted in energy intake and weight gain similar to the ones observed for C diet, contrarily to HF one.

*Glucose, insulin, leptin and NEFA plasma levels of 9-week-old male offspring*

In C-fed rats after weaning, glycaemic response during OGTT was higher in HF-C and HF*n*-3-C than in C-C ( $P = 0.003$ ), and not different between HF-C and HF*n*-3-C rats (Fig. 4(a)). The AUC of glycaemia was higher in HF-C and HF*n*-3-C rats than in C-C ones ( $P = 0.028$  and  $0.021$ , respectively), and not different between HF-C and HF*n*-3-C pups (Table 5). Insulinaemia was higher at time 15 min in HF-C rats than in C-C ones ( $P = 0.027$ ; Fig. 4(c)). AUC of insulinaemia was higher in HF-C than in C-C and in HF*n*-3-C rats ( $P = 0.03$  and  $0.01$ , respectively), and similar between C-C and HF*n*-3-C rats (Table 5).

In HF-fed rats, glycaemic response was higher at time 15 and 30 min in HF-HF than in C-HF and HF*n*-3-HF ones, and not different between C-HF and HF*n*-3-HF rats (Fig. 4(b)). AUC of glycaemia was higher in HF-HF rats than in C-HF and HF*n*-3-HF ones ( $P = 0.019$  and  $0.023$ , respectively), and different between C-HF and HF*n*-3-HF rats ( $P = 0.036$ ; Table 5). In HF-fed male offspring, insulinaemia during the OGTT was higher from time 15 min to time 45 min in HF-HF rats than in C-HF and HF*n*-3-HF ones ( $P = 0.001$  and  $0.0006$ , respectively) (Fig. 4(d)); it was not different between C-HF and HF*n*-3-HF rats (Fig. 4(d)). In HF-fed rats, AUC of insulinaemia was higher in HF-HF rats than in C-HF and HF*n*-3-HF ones ( $P = 0.007$  and  $0.006$ , respectively) and was not different between C-HF and HF*n*-3-HF rats (Table 5). These results evidence that offspring born from HF diet-fed dams always present a higher AUC of insulinaemia independent of their post-weaning diet. This was not observed for HF*n*-3 fed dams whose offspring display values similar to the

ones of rats born to C-fed dams (Table 5). HOMA-IR was lower in HF*n*-3-C pups as compared with HF-C pups and lower HOMA-IR in HF*n*-3-HF adult pups as compared with HF-HF pups (Table 5). Basal plasma NEFA concentrations were not different among the three groups of C-fed rats. Contrarily, they were higher in HF-HF pups than in C-HF and in HF*n*-3-HF pups ( $P = 0.0029$  and  $0.022$ , respectively). They were not different between C-HF and HF*n*-3-HF (Table 5).

Basal plasma leptin levels were not different among all C-fed groups (Table 5). In HF-fed male rat offspring, leptin concentration was higher in C-HF and HF-HF rats than in HF*n*-3-HF ones ( $P = 0.040$  and  $0.035$ , respectively). They were not different between C-HF and HF-HF rats (Table 5).

Liver weight (expressed as percentage of body weight) was not different among the twelve groups. However, peri-epididymal AT (expressed as percentage of body weight) was more important in C-HF and HF-HF rats than in their C-C and HF-C counterparts ( $P < 0.05$ ; Table 5). This was not observed between HF*n*-3-HF and HF*n*-3-C rats (Table 5). Moreover, in HF*n*-3-HF rats, peri-epididymal AT mass was decreased by 14 and 22% as compared with C-HF and HF-HF, respectively (Table 5).

*Physiological characteristics of male offspring at sacrifice*

As described in the ‘Materials and methods’ section, at 10 weeks, each of the six groups was randomly divided into two subgroups: one receiving an intra-peritoneal saline injection and the other an intra-peritoneal insulin injection in order to activate insulin signalling (4 IU/kg of insulin).

As observed at time 0 during OGTT, plasma leptin concentration in the saline injected groups was higher in C-HF and HF-HF rats than in HF*n*-3-HF ones ( $P = 0.037$  and  $0.042$ , respectively) but was not different between C-HF and HF-HF ones (Table 6).

After saline injection, plasma NEFA concentration was higher in all HF-fed rats than in all C-fed rats groups ( $P < 0.05$ ; Table 6). Following insulin, NEFA concentrations were decreased in all C-fed and HF-fed rats ( $P < 0.05$ ; Table 6).

**Table 5.** Morphometric and biochemical characteristics of rat male offspring at 9 weeks old after 6 weeks of diet (Mean values with their standard error of the means)

Dam's diet	Chow		HF		HF <i>n</i> -3		Chow		HF		HF <i>n</i> -3	
	Chow (n 11)	C-C	Chow (n 14)	HF-C	Chow (n 11)	HF <i>n</i> -3-C	HF (n 12)	C-HF	HF (n 14)	HF-HF	HF (n 10)	HF <i>n</i> -3-HF
Offspring male rats' diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Offspring male rats' group name												
Body weight (g)	312	3 <sup>a</sup>	352	8 <sup>b</sup>	290	14 <sup>a</sup>	360	9 <sup>b</sup>	365	10 <sup>b</sup>	317	12 <sup>a</sup>
Body weight gain (g)	277	3 <sup>a</sup>	306	7 <sup>b</sup>	257	12 <sup>a</sup>	322	9 <sup>b</sup>	317	9 <sup>b</sup>	275	10 <sup>a</sup>
Cumulative food intake (kcal)	2309	72 <sup>a</sup>	2805	64 <sup>b</sup>	2099	139 <sup>a</sup>	2967	109 <sup>b</sup>	3315	101 <sup>c</sup>	2370	108 <sup>a</sup>
Leptin (ng/ml)	4.05	0.40 <sup>a</sup>	3.47	0.52 <sup>a</sup>	3.59	0.37 <sup>a</sup>	7.65	0.52 <sup>b</sup>	6.74	0.59 <sup>b</sup>	5.67	0.64 <sup>c</sup>
NEFA (mM)	0.96	0.05 <sup>a</sup>	1.03	0.07 <sup>a</sup>	0.96	0.05 <sup>a</sup>	1.17	0.03 <sup>a</sup>	1.31	0.06 <sup>b</sup>	1.12	0.07 <sup>a</sup>
Glucose at T0 (mg/dl)	71.33	2.65 <sup>a</sup>	89.36	3.61 <sup>b</sup>	88.60	3.32 <sup>b</sup>	74.05	2.34 <sup>a</sup>	74.14	3.04 <sup>a</sup>	73.29	4.50 <sup>a</sup>
Insulin at T0 (ng/ml)	1.08	0.39	1.04	0.17	0.89	0.13	1.06	0.21	1.27	0.30	0.85	0.14
HOMA-IR	5.33	1.56 <sup>a</sup>	8.57	1.25 <sup>b</sup>	5.59	0.94 <sup>a</sup>	5.56	1.24 <sup>a</sup>	6.40	1.37 <sup>a,b</sup>	5.18	0.75 <sup>a</sup>
AUC insulinaemia (ng/ml per 2h)	169	29 <sup>a</sup>	198	21 <sup>b</sup>	156	19 <sup>a</sup>	164	21 <sup>a</sup>	296	52 <sup>c</sup>	110	22 <sup>a</sup>
Liver (g)	9.17	0.32 <sup>a</sup>	9.87	0.30 <sup>b</sup>	9.15	0.37 <sup>a</sup>	10.63	0.47 <sup>b</sup>	10.94	0.38 <sup>b</sup>	9.87	0.43 <sup>a,b</sup>
Liver (% body weight)	2.93	0.10	2.81	0.04	2.99	0.05	2.94	0.08	3.00	0.07	2.88	0.07
Peri-epididymal adipose tissue (g)	3.96	0.45 <sup>a</sup>	5.46	0.43 <sup>b</sup>	3.07	0.24 <sup>a</sup>	5.33	0.39 <sup>b</sup>	6.09	0.59 <sup>b</sup>	3.96	0.34 <sup>a</sup>
Peri-epididymal adipose tissue (% body weight)	1.27	0.14 <sup>a</sup>	1.53	0.10 <sup>b</sup>	1.30	0.05 <sup>a</sup>	1.57	0.08 <sup>b</sup>	1.64	0.12 <sup>b</sup>	1.42	0.09 <sup>a,b</sup>

The first letter of the group corresponds to the dam's diet and the second one to the pup's one after weaning. HOMA-IR (homeostasis model assessment of insulin resistance) score was calculated using the formula: fasting insulin (μIU/ml) × (fasting blood glucose (mmol/l)/22.5). The number of rats per group is indicated in the table heading. Results for each parameter were analysed by two-way ANOVA. For each row, data points with differing superscript letters (a, b, c, d) differ from each other by  $P < 0.05$  or less by post hoc test after intergroup differences were found by two-way ANOVA.

N. Guriec *et al.*

**Akt expression and Ser<sup>473</sup> phosphorylation in liver, muscle and peri-epididymal adipose tissue of male offspring at sacrifice**

To assess whether HF*n*-3 dams feeding during gestation and lactation might improve insulin signalling in male offspring, the phosphorylation of Akt on Ser<sup>473</sup> was determined after an insulin bolus at sacrifice, as performed for dams.

When compared with actin by western blot, Akt protein abundance did not vary between any pups' groups in the three tissues (Fig. 5). However, differences occurred regarding to its phosphorylation on Ser<sup>473</sup>.

In liver, a significant Ser<sup>473</sup>Akt phosphorylation occurred in C-C ( $P < 0.05$ ) and in HF*n*-3-C rats ( $P < 0.05$ ), while it was blunted in HF-C rats (Fig. 5(a) and (c)). Ser<sup>473</sup>Akt phosphorylation was decreased by 80% in HFC and 53% HF*n*-3-C male offspring as compared with C-C rats ( $P = 0.003$  and  $0.007$ , respectively, Fig. 5(c)). In addition, phosphorylation was significantly higher in HF*n*-3-C rats than in HF-C ones ( $P = 0.01$ ; Fig. 5(c)). Similarly, when male offspring born to HF*n*-3 dams were fed HF post-weaning, Ser<sup>473</sup>Akt phosphorylation was decreased by only 20% compared with C-HF diet fed rats ( $P = 0.03$ ), which is far less than in HF-HF rats for which no phosphorylation occurred (Fig. 5(c)). Moreover, Ser<sup>473</sup>Akt phosphorylation in HF*n*-3-HF rats was similar to that of HF-C diet rats (Fig. 5(c)).

In muscle, Ser<sup>473</sup>Akt phosphorylation increased in all C-fed groups in response to insulin (Fig. 5(a) and (c)). Ser<sup>473</sup>Akt phosphorylation was higher in C-C than in HF-C rats ( $P = 0.033$ ) and in a lesser extent higher in C-C rats than in HF*n*-3-C ones ( $P = 0.048$ ; Fig. 5(c)). Notably, Ser<sup>473</sup>Akt phosphorylation was higher in HF*n*-3-C than in HF-C rats ( $P = 0.038$ ; Fig. 5(c)).

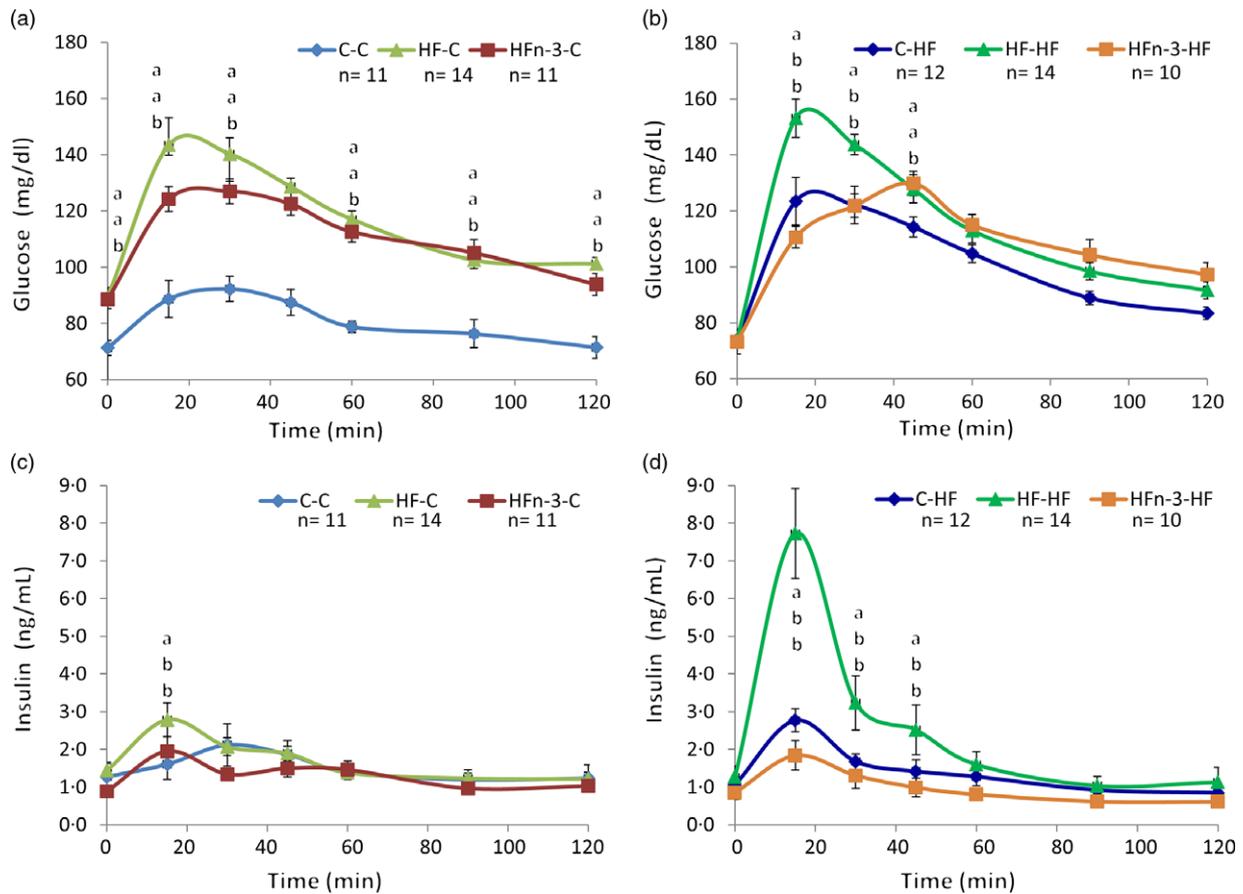
In peri-epididymal AT, Ser<sup>473</sup>Akt was phosphorylated in response to the insulin bolus in C-C rats (by 3 folds in comparison with vehicle,  $P = 0.034$ ) and in HF*n*-3-C pups (by 4.5 folds in comparison with vehicle,  $P = 0.021$ ) but not in HF-C pups (Fig. 5(a) and (c)). In this tissue, Ser<sup>473</sup>Akt phosphorylation was significantly higher in HF*n*-3-C than in C-C pups ( $P = 0.040$ ; Fig. 5(a) and (c)).

When male offspring born to C, HF, HF*n*-3 diet were fed with a HF diet after weaning, Ser<sup>473</sup>Akt was not phosphorylated following insulin application, in muscle and peri-epididymal AT (Fig. 5(b) and (c)).

**Discussion**

The aim of this study was to test the hypothesis that feeding dams a HF diet enriched in *n*-3 FA during gestation and lactation would prevent metabolic alterations observed when fed a HF diet, as far as the adult male offspring is fed a C diet after weaning.

We show that adult offspring exposed to LC *n*-3PUFA during gestation and lactation were protected against excessive weight gain and energy intake, obesity, glucose intolerance (GI) and IR when C or HF fed after weaning as compared with offspring of HF diet fed dams. The discrepancy observed between this protective effect in rats (our study and others) and the lack of effect in humans can be explained by species-specific differences in terms of defined windows of development of



**Fig. 4.** Oral glucose tolerance test in 9-week-old male rat offspring born to C, HF or HF*n*-3 dams and fed C or HF for 6 weeks after weaning, after a 12 h fast. This figure related to Table 4. The first letter corresponds to dam's diet and the second one to pup's one after weaning. C means chow, H means high fat, HF*n*-3 means high-fat *n*-3 enriched diet. (a) Glucose plasma levels when C diet post-weaning; (b) glucose plasma levels when HF diet post-weaning; (c) insulin plasma levels when C diet post-weaning; (d) insulin plasma levels when HF diet post-weaning. Data are mean values with their standard error of the means (*n* 10–14/group). Data were analysed using a repeated-measures one-way ANOVA followed by post hoc test. For each time point, data with differing superscripts differ from each other by *P* < 0.05 or less when assessed by one-way ANOVA followed by post hoc test.

AT and beta cells. In addition, the model used in our study (a very HF diet) in dams and the amount of LC *n*-3 PUFA given are not expandable to human diet.

After 6 weeks of diet and before pregnancy, body weight of dams was not different among the groups even though their energy intake was higher in HF than in C and HF*n*-3 dams. The lack of body weight difference in HF dams although slightly higher than in the other two groups may result from a lack of statistical power. The absence of body weight difference in HF dams has been previously described in the literature. Female rats or mice are notoriously known to be resistant to HF diet feeding contrary to male rats<sup>(41)</sup>. Oestrogens have been proposed to exert a protective effect against IR and the metabolic syndrome since the prevalence of these symptoms increases in menopausal women<sup>(42)</sup>.

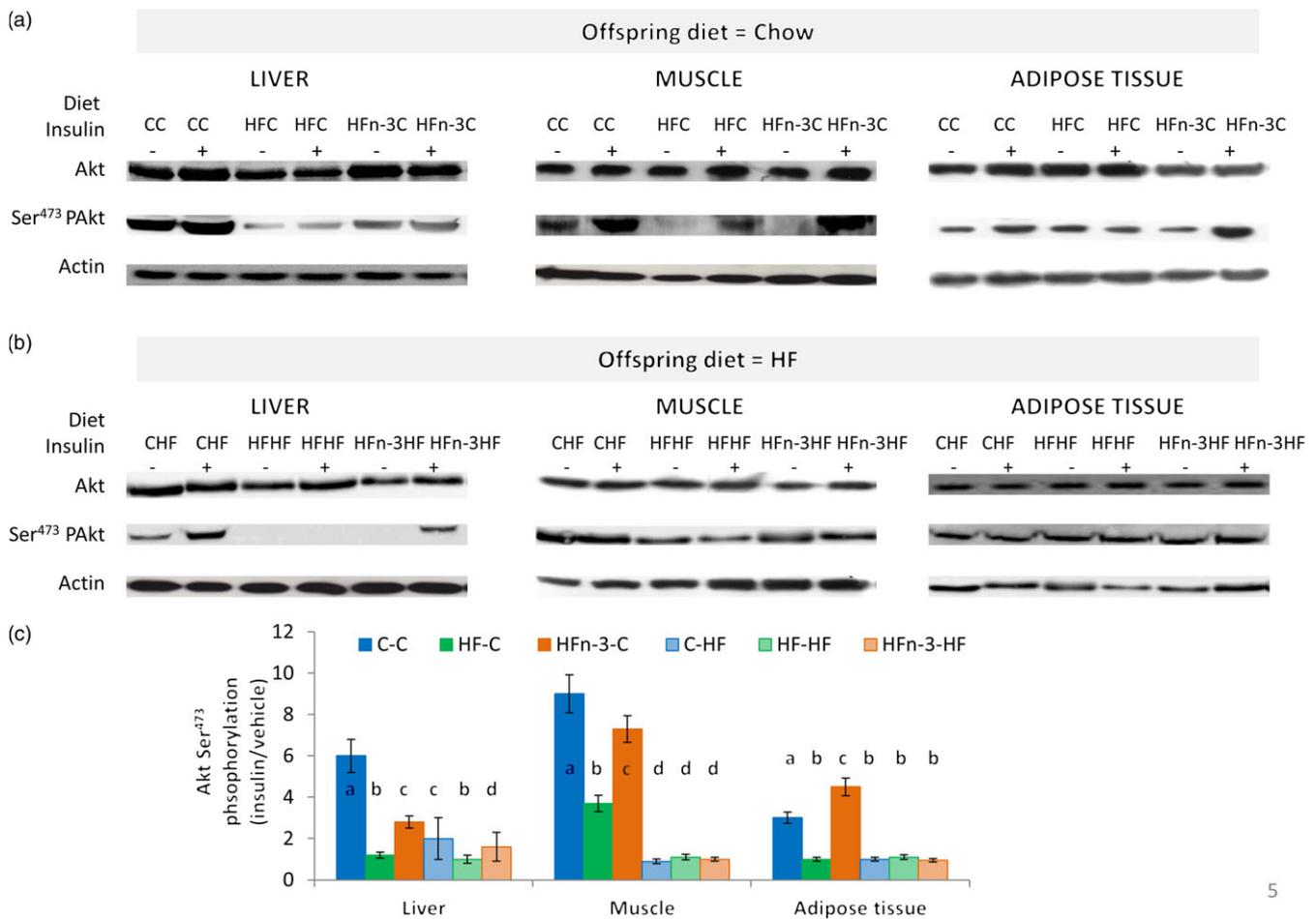
As expected, and after 6 weeks on HF diet, the glycaemic and insulinaemic responses and the NEFA plasma levels were higher in HF than in C dams, reflecting GI and IR of both glucose metabolism and lipolysis. These alterations were not observed in HF*n*-3 rats, confirming their preventive effect towards IR in rodents as reported in many studies<sup>(14)</sup>. Although higher in

HF*n*-3 than in C dams, plasma leptin concentrations were lower in HF*n*-3 than in HF dams, related to a probable lower fat mass and/or reduced leptin expression in AT as previously observed<sup>(43,44)</sup>. These results are also in agreement with those obtained the day of sacrifice of dams.

At sacrifice and after 12 weeks of diet, HF diet hampered lipid mobilisation in response to insulin, as evidenced by NEFA plasma concentrations. LC *n*-3 PUFA prevented this alteration as HF*n*-3 dams displayed similar NEFA plasma concentration as C dams following insulin bolus, reflecting an increased insulin sensitivity of lipolysis by LC *n*-3 PUFA. Consistent with these results, the defect of Akt Ser<sup>473</sup> phosphorylation induced by the HF diet was completely prevented in HF*n*-3 dams in liver and muscle and partially in AT as previously observed<sup>(45,46)</sup>.

Dam's diet did not influence the number of pups per litter or the sex ratio in our experiments as previously reported<sup>(47,48)</sup>. Pups born to HF dams were heavier at birth and during the lactation period than those born to C dams, as previously reported<sup>(46,47)</sup>. At birth, body weight of male offspring of HF*n*-3 dams was similar to the body weight of male offspring born to C dams, as previously reported<sup>(48)</sup>. This effect was also





**Fig. 5.** Akt and Ser<sup>473</sup> Akt phosphorylation in the liver, muscle and peri-epididymal adipose tissue in response to insulin at sacrifice after 12 h fasting in 10-week-old male rats fed C or HF diet after weaning. This figure related to Table 5. The first letter corresponds to dam's diet and the second one to the offspring's one after weaning. C means chow, H means high fat, HFn-3 means high-fat *n*-3 enriched diet. Insulin was injected intra-peritoneally at 4 µg/kg 10 min before euthanasia. (a) Ser<sup>473</sup> Akt phosphorylation in liver, muscle and adipose tissue of C-fed offspring; (b) western blots for Akt and Ser<sup>473</sup> Akt phosphorylation in liver, muscle and adipose tissue of HF diet fed offspring. To ensure equal protein loading and transfer, membranes were re-probed for β-actin; (c) quantification of the Ser<sup>473</sup> Akt phosphorylation ratio insulin/vehicle. Data are mean values with their standard error of the means (*n* 5–7/group). Data were analysed using a two-way ANOVA (factors: dams diet, offspring diet) followed by post hoc test. Fig. 5(c): For each tissue, data with differing superscripts differ from each other by *P* < 0.05 or less when analysed using a two-way ANOVA followed by post hoc test. For the liver: dams diet, *F* = 5.24, *P* = 0.03; offspring diet, *F* = 3.67, *P* = 0.04; interaction dams diet × offspring diet, *F* = 3.16, *P* = 0.04. For the muscle: dams diet, *F* = 10.73, *P* = 0.01; offspring diet, *F* = 9.35, *P* = 0.01; interaction dams diet × offspring diet, *F* = 7.83, *P* = 0.01. For the adipose tissue: dams diet, *F* = 2.06, *P* = 0.03; offspring diet, *F* = 1.87, *P* = 0.04; interaction dams diet × offspring diet, *F* = 1.58, *P* = 0.03.

peptides<sup>(59,60)</sup>. Furthermore, it is of interest to note that an elevated sensation of fullness following a diet rich in LC *n*-3 PUFA has been reported in overweight and obese patients<sup>(61)</sup>.

The adult male offspring of HF dams displayed a higher glycaemic and insulinaemic response to OGTT than those born to C dams, both after being fed the C or the HF diet as previously reported<sup>(62,63)</sup>. Offspring of HFn-3 diet fed dams displayed an insulinaemic response similar to that of offspring born to C dams, while their glycaemic response was intermediate between that of offspring of C and of HF dams. The fact that we did not perform an insulin tolerance test to assess insulin sensitivity is a limitation to formally assert that IR has been prevented. However, the lower HOMA-IR observed in HFn-3-C pups as compared with HF-C pups and the lower HOMA-IR observed in HFn-3-HF adult pups as compared with HF-HF pups (Table 5) may suggest that, when given a HF diet without LC *n*-3 PUFA, IR of adult pups has been at least partially prevented by LC *n*-3 PUFA given to dams.

To assess the effectiveness of insulin response in male offspring, insulin signalling activation was measured through the expression of Akt protein and its phosphorylation on Ser<sup>473</sup>. Expression of Akt was not altered in any of our experiments. The amplitude of Akt phosphorylation in offspring of C dams fed a C diet was the highest in muscle as compared with liver and peri-epididymal AT. The post-weaning HF diet profoundly depressed Akt phosphorylation in the three tissues in offspring of C dams in agreement with previous results<sup>(63)</sup>. Ser<sup>473</sup> Akt phosphorylation was deeply depressed in liver and AT and to a lesser extent in muscle when offspring of HF dams were fed the C diet, in comparison with male offspring of C dams fed a C diet after weaning indicating that insulin signalling is hampered in C-fed HF dam male offspring, as previously observed<sup>(45,46,52)</sup>. The HF diet after weaning of HF pups further amplified the depression of Akt phosphorylation in muscle. Ser<sup>473</sup> Akt phosphorylation in offspring of HFn-3 dams fed a C diet after weaning

was less depressed in liver and muscle as compared with what was observed for offspring of HF dams fed a C diet, indicating partial protection of insulin signalling in HF*n*-3 male offspring fed a C diet. HF-diet feeding post-weaning of HF*n*-3 male offspring reversed this protective effect, agreeing with their IR. However, Ser<sup>473</sup>Akt phosphorylation in liver of HF*n*-3-HF rats was similar to that of HF*n*-3-C rats, which might contribute to the improvement of their glucose tolerance in comparison with that of HF-HF ones. Moreover, Ser<sup>473</sup>Akt phosphorylation in male HF*n*-3-C rats was also greater than that of C-C and HF-C rats in peri-epididymal AT, which demonstrates a tissue-specific protective effect of LC *n*-3 diet towards fetal programming of IR. This tissue-specific effect of LC *n*-3 PUFA confirms our previous observations made in a different model of IR<sup>(13,45)</sup>. A protective effect towards fetal programming of IR in liver has also been reported by Wang *et al.*<sup>(30)</sup>. In this study, no other insulin sensitive tissues were assessed, thus preventing us to determine whether *n*-3 PUFA also exerted other tissue-specific effect. Similarly, in the transgenic model Fat1 C57BL6 mice, characterised by a very high content of LC *n*-3 PUFA in tissues, excessive placental and liver growth during pregnancy was prevented, and better insulin sensitivity was observed as compared with wild-type mice. This was associated with a lower macrophage infiltration of AT in adulthood<sup>(29)</sup>. However, in our work, when fed a HF diet after weaning, offspring of HF*n*-3 dams displayed a depression of Ser<sup>473</sup>Akt phosphorylation in muscle and peri-epididymal AT, similar to that of HF-HF rats, so that in these dietary conditions LC *n*-3 PUFA were no more protective. A possible explanation may be a lower amount of LC *n*-3 PUFA incorporated into tissues in adulthood (10 weeks after weaning) than in other studies<sup>(13,64–67)</sup>, inasmuch as on difference with Ramalingham *et al.* study<sup>(68)</sup>, our rats did not received LC *n*-3 PUFA in their diets after weaning.

Several mechanisms could have contributed to the protective effect of LC *n*-3 PUFA towards fetal imprinting. Maternal oocyte quality and conceptus metabolism are altered in rodents that are obese or fed diets with altered nutrient composition pre-gestationally. Similarly, in humans, women who were obese had lower levels of SFA and polyunsaturated *n*-3 FA across all studied stages of oocyte development, and overweight women had lower *n*-3 FA in Metaphase II oocytes, which can contribute to alter embryo development and metabolism at the cell signalling level<sup>(69)</sup>. LC *n*-3 PUFA given to infertile women improve oocyte quality<sup>(70)</sup>, so that, beyond their positive effect on fertility, they could also participate to improve fetal programming in obese.

The placenta contributes to modulate the risks of diseases during adulthood by modulating its metabolism<sup>(71)</sup>. Placental size, shape and transport function are important for fetal growth and development. The delivery of maternal LC-PUFA to the fetus is largely mediated via the placental transport, metabolic and adaptive functionalities. Supplementation of *n*-3 PUFA is beneficial for the obese and overweight women by maintaining optimal FA levels during gestation by inhibiting the placental ability to esterify and store lipids<sup>(72)</sup>. Dietary *n*-3 PUFA play a key role in various placental functions, with particular focus on placental inflammation<sup>(73)</sup>, and oxidative stress, factors implicated in development of IR.

LC *n*-3 PUFA can also play a protective role via their epigenetic effect on non-coding RNA, methylation of DNA and histone modifications<sup>(74)</sup>. Hepatic IR results for a part from several epigenetic alterations<sup>(75)</sup>, as obesity-associated type 2 diabetes<sup>(76)</sup>. In rodents, being overweight and having excessive fat or sugar absorption are associated with alterations in DNA methylation patterns. These modifications affect the promoter regions of several genes, including leptin promoter<sup>(77)</sup>, NADH dehydrogenase (ubiquinone)1 subcomplex subunit 6 (ND UFB6) and FA synthase, which play an important role on energy homeostasis and obesity<sup>(78)</sup>.

Rats' visceral adipocytes' CpG 15 has been shown to be hypomethylated, and numerous CpG sites in the leptin promoter region were hypermethylated as a result of a HF, high-sucrose diet<sup>(79)</sup>. Changing to a normal energetic diet also reversed the HF sucrose-induced DNA methylation alterations at these CpG sites. Additionally, these dietary-related modifications have been linked to hypermethylated CpG on the promoters of the peroxisome proliferator-activated receptor (PPAR C1A) and FA synthase, as well as a CpG site of the sterol regulatory element binding transcription factor 1 (SREB F1), all transcription factors known to be targets of anti-lipogenic effects of LC *n*-3 PUFA in liver<sup>(80)</sup>.

The levels of DNA methylation in neonatal blood are associated with IR in 5-year-old children<sup>(81)</sup>. In the models of maternal HF diet in mice, offspring display epigenetic modifications in the liver, including widespread hypermethylation and altered gene expression along with changes in the enrichment of active promoter histone marks<sup>(82,83)</sup>. Maternal HF diet also led to the altered expression of genes encoding the epigenetic machinery in offspring, particularly those in the histone acetylation pathway such as SIRT1<sup>(83)</sup>. SIRT1 overexpression has been reported to attenuate the effects of a maternal HF diet in offspring and protect against hepatic steatosis and IR<sup>(84)</sup>. In the fat-1 model, SIRT1 is overexpressed<sup>(85)</sup>.

It has been shown that LC *n*-3 PUFA given to dams or to mothers had epigenetic effects<sup>(86–88)</sup>. MicroRNA-143 (miR-143) seems to stimulate adipocytes' differentiation and is correlated with obese condition in mice fed with a HF diet. The expression of miR-143 and PPAR is increased by DHA, suggesting a potential role in AT differentiation<sup>(89)</sup>. One animal experiment evaluated DHA epigenetic impact on histone acetylation, possibly correlated with the MetS pathways. This work reported that *n*-3 FA have a beneficial effect on dyslipidaemia-induced epigenetic changes in maternal placenta and fetal liver of weanling female Wistar rats fed with control and different HF diets (HF lard or HF fish oil). Maternal dyslipidaemia caused a 4-75-fold increase in fetal liver TAG accumulation with a 78% decrease in the DNA-binding ability of PPAR- $\alpha$ . The combination EPA + DHA had a profound effect on modulating DNA methylation and histone acetylation in placenta and fetal liver<sup>(90)</sup>. A randomised controlled trial showed that prenatal DHA supplementation (800 mg/d given in pregnant women) induced a difference of methylation in twenty-one regions at birth some of which persisted until age of 5 years<sup>(91)</sup>. Altogether, it can be proposed that LC *n*-3 PUFA given to dams in our study could have exerted part of their protective effect through repairing epigenetic



alteration induced by HF diet on some transcription factors, especially those implicated in liver/AT lipogenesis, inflammation and reticulum endoplasmic stress.

Another mechanism of the protective effect of LC *n*-3 PUFA could have been through the reversal of 'whitening' of brown adipose tissue (BAT) towards browning. Indeed, very recently, Hao *et al.*<sup>(92)</sup> have shown that a HF diet induced a 'whitening' of AT due to large lipid droplet accumulation within BAT cells, associated with obesity in wild-type mice, whereas the changes in body fat mass and BAT morphology were significantly alleviated in fat-1 mice. In addition, Tsuji *et al.*<sup>(93)</sup> demonstrated that the transplantation of BAT from fat-1 mice to wild-type mice fed a HF diet counteracted the metabolic abnormalities such as GI and increased energy expenditure. Fan *et al.*<sup>(94)</sup> have shown that LC *n*-3 PUFA given to dams promoted fetal BAT development through epigenetic modifications. The offspring received maternal *n*-3 PUFA nutrition exhibited a significant increase in whole-body energy expenditure. These data are in accordance with other studies in mice<sup>(95,96)</sup>.

### Conclusion

Altogether, our results demonstrate that LC *n*-3 PUFA given to HF diet-fed rats 6 weeks before mating and throughout gestation and lactation prevent excess body weight gain, and GI in the offspring at birth and until weaning. This protective effect towards obesity persisted at adulthood even when the offspring did no longer receive LC *n*-3 PUFA into their diet. The lower AUC of insulinaemia during OGTT and lower HOMA-IR of adult pups after a HF diet without of LC *n*-3 PUFA may suggest that IR was at least partially prevented by the LC *n*-3 PUFA given to dams. Furthermore, when fed a chow diet after weaning, the alteration of insulin signalling was completely prevented in AT, and partially prevented in liver and muscle, in adult male offspring of HF *n*-3 fed dams as compared with male offspring of HF-fed dam.

### Acknowledgements

The authors would like to acknowledge Séverine Loisel and Christelle Goanvec for scientific discussions. The authors also thank Marie-Françoise Scoazec and Manuel Feillant for their technical daily support and advices with the animals.

This work was financed by a grant from Region Bretagne (JD) and a grant from the ALFEDIAM/Sanofi-Aventis (CLF).

C. L. F. and N. G. contributed to conceptualisation. C. L. F. and J. D. acquired funding. N. G. and C. L. F. contributed to methodology, investigation and formal analysis. NG wrote the original draft. N. G., C. L. F. and J. D. reviewed and edited the final version. All authors contributed to the article and approved the submitted version.

The authors have no conflict of interest related to this paper.

### References

1. WHO (2021) Obesity and Overweight. <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (accessed 19 November 2022).
2. World Obesity (2022) World Obesity Atlas 2022. [https://www.worldobesityday.org/assets/downloads/World\\_Obesity\\_Atlas\\_2022\\_WEB.pdf](https://www.worldobesityday.org/assets/downloads/World_Obesity_Atlas_2022_WEB.pdf) (accessed 19 November 2022).
3. IDF Diabetes Atlas (2022) Diabetes around the World in 2021. <https://diabetesatlas.org> (accessed 19 November 2022).
4. Roden M & Shulman GI (2019) The integrative biology of type 2 diabetes. *Nature* **576**, 51–60.
5. Moreno-Fernandez J, Ochoa JJ, Lopez-Frias M, *et al.* (2020) Impact of early nutrition, physical activity and sleep on the fetal programming of disease in the pregnancy: a narrative review. *Nutrients* **12**, 3900.
6. Fall CHD & Kumaran K (2019) Metabolic programming in early life in humans. *Philos Trans R Soc Lond B Biol Sci* **374**, 20180123.
7. Hieronimus B & Ensenauer R (2021) Influence of maternal and paternal pre-conception overweight/obesity on offspring outcomes and strategies for prevention. *Eur J Clin Nutr* **75**, 1735–1744.
8. Schoonejans JM & Ozanne SE (2021) Developmental programming by maternal obesity: lessons from animal models. *Diabet Med* **38**, e14694.
9. Menjivar M & Zambrano E (2020) Maternal interventions to prevent adverse fetal programming outcomes due to maternal malnutrition: evidence in animal models. *Placenta* **102**, 49–54.
10. Hughes AN & Oxford JT (2014) A lipid-rich gestational diet predisposes offspring to nonalcoholic fatty liver disease: a potential sequence of events. *Hepat Med* **6**, 15–23.
11. Mennitti LV, Oliveira JL, Morais CA, *et al.* (2015) Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. *J Nutr Biochem* **26**, 99–111.
12. Christoforou ER & Sferruzzi-Perri AN (2020) Molecular mechanisms governing offspring metabolic programming in rodent models of in utero stress. *Cell Mol Life Sci* **77**, 4861–4898.
13. Taouis M, Dagou C, Ster C, *et al.* (2002) *n*-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. *Am J Physiol Endocrinol Metab* **282**, E664–E671.
14. Delarue J (2020) Are marine *n*-3 fatty acids protective towards insulin resistance? From cell to human. *Proc Nutr Soc* **6**, 1–11.
15. Xia Y, Wang Y, Cui M, *et al.* (2021) Efficacy of *n*-3 fatty acid supplementation on cardiovascular risk factors in patients with polycystic ovary syndrome: a systematic review and meta-analysis. *Ann Palliat Med* **10**, 6425–6437.
16. Gao L, Lin L, Shan N, *et al.* (2020) The impact of *n*-3 fatty acid supplementation on glycemic control in patients with gestational diabetes: a systematic review and meta-analysis of randomized controlled studies. *J Matern Fetal Neonatal Med* **33**, 1767–1773.
17. Jiang L, Gao C, Yan P, *et al.* (2020) *n*-3 fatty acids plus vitamin for women with gestational diabetes or prediabetes: a meta-analysis of randomized controlled studies. *J Matern Fetal Neonatal Med* **35**, 1–8.
18. Delpino FM, Figueiredo LM, da Silva BGC, *et al.* (2021) *n*-3 supplementation and diabetes: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr* **62**, 1–14.
19. Shrestha N, Sleep SL, Cuffe JSM, *et al.* (2020) Role of *n*-6 and *n*-3 fatty acids in fetal programming. *Clin Exp Pharmacol Physiol* **47**, 907–915.
20. Duttaroy AK (2009) Transport of fatty acids across the human placenta: a review. *Prog Lipid Res* **48**, 52–61.
21. Campbell FM, Gordon MJ & Dutta-Roy AK (1996) Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. *Mol Cell Biochem* **155**, 77–83.
22. Hanebutt FL, Demmelmair H, Schiessl B, *et al.* (2008) Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clin Nutr* **27**, 685–693.

23. Nishimura RY, Barbieiri P, Castro GS, *et al.* (2014) Dietary polyunsaturated fatty acid intake during late pregnancy affects fatty acid composition of mature breast milk. *Nutrition* **30**, 685–689.
24. Larqué E, Pagán A, Prieto MT, *et al.* (2014) Placental fatty acid transfer: a key factor in fetal growth. *Ann Nutr Metab* **64**, 247–253.
25. Demmelmair H & Koletzko B (2021) Perinatal polyunsaturated fatty acid status and obesity risk. *Nutrients* **13**, 3882.
26. Mark PJ, Wyrwoll CS, Zulkafli IS, *et al.* (2014) Rescue of glucocorticoid-programmed adipocyte inflammation by *n*-3 fatty acid supplementation in the rat. *Reprod Biol Endocrinol* **12**, 39.
27. Bringhenti I, Schultz A, Rachid T, *et al.* (2011) An early fish oil-enriched diet reverses biochemical, liver and adipose tissue alterations in male offspring from maternal protein restriction in mice. *J Nutr Biochem* **22**, 1009–1014.
28. Chicco A, Creus A, Illesca P, *et al.* (2016) Effects of post-suckling *n*-3 polyunsaturated fatty acids: prevention of dyslipidemia and liver steatosis induced in rats by a sucrose-rich diet during pre- and post- natal life. *Food Funct* **7**, 445–454.
29. Heerwagen MJ, Stewart MS, de la Houssaye BA, *et al.* (2013) Transgenic increase in *n*-3/*n*-6 Fatty Acid ratio reduces maternal obesity- associated inflammation and limits adverse developmental programming in mice. *PLoS One* **8**, e67791.
30. Wang DD, Wu F, Zhang LY, *et al.* (2021) Effects of dietary *n*-3 PUFA levels in early life on susceptibility to high-fat-diet-induced metabolic syndrome in adult mice. *J Nutr Biochem* **89**, 108578.
31. Ibrahim A, Basak S, Ehtesham NZ, *et al.* (2009) Impact of maternal dietary fatty acid composition on glucose and lipid metabolism in male rat offspring aged 105 d. *Br J Nutr* **102**, 233–241.
32. Hussain A, Nookaew I, Khoomrung S, *et al.* (2013) A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice. *Acta Physiol* **209**, 220–234.
33. Sardinha FL, Fernandes FS, Tavares do Carmo MG, *et al.* (2013) Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. *Am J Physiol Regul Integr Comp Physiol* **304**, R313–R320.
34. Vahdaninia M, Mackenzie H, Dean T, *et al.* (2018) The effectiveness of  $\omega$ -3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. *Eur J Nutr* **58**, 2597–2613.
35. Meyer DM, Brei C, Bader BL, *et al.* (2020) Evaluation of maternal dietary *n*-3 LCPUFA supplementation as a primary strategy to reduce offspring obesity: lessons from the INFAT trial and implications for future research. *Front Nutr* **7**, 156.
36. Amirani E, Asemi Z, Asbaghi O, *et al.* (2020) The effects of *n*-3 fatty acids supplementation on metabolic status in pregnant women: a systematic review and meta-analysis of randomized controlled trials. *J Diabetes Metab Disord* **19**, 1685–1699.
37. Garmendia ML, Casanello P, Flores M, *et al.* (2021) The effects of a combined intervention (docosahexaenoic acid supplementation and home-based dietary counseling) on metabolic control in obese and overweight pregnant women: the MIGHT study. *Am J Obstet Gynecol* **224**, 526.e1–526.e25.
38. Maslova E, Hansen S, Strøm M, *et al.* (2018) Fish intake in pregnancy and offspring metabolic parameters at age 9–16-decs gestational diabetes modify the risk? *Nutrients* **10**, 1534.
39. Monthe-Dreze C, Penfield-Cyr A, Smid MC, *et al.* (2018) Maternal pre-pregnancy obesity attenuates response to *n*-3 fatty acids supplementation during pregnancy. *Nutrients* **10**, 1908.
40. Alvarez D, Muñoz Y, Ortiz M, *et al.* (2021) Impact of maternal obesity on the metabolism and bioavailability of polyunsaturated fatty acids during pregnancy and breastfeeding. *Nutrients* **13**, 19.
41. Pettersson US, Waldén TB, Carlsson PO, *et al.* (2012) Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. *PLoS One* **7**, e46057.
42. Shi H & Clegg DJ (2009) Sex differences in the regulation of body weight. *Physiol Behav* **97**, 199–204.
43. Sharma P & Agnihotri N (2020) Fish oil and corn oil induced differential effect on beiging of visceral and subcutaneous white adipose tissue in high-fat-diet-induced obesity. *J Nutr Biochem* **84**, 108458.
44. Ukropec J, Reseland JE, Gasperikova D, *et al.* (2003) The hypotriglyceridemic effect of dietary *n*-3 FA is associated with increased beta-oxidation and reduced leptin expression. *Lipids* **38**, 1023–1029.
45. Le Foll C, Corporeau C, Le Guen V, *et al.* (2007) Long-chain *n*-3 polyunsaturated fatty acids dissociate phosphorylation of Akt from phosphatidylinositol 3'-kinase activity in rats. *Am J Physiol Endocrinol Metab* **292**, E1223–E1230.
46. Nasu-Kawaharada R, Nakamura A, Kakarla SK, *et al.* (2013) A maternal diet rich in fish oil may improve cardiac Akt-related signaling in the offspring of diabetic mother rats. *Nutrition* **29**, 688–692.
47. Zheng J, Xiao X, Zhang Q, *et al.* (2014) Maternal high-fat diet modulates hepatic glucose, lipid homeostasis and gene expression in the PPAR pathway in the early life of offspring. *Int J Mol Sci* **15**, 14967–14983.
48. George G, Draycott SAV, Muir R, *et al.* (2019) The impact of exposure to cafeteria diet during pregnancy or lactation on offspring growth and adiposity before weaning. *Sci Rep* **9**, 14173.
49. Korotkova M, Gabrielsson B, Lönn M, *et al.* (2002) Leptin levels in rat offspring are modified by the ratio of linoleic to  $\alpha$ -linolenic acid in the maternal diet. *J Lipid Res* **43**, 1743–1749.
50. Rolls BA, Gurr MI, Van Duijvenvoorde PM *et al.* (1986) Lactation in lean and obese rats: effect of cafeteria feeding and of dietary obesity on milk composition. *Physiol Behavior* **38**, 185–190.
51. Priego T, Sanchez J, García AP, *et al.* (2013) Maternal dietary fat affects milk fatty acid profile and impacts on weight gain and thermogenic capacity of suckling rats. *Lipids* **48**, 481–495.
52. Shankar K, Kang P, Harrell A, *et al.* (2010) Maternal overweight programs insulin and adiponectin signaling in the offspring. *Endocrinology* **151**, 2577–2589.
53. Bautista CJ, Montaña S, Ramirez V, *et al.* (2016) Changes in milk composition in obese rats consuming a high-fat diet. *Br J Nutr* **115**, 538–546.
54. Zambrano E, Rodríguez-González GL, Reyes-Castro LA, *et al.* (2021) DHA supplementation of obese rats throughout pregnancy and lactation modifies milk composition and anxiety behavior of offspring. *Nutrients* **13**, 4243.
55. Sun B, Purcell RH, Terrillion CE, *et al.* (2012) Maternal high-fat diet during gestation or suckling differentially affects offspring leptin sensitivity and obesity. *Diabetes* **61**, 2833–2841.
56. Rostami H, Samadi M, Yuzbashian E, *et al.* (2017) Habitual dietary intake of fatty acids is associated with leptin gene expression in subcutaneous and visceral adipose tissue of patients without diabetes. *Prostaglandins Leukot Essent Fatty Acids* **126**, 49–54.



57. Ferezou-Viala J, Roy AF, Serougne D, *et al.* (2007) Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol* **293**, R1056–R1062.
58. Cheng L, Hu T, Shi H, *et al.* (2020) DHA reduces hypothalamic inflammation and improves central leptin signaling in mice. *Life Sci* **257**, 118036.
59. Chang GQ, Gaysinskaya V, Karatayev O, *et al.* (2008) Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. *J Neurosci* **28**, 12107–12119.
60. Schipper L, Bouyer K, Oosting A, *et al.* (2013) Postnatal dietary fatty acid composition permanently affects the structure of hypothalamic pathways controlling energy balance in mice. *Am J Clin Nutr* **98**, 1395–1401.
61. Parra D, Ramel A, Bandarra N, *et al.* (2008) A diet rich in long chain *n*-3 fatty acids modulates satiety in overweight and obese volunteers during weight loss. *Appetite* **51**, 676–680.
62. Howie GJ, Deborah M, Sloboda DM, *et al.* (2013) Timing of maternal exposure to a High Fat diet and development of obesity and hyperinsulinemia in male rat offspring: same metabolic phenotype, different developmental pathways? *J Nutr Metab* **2013**, 517384.
63. Latouche C, Heywood SE, Henry SL, *et al.* (2014) Maternal over-nutrition program changes in the expression of skeletal muscle genes that are associated with insulin resistance and defects of oxidative phosphorylation in adult male rat offspring. *J Nutr* **144**, 237–244.
64. Storlien LH, Kraegen EW, Chisholm DJ, *et al.* (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* **237**, 885–888.
65. Podolin DA, Gayles EC, Wei Y, *et al.* (1998) Menhaden oil prevents but does not reverse sucrose-induced insulin resistance in rats. *Am J Physiol* **274**, R840–R848.
66. D'Alessandro ME, Chicco A & Lombardo YB (2013) Fish oil reverses the altered glucose transporter, phosphorylation, insulin receptor substrate-1 protein level and lipid contents in the skeletal muscle of sucrose-rich diet fed rats. *Prostaglandins Leukot Essent Fatty Acids* **88**, 171–177.
67. Neschen S, Moore I, Regittnig W, *et al.* (2002) Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. *Am J Physiol Endocrinol Metab* **282**, E395–E401.
68. Ramalingam L, Menikdiwela KR, Clevenger S, *et al.* (2018) Maternal and postnatal supplementation of fish oil improves metabolic health of mouse male offspring. *Obesity* **26**, 1740–1748.
69. Matorras R, Exposito A, Ferrando M, *et al.* (2020) Oocytes of women who are obese or overweight have lower levels of *n*-3 polyunsaturated fatty acids compared with oocytes of women with normal weight. *Fertil Steril* **113**, 53–61.
70. Abodi M, De Cosmi V, Parazzini F, *et al.* (2022) *n*-3 fatty acids dietary intake for oocyte quality in women undergoing assisted reproductive techniques: a systematic review. *Eur J Obstet Gynecol Reprod Biol* **275**, 97–105.
71. Sferruzzi-Perri AN & Camm EJ (2016) The programming power of the placenta. *Front Physiol* **14**, 33.
72. Calabuig-Navarro V, Puchowicz M, Glazebrook P, *et al.* (2016) Effect of  $\omega$ -3 supplementation on placental lipid metabolism in overweight and obese women. *Am J Clin Nutr* **103**, 1064–1072.
73. Jones ML, Mark PJ & Waddell BJ (2014) Maternal dietary *n*-3 fatty acids and placental function. *Reproduction* **147**, R143–52.
74. Amatruda M, Ippolito G, Vizzuso S, *et al.* (2019) Epigenetic effects of *n*-3 LCPUFAs: a role in pediatric metabolic syndrome. *Int J Mol Sci* **29**, 2118.
75. Maude H, Sanchez-Cabanillas C & Cebola I (2021) Epigenetics of hepatic insulin resistance. *Front Endocrinol* **12**, 681356.
76. Ibrahim HIM (2022) Epigenetic regulation of obesity-associated type 2 diabetes. *Medicina* **58**, 1366.
77. Milagro FI, Campión J, García-Díaz DF, *et al.* (2009) High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. *J Physiol Biochem* **65**, 1–9.
78. Lomba A, Martínez JA, García-Díaz DF, *et al.* (2010) Weight gain induced by an isocaloric pair-fed high fat diet: a nutriepigenetic study on FASN and NDUFB6 gene promoters. *Mol Genet Metab* **101**, 273–278.
79. Uriarte G, Paternain L, Milagro FI, *et al.* (2013) Shifting to a control diet after a high-fat, high-sucrose diet intake induces epigenetic changes in retroperitoneal adipocytes of Wistar rats. *J Physiol Biochem* **69**, 601–611.
80. Jump DB (2008) *n*-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr Opin Lipidol* **19**, 242–247.
81. van Dijk SJ, Peters TJ, Buckley M, *et al.* (2018) DNA methylation in blood from neonatal screening cards and the association with BMI and insulin sensitivity in early childhood. *Int J Obes* **42**, 28–35.
82. Seki Y, Suzuki M, Guo X, *et al.* (2017) In utero exposure to a high-fat diet programs hepatic hypermethylation and gene dysregulation and development of metabolic syndrome in male mice. *Endocrinology* **158**, 2860–2872.
83. Suter MA, Ma J, Vuguin PM, *et al.* (2014) In utero exposure to a maternal high-fat diet alters the epigenetic histone code at genes involved in both metabolism and liver development in a murine model. *Am J Obstet Gynecol* **210**, 463.e1–463.e11.
84. Li Y, Xu S, Giles A, *et al.* (2011) Hepatic overexpression of SIRT1 in mice attenuates endoplasmic reticulum stress and insulin resistance in the liver. *FASEB J* **25**, 1664–1679.
85. Rahman M, Halade GV, Bhattacharya A, *et al.* (2009) The fat-1 transgene in mice increases antioxidant potential, reduces pro-inflammatory cytokine levels, and enhances PPAR- $\gamma$  and SIRT-1 expression on a calorie restricted diet. *Oxid Med Cell Longev* **2**, 307–316.
86. Burdge GC & Lillycrop KA (2014) Fatty acids and epigenetics. *Curr Opin Clin Nutr Metab Care* **17**, 156–161.
87. Basak S, Vilasagaram S & Duttaroy AK (2020) Maternal dietary deficiency of *n*-3 fatty acids affects metabolic and epigenetic phenotypes of the developing fetus. *Prostaglandins Leukot Essent Fatty Acids* **158**, 102109.
88. Basak S & Duttaroy AK (2022) Maternal PUFAs, placental epigenetics, and their relevance to fetal growth and brain development. *Reprod Sci* (Epublication ahead of print version 8 June 2022).
89. McGregor RA & Choi MS (2011) microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med* **11**, 304–316.
90. Ramaiyan B & Talahalli RR (2018) Dietary unsaturated fatty acids modulate maternal dyslipidemia-induced DNA methylation and histone acetylation in placenta and fetal liver in rats. *Lipids* **53**, 581–588.
91. van Dijk SJ, Zhou J, Peters TJ, *et al.* (2016) Effect of prenatal DHA supplementation on the infant epigenome: results from a randomized controlled trial. *Clin Epigenet* **8**, 114.
92. Hao L, Nie YH, Chen CY, *et al.* (2022) *n*-3 polyunsaturated fatty acids protect against high-fat diet-induced morphological and functional impairments of brown fat in transgenic fat-1 mice. *Int J Mol Sci* **23**, 11903.



93. Tsuji T, Bussberg V, MacDonald AM, *et al.* (2022) Transplantation of brown adipose tissue with the ability of converting *n*-6 to *n*-3 polyunsaturated fatty acids counteracts high-fat-induced metabolic abnormalities in mice. *Int J Mol Sci* **23**, 5321.
94. Fan R, Toney AM, Jang Y *et al.* (2018) Maternal *n*-3 PUFA supplementation promotes fetal brown adipose tissue development through epigenetic modifications in C57BL/6 mice. *Biochim Biophys Acta, Mol Cell Biol Lipids* **1863**, 1488–1497.
95. Yue H, Liu W, Zhang W, *et al.* (2021) Dietary low ratio of *n*-6/*n*-3 polyunsaturated fatty acids improve type 2 diabetes mellitus via activating brown adipose tissue in male mice. *J Food Sci* **86**, 1058–1065.
96. Wei W, Hu M, Huang J, *et al.* (2021) Anti-obesity effects of DHA and EPA in high fat-induced insulin resistant mice. *Food Funct* **12**, 1614–1625.