Perinatal exposure of rats to a maternal diet with varying protein quantity and quality affects the risk of overweight in female adult offspring

Carlin, Gabrielle; Chaumontet, Catherine; Blachier, Francois; Barbillon, Pierre; Darcel, Nicolas; Delteil, Corine; van der Beek, Eline M.; Kodde, Andrea; van de Heijning, Bert J. M.; Tome, Daniel

Published in:
Journal of Nutritional Biochemistry

DOI:
10.1016/j.jnutbio.2019.108333

Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 79 (2020) 108333

Journal of Nutritional Biochemistry

Perinatal exposure of rats to a maternal diet with varying protein quantity and quality affects the risk of overweight in female adult offspring☆

Gabrielle Carlin[a], Catherine Chaumontet[a], François Blachier[a], Pierre Barbillon[b], Nicolas Darcel[a], Corine Delteil[a], Eline M. van der Beek[c,d], Andrea Kodde[c], Bert J.M. van de Heijning[c], Daniel Tomé[a], Anne-Marie Davila[a,*]

[a]Université Paris-Saclay, AgroParisTech, INRAE, UMR PNCA, 75005, Paris, France
[b]Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA-Paris, 75005, Paris, France
[c]Danone Nutricia Research, Utrecht, the Netherlands
[d]Department of Pediatrics, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands

Received 9 April 2019; received in revised form 30 November 2019; accepted 30 December 2019

Abstract

The maternal protein diet during the perinatal period can program the health of adult offspring. This study in rats evaluated the effects of protein quantity and quality in the maternal diet during gestation and lactation on weight and adiposity in female offspring. Six groups of dams were fed a high-protein (HP; 47% protein) or normal-protein (NP; 19% protein) isocaloric diet during gestation ($_G$) using either cow's milk (**M**), pea (**P**) or turkey (**T**) proteins. During lactation, all dams received the NP diet (protein source unchanged). From postnatal day (PND) 28 until PND70, female pups (n=8) from the dam milk groups were exposed to either an NP milk diet (NP$\mathbf{M}_W$) or to dietary self-selection (DSS). All other pups were only exposed to DSS. The DSS design was a choice between five food cups containing HP**M**, HP**P**, HP**T**, carbohydrates or lipids. The weights and food intakes of the animals were recorded throughout the study, and samples from offspring were collected on PND70. During the lactation and postweaning periods, body weight was lower in the pea and turkey groups (NP_G and HP_G) *versus* the milk group (P<.0001). DSS groups increased their total energy and fat intakes compared to the NP$\mathbf{M}_W$ group (P<.0001). In all HP_G groups, total adipose tissue was increased (P=.03) associated with higher fasting plasma leptin (P<.05). These results suggest that the maternal protein source impacted offspring body weight and that protein excess during gestation, irrespective of its source, increased the risk of adiposity development in female adult offspring.

Keywords: Perinatal programming; High-protein; Protein quality; Growth; Adiposity

1. Introduction

Epidemiological and experimental data have shown that nutrition is a perinatal programming factor that can modulate tissue and organ development (*i.e.*, pancreas, liver, brain, gut), and the risk of later metabolic diseases including obesity and type 2 diabetes [1–4]. Several studies have reported that manipulating energy macronutrients during the perinatal period had long-lasting effects on offspring health [5]. *In utero* exposure to energy or macronutrient restriction [6] or excess [3] during gestation can program food intake control pathways and induce hyperphagia. As for protein, a low-protein (LP) diet during gestation affects offspring food preferences and body weight [7, 8], while a high-protein (HP) diet during the same period (although less studied) also induced a predisposition to metabolic and food intake disorders among offspring [9–12]. In a previous study in rats, a maternal HP diet (55% energy) during gestation *versus* a normal-protein (NP) diet (20% energy) modified insulin signaling and induced increased adiposity in female offspring at an adult age (15 weeks old) in a model of macronutrient dietary self-selection (DSS) during the postweaning period [13].

As well as the protein content in the diet, protein quality in the maternal diet during pregnancy and lactation has been suspected to affect the risk of developing metabolic diseases in offspring [14]. In both human [15–17] and animal models [18–20], the maternal diet has been shown to exert a direct impact on the placental flow of nutrients during gestation and can modulate milk composition during lactation. Sufficient levels of protein and amino acids during gestation and lactation are nutritionally programming factors for optimal growth and health in offspring [21, 22]. An amino acid deficiency

☆ Declaration of interest: Gabrielle Carlin, Catherine Chaumontet, François Blachier, Pierre Barbillon, Nicolas Darcel, Corine Delteil, Daniel Tomé and Anne-Marie Davila have no conflicts of interest. Andrea Kodde, Bert J.M. van de Heijning and Eline M. van der Beek are employees of DNR.

* Corresponding author at: 16, rue Claude Bernard, 75005, Paris. Tel.: +33 1 44 08 86 76.
E-mail address: davila@agroparistech.fr (A.-M. Davila).

https://doi.org/10.1016/j.jnutbio.2019.108333

due to protein malnutrition during gestation in rats induced gene hypermethylation of the fetal liver [23]. When compared to animal protein, plant-based protein is characterized by poorer digestibility [21] and a lower content in essential amino acids (EAAs), notably methionine, lysine and tryptophan [24]. A normal-protein diet (19% energy) containing soy protein compared to casein during gestation and lactation in rats induced a higher food intake and body weight in offspring at 15 weeks [25]. By contrast, during pregnancy in humans, animal protein (particularly meat protein) compared to plant protein increased the risk of overweight in offspring (notably female) at the age of 20 years [26].

The present work analyzed the impact of different protein sources in variable quantity in the maternal diet using the mostly consumed animal-protein sources (including milk and poultry such as turkey) and plant-protein sources (including pulses such as peas) that are available in an acceptable purified form on the market. Specifically, this study in rats therefore evaluated the consequences of varying protein quantity (NP *vs.* HP) and quality (pea- and turkey-derived protein compared to milk protein, the protein source in semisynthetic rodent chow) in the maternal diet during gestation with respect to food intake, body weight, adiposity, certain plasma hormone levels and gene expression in the liver in female offspring subjected to macronutrient DSS during the postweaning period.

2. Materials and methods

2.1. Animals and diets

The animal experiments complied with the European guidelines on animal experimentation as validated by INRA's Ethics Committee on Animal Experimentation in Jouy-en-Josas (COMETHEA) and approved by the French Ministry of Research (registration number: APAFIS#3988-2016011910059852). The procedures used during this experimental work on animals had previously been described [13]. Wistar rats ("dams," HsdHan:WIST, Envigo, France) were studied and maintained under controlled conditions (22°C±1°C, 12 h-light/12 h-dark cycle with lights on at 8:00 am) with free access to food and water throughout the study. Protein powders from milk (M) (89% protein; Ingredia, France), pea (P) (78.5% protein; Roquette, France) and turkey (T) (77.5% protein; Proliver, Belgium) (Supplementary Table 1) were used to formulate the different diets (Table 1). The specific characteristics of the diets, including their EAA and fatty acid composition, were recorded according to properties of their protein sources. A diet containing a normal level of milk protein (NP **Milk**) was used as a control, nearly aligned with the AIN-93G diet for rodents [27].

Table 1
Diet composition

	NP**M**	NP**P**	NP**T**	HP**M**	HP**P**	HP**T**	**C**	**L**
Composition								
Metabolizable energy (kcal/g)	3.8	3.8	3.8	3.8	3.8	3.8	3.6	8.1
Protein (% energy)	19	19	19	47	47	47	-	-
Carbohydrate (% energy)	65	65	65	37	37	37	100	-
Lipid (%energy)	16	16	16	16	16	16[a]	-	100
Saturated fat (% of lipid)	16	15	20	19	16	39	-	-
Monounsaturated fat (% of lipid)	25	27	27	26	27	41	-	-
Polyunsaturated fat (% of lipid)	58	58	53	54	57	19	-	-
Polyunsaturated fat n-6 (% of lipid) (almost or totally Linoleic)	54	53	48	50	52	17		
Polyunsaturated fat n-3 (% of lipid) (almost or totally α-Linolenic)	4.9	4.5	4.9	4.0	4.9	1.6		
Ingredients (g/kg)								
Cow's milk protein powder	220	-	-	505	-	-	-	-
Pea protein powder	-	225	-	-	540	-	-	-
Turkey protein powder	-	-	230	-	-	560	-	-
Cornstarch	540	545	555	295	285	300	776.4	-
Sucrose	76.7	77.7	77.7	42.7	42.7	42.7	126.3	-
Soybean oil	66	55	40	60	35	0.0[a]	-	902.7
Mineral mix (AIN-93-MX)	35	35	35	35	35	35	35	35
Vitamin mix (AIN-93-VX)	10	10	10	10	10	10	10	10
Cellulose	50	50	50	50	50	50	50	50
Choline	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Histidine	0.52	0.45	0.35	1.19	1.07	0.85	-	-
Isoleucine	0.89	0.79	0.55	2.05	1.88	1.34	-	-
Leucine	1.85	1.49	1.09	4.24	3.56	2.62	-	-
Lysine	1.52	1.34	1.09	3.48	3.21	2.62	-	-
Threonine	0.89	0.77	0.75	2.05	1.84	1.82	-	-
Tryptophan	0.15	0.08	0.09	0.33	0.20	0.21	-	-
Valine	1.08	0.83	0.71	2.48	1.99	1.71	-	-
Methionine + cysteine	0.60	0.30	0.44	1.38	0.73	1.07	-	-
Phenylalanine + tyrosine	1.95	1.66	1.09	4.48	3.97	2.62	-	-
Arginine	0.66	1.61	1.44	1.53	3.87	3.48	-	-
Alanine	0.62	0.74	1.33	1.43	1.78	3.21	-	-
Asparagine	1.43	2.08	1.44	3.29	4.99	3.48	-	-
Glutamine	4.32	3.25	2.46	9.92	7.79	5.94	-	-
Glycine	0.35	0.74	2.73	0.81	1.78	6.58	-	-
Proline	1.83	0.64	1.60	4.20	1.53	3.85	-	-
Serine	1.06	0.91	0.66	2.43	2.19	1.60	-	-

NP, normal protein (control); HP, high protein; M, milk; P, pea; T, turkey; C, carbohydrate; L, lipid; NP**M**, normal-protein diet composed of milk protein; HP**M**, high-protein diet composed of milk protein during gestation; NP**P**, normal-protein diet composed of pea protein; HP**P**, high-protein diet composed of pea protein; NP**T**, normal-protein diet composed of turkey protein; HP**T**, high-protein diet composed of turkey protein.

[a] Lipid in the HP**T** diet only came from protein powder.

2.2. Experimental design

Under the experimental design (Fig. 1), 24 pregnant 6-week-old females were received and housed in groups of three. After 1 week of habituation, the dams were housed individually and mated.

2.2.1. Gestation

As from mating and throughout gestation ($_G$), the dams were assigned randomly to six groups (n=3 or 4) and fed an NP diet (19 En% protein, 16 En% fat, 65 En% carbohydrate) or an isocaloric (3.8 kcal/g) HP diet (47 En% protein, 16 En% fat, 37 En% carbohydrate) containing either cow's milk total protein, pea protein or turkey protein (NP **[M, P or T]**$_G$ and HP **[M, P or T]**$_G$ groups).

2.2.2. Lactation

During lactation (until PND21), all dams were fed the NP diet, where the protein source remained unchanged **[M, P or T]**.

2.2.3. Birth

At birth, female pups were selected because they showed a higher sensitivity to early fetal environmental manipulations compared to males [12, 28]. The litters were normalized to eight pups.

2.2.4. Postweaning period

One week after weaning ($_W$) and acclimation, from postnatal day (PND) 28 until PND70, female pups from the six maternal diet groups were placed in groups of eight (n=8 per group): one pup group from NP**M**$_G$ and one from HP**M**$_G$ dams were exposed to an NP control milk diet (NP**M**$_W$) (*i.e.* the NP**M**$_G$-NP**M**$_W$ and the HP**M**$_G$-NP**M**$_W$ group), and six pup groups (also n=8 per group) received the NP and HP protein diets containing milk, pea or turkey protein and were subjected to DSS feeding (*i.e.*, three NP **[M, P, or T]**$_G$-DSS and three HP **[M, P, or T]**$_G$-DSS groups). DSS involved the use of five cups containing either the HP milk protein diet (HP**M**), the HP pea protein diet (HP**P**), the HP turkey protein diet (HP**T**), carbohydrates only (C) (100%) or lipids only (L) (100%).

2.3. Animal data collection and sampling

During gestation and lactation, the weight and food intake of the dams were recorded daily. Each pup was weighed daily from birth to PND70, and their food intake was monitored daily from PND28 to PND70. On PND70, after an overnight fast, a fasting blood sample was collected *via* a tail vein puncture. The animals were then fed a calibrated meal (3 g dry matter) during the light phase that was made up according to the macronutrient intake the previous week of each animal when they had been put on DSS. The calibrated meal was removed after 30 min, and then 60 min after removal, the adult pups were anesthetized under isoflurane balanced with nitrous oxide and decapitated. Body composition was assessed by weighing the carcass, organs and fat tissues, both subcutaneous and visceral (including periovarian, retroperitoneal and mesenteric). The periovarian adipose tissue and liver were sampled, snap-frozen in liquid nitrogen and stored at −80°C. Blood samples collected in the fed state (portal and trunk blood) were centrifuged (3000 ×*g*, 4°C, 15 min) and the plasma stored directly at −80°C.

2.4. Characteristics of adipocytes

An analysis of adipocytes was performed on approximatively 20 mg of periovarian adipose tissue during dissection directly after sampling, as previously described [29]. The sample was digested in 500 µl Krebs buffer with collagenase (10 mg/ml) at 37°C for 15 min and placed on a microscope slide. Images of the adipocytes were captured using a microscope equipped with a camera (Axioimager.Z1 and Axiocam MRc, Zeiss, Germany). The perimeters of approximately 500 adipocytes were calculated using ImageJ software (USA) to define the mean adipocyte weight with a triglyceride density of 0.92. Approximately 30 mg of periovarian tissue was sampled and stored at −80°C for the subsequent quantification of triglycerides (TG) quantification using a colorimetric enzymatic assay (TRIGS kit, Randox). The numbers of cells were estimated by dividing the triglyceride content in tissue by the mean weight of adipocytes.

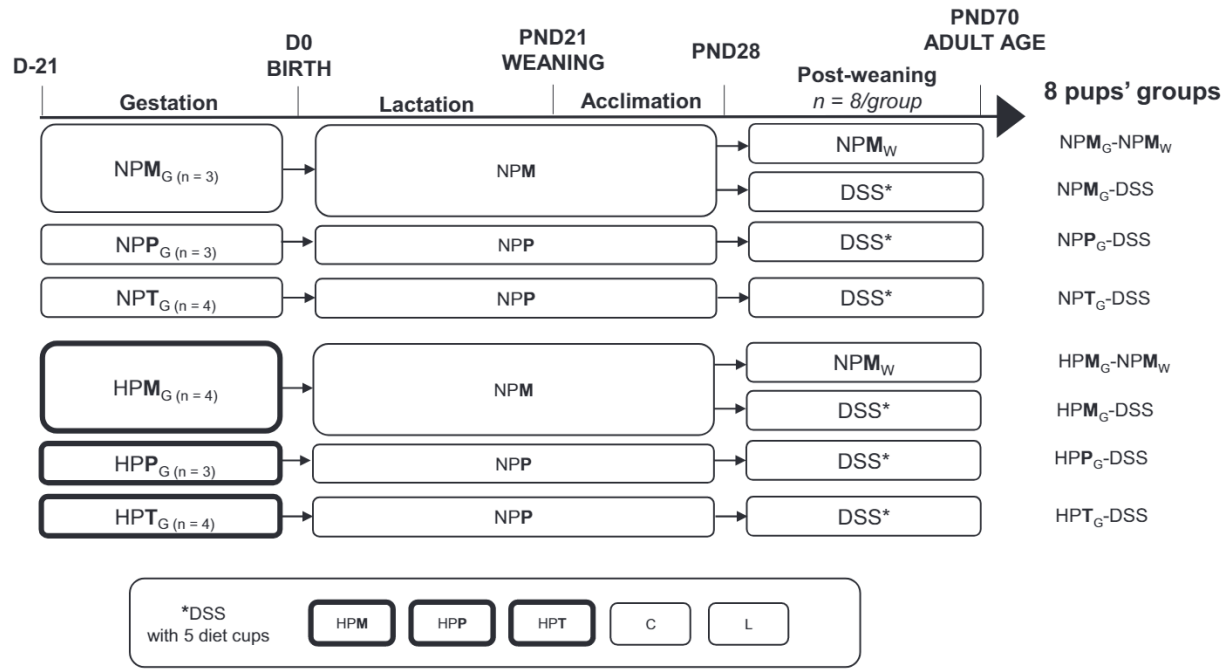

Fig. 1. Experimental design. During gestation ($_G$), the dams received an NP$_G$ or HP$_G$ diet and the protein sources used were either milk (**M**), pea (**P**) or turkey (**T**). During lactation, the dams received the NP diet containing protein from the same source as during gestation (**M**, **P** or **T**). After weaning ($_W$) and 1 week of acclimation, from PND28 to PND70 (adult stage), two pup groups were formed from the two **M** dam groups (NPM$_G$ and HPM$_G$): one received the NP**M**$_W$ control diet and the other the DSS option that comprised five separate food cups: HP**M**, HP**P**, HP**T**, carbohydrates (C) and lipids (L). Pups from the **P** and **T** dam groups were exposed to the DSS option during the postweaning period.

2.5. Biochemical assays

Blood fasting glucose levels were measured immediately with a glucose meter (Accu-Check Go, Roche Diagnostic). Plasma samples were stored at −80°C for the determination of metabolites. Fed glucose and triglyceride levels in plasma were determined with a colorimetric enzymatic assay (GLU-PAP kit, TRIGS kit, Randox). Plasma insulin levels were measured using an FIA immunoassay (Insulin FIA, Mercodia), and those of plasma leptin were measured with a Luminex assay (RMHMAG-84K-05, Rat Metabolic Hormone Magnetic Bead Mutiplex Assay, Merck-Millipore). The TG content of the liver was measured using a colorimetric enzymatic assay (TRIGS kit, Randox) after extraction with a buffer solution (NaCl, Tris HCl and Triton X100) from 80 mg samples.

2.6. Gene expression determined by quantitative PCR

Total RNA was extracted from the liver (100 mg) using 1 ml Trizol Reagent (Invitrogen). Concentrations of total RNA were determined by measuring absorbance at 260 nm with a Nanodrop spectrophotometer (Isogen, the Netherlands). cDNA synthesis was performed on 400 ng total RNA by reverse transcriptase using a high-capacity DNA kit (Applied Biosystems, CA, USA). Real-time PCR was performed on a StepOnePlus real-time PCR system (Applied Biosystems) using the SYBR green fast Reagent PCR master mix (Applied Biosystems) under the following conditions: 10 min at 95°C and 40 cycles of 95°C for 15 s and 1 min at 60°C. For each run, a calibration range was used to estimate efficiency, and a melting curve was determined to confirm the absence of contamination. The primer sequences are detailed in Supplementary Table 2. Ribosomal 18S was used to normalize the abundance of mRNA in genes of interest. Gene expression was presented as an arbitrary unit using the NP$\mathbf{M}_G$-NP$\mathbf{M}_W$ group as the reference sample (gene expression=1) according to the following formula: $2^{-\Delta\Delta Ct}$ with $\Delta\Delta Ct$ = (Ct sample gene of interest − Ct sample 18S) − (Ct reference sample gene of interest − Ct reference sample 18S).

2.7. Statistical analysis

2.7.1. Data on dams during gestation and lactation (model 1)

Two-way analysis of variance (ANOVA) was performed to determine the effects of protein quantity during gestation (quantity factor), the protein source during gestation and lactation (source factor) and interactions between the diets. For the quantity factor, the baseline value was NP diet for the study. For the source factor, the baseline value was the M source for the study. For repeated measurements, time was added as a repeated factor (model 1t). The dam factor was also added as a random factor in order to confirm that the animal was the same at each time.

2.7.2. Data on pups after weaning, all groups (model 2)

Three-way ANOVA was performed to determine the effects of the dam diet during gestation (protein “quantity” and “source” factors), the postweaning diet (postweaning factor) and interactions between the diets. In this design, the NP diet during the postweaning period was only imposed on pups from dams receiving milk protein, excluding interactions between the source and postweaning factors. Only the interaction between the quantity and postweaning factors was possible in the model. For repeated measurements, time was added as a repeated factor (model 2t). The pup factor was added as a random factor in order to confirm that the pup was the same at each time.

2.7.3. Data on pups after weaning, DSS groups (model 3)

Two-way ANOVA was performed to determine the effects of the dam diet during gestation (protein “quantity” and “source” factors) and the interactions between maternal diet factors.

In the postweaning models, a random factor was added to include correlations between pups from the same litter. Pairwise comparisons were adjusted multiple comparisons using a Tukey *post hoc* test. Statistical analyses were performed using R studio 1.1.383, and the differences between groups were considered to be significant at $P<.05$. All data are expressed as means±S.E.M.

3. Results

3.1. Dams: protein quantity and quality during gestation and lactation affected their energy and nutrient intake

The average daily intakes of total energy, total lipid, total protein and total EAA by dams during gestation and lactation are presented in Fig. 2. Individual EAA and polyunsaturated fatty acid (PUFA) intakes are reported in Tables 2A and B, respectively.

3.1.1. Energy

The daily energy intake was significantly higher during lactation than during gestation ($P<.0001$). During both periods, the HP groups generally displayed a lower overall daily energy intake (mean of the period) compared to NP groups (quantity, gestation $P=.0002$; lactation $P=.0003$). During lactation, animals in the pea groups had a significantly lower daily energy intake compared to other source groups (source, $P=.0007$) (Fig. 2).

3.1.2. Protein

During gestation, daily protein and EAA intakes were higher in HP groups than in NP groups (quantity, $P<.0001$); the EAA intake was significantly lower in the pea and turkey groups (source, $P\leq.0003$) and specifically in the HP groups (Fig. 2). For histidine, isoleucine, leucine and phenylalanine+tyrosine: HP$\mathbf{M}_G$ > HP$\mathbf{T}_G$ > HP$\mathbf{P}_G$; for tryptophan and methionine+cysteine: HP$\mathbf{M}_G$ > HP$\mathbf{P}_G$ > HP$\mathbf{T}_G$; $P<.05$), and daily methionine+cysteine intake was also decreased in the NP$\mathbf{P}_G$ group compared to the NP$\mathbf{M}_G$ and NP$\mathbf{T}_G$ groups ($P<.05$) (Table 2A).

During lactation, with all the dams receiving NP diets only, the daily protein intake was increased in the NP compared to HP gestation groups and decreased in the pea groups compared to the milk and turkey groups (quantity, $P=.0003$; source, $P=.0007$). The daily EAA intake was significantly lower in the pea and turkey groups compared to the milk groups (source, $P<.0001$) (Fig. 2), and the daily methionine +cysteine intake was significantly higher in the milk group compared to the pea and turkey groups and significantly higher in the turkey group compared to the pea group (NP$\mathbf{M}_G$ and HP$\mathbf{M}_G$ > NP$\mathbf{P}_G$ and HP$\mathbf{P}_G$ > NP$\mathbf{T}_G$ and HP$\mathbf{T}_G$, $P<.05$) (Table 2A).

3.1.3. Lipids

During gestation, the daily lipid intake was lower in the HP groups *versus* the NP groups (quantity, $P=.0002$) (Fig. 2). Similar observations were made regarding daily intakes of total PUFA, PUFA n-6 and PUFA n-3 (quantity, $P<.0001$). A more marked decrease was seen in the HP$\mathbf{T}_G$ group compared to the NP$\mathbf{T}_G$ group and also to other groups (source, $P<.0001$; quantity × source, $P<.0001$) (Table 2B).

During lactation, the average daily lipid intake was decreased in the HP compared to the NP gestation groups (quantity, $P=.0003$) and also decreased in the pea compared to the milk and turkey groups (source, $P=.0007$) (Fig. 2). The same observations were made with respect to daily intakes of total PUFA, PUFA n-6 and PUFA n-3 (quantity, $P\leq.0004$) (Table 2B).

Finally, weight gain did not differ between the dams in the six dietary groups during gestation and lactation (data not shown).

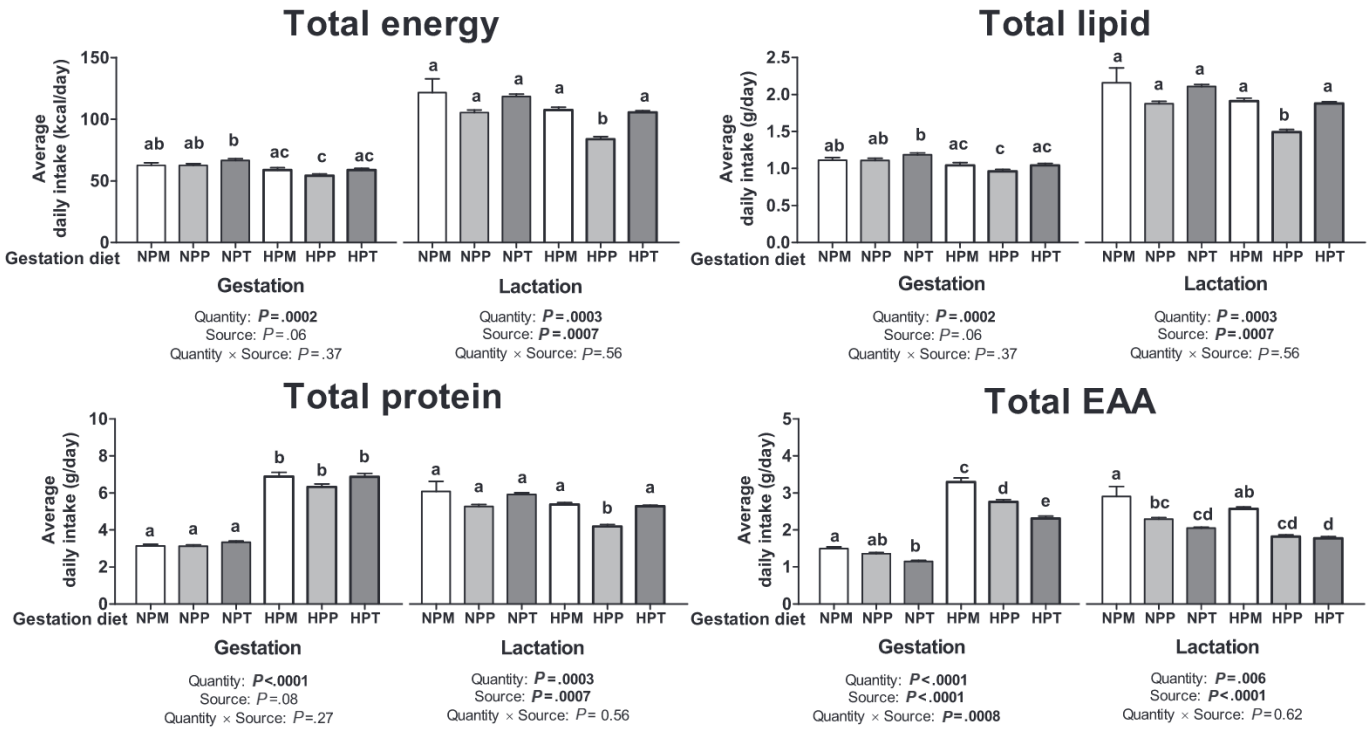

Fig. 2. Average daily intakes by dams during gestation and lactation of total energy, protein, EAA and lipids. Data were tested using statistical model 1. Quantity: protein quantity during gestation factor. Source: protein source during gestation and lactation factor. Data are means±S.E.M. [a,b,c,d] Mean values with different letters were significantly different ($P<.05$) and tested separately for gestation and lactation.

3.2. Offspring: the maternal protein source affected weight gain and food intake in offspring during lactation and the postweaning period in the event of DSS

Birth data are presented in Table 3. Dams in the turkey diet groups (NP**T**$_G$ and HP**T**$_G$) and even more those in the pea diet groups (NP**P**$_G$ and HP**P**$_G$) had significantly lighter litters (source, $P=.003$) with fewer pups in the litter (source, $P=.03$) than dams in the milk protein groups (NP**M**$_G$ and HP**M**$_G$). However, the average weight of pups in the litter was not affected (source, $P=.21$). The weight gain of litters (Fig. 3A) was significantly lower throughout lactation in the turkey and pea dam groups *versus* the milk dam groups (source, $P<.0001$; source × time, $P<.0001$). As from PND19, litters in the NP**M**$_G$ group weighed significantly more than those in the HP**M**$_G$ group (quantity × source × time, $P<.001$). On PND21, the weaned litter weight was significantly lower in the pea and turkey groups compared to the milk groups (source, $P<.0001$) (Table 3).

On PND28, pups from dams receiving the pea and turkey diets had a significantly lower weight than those from dams receiving milk diets during gestation and lactation (source, $P<.0001$) (Table 4). The

Table 2A
EAA intakes (mg/day) during gestation and lactation in each dam group

Gestation diet		NPM$_G$	NPP$_G$	NPT$_G$	HPM$_G$	HPP$_G$	HPT$_G$	*P*		
Dams		*n*=3	*n*=3	*n*=4	*n*=4	*n*=3	*n*=4	Quantity	Source	Quantity × source
Histidine	Gestation	82.4±2.5*[a]	78.8±1.9[a]	66.2±1.4[a]	181.1±5.8[b]	159.7±3.8[c]	136.5±3.6[d]	**<.0001**	**<.0001**	**<.0001**
	Lactation	159.8±14.6[a]	133.0±2.3[b]	117.7±1.8[b,c]	141.2±3.0[a]	105.7±2.6[c]	104.9±1.4[c]	**.001**	**<.0001**	.50
Isoleucine	Gestation	141.8±4.4[a]	138.9±3.4[a]	103.7±2.1[a]	311.6±10.1[b]	281.4±6.6[c]	213.6±5.6[d]	**<.0001**	**<.0001**	**<.0001**
	Lactation	275.0±25.2[a]	234.4±4.1[b,d]	184.3±4.5[c]	243.0±5.2[a,d]	186.3±4.5[b,c]	164.2±2.1[c]	**.001**	**<.0001**	.42
Leucine	Gestation	293.3±9.0[a]	262.7±6.4[a]	203.0±4.2[a]	644.8±20.8[b]	532.2±12.5[c]	418.4±11.0[d]	**<.0001**	**<.0001**	**<.0001**
	Lactation	569.0±52.1[a]	443.3±7.8[b]	360.7±5.4[b,c]	502.7±10.7[a]	352.4±8.5[b,c]	321.5±4.2[c]	**.002**	**<.0001**	.48
Lysine	Gestation	240.6±7.4[a]	236.4±7.4[a]	203.0±4.2[a]	528.8±17.1[b]	479.0±11.3[c]	418.4±11.0[d]	**<.0001**	**<.0001**	**.0004**
	Lactation	466.7±42.8[a]	399.0±7.0[b,c]	360.7±5.4[b]	412.3±8.8[a,c]	317.2±7.7[b]	321.5±4.2[b]	**.001**	**<.0001**	.50
Methionine + cysteine	Gestation	95.7±2.9[a]	53.7±1.3[b]	83.0±1.7[a]	210.3±6.8[c]	108.9±2.6[d]	171.1±4.5[e]	**<.0001**	**<.0001**	**<.0001**
	Lactation	185.6±17.0[a]	90.7±1.6[b]	147.5±2.2[c]	164.0±3.5[a]	72.1±1.7[b]	131.5±1.7[c]	**.003**	**<.0001**	.84
Phenylalanine + tyrosine	Gestation	310.0±9.5[a]	292.9±7.1[a]	203.0±4.2[a]	681.6±22.0[b]	593.5±14.0[c]	354.4±60.0[d]	**<.0001**	**<.0001**	**<.0001**
	Lactation	601.4±55.1[a]	494.4±8.7[b,c]	360.7±5.4[d]	531.4±11.3[a,b]	392.9±9.5[c]	273.4±46.9[d]	**.002**	**<.0001**	.40
Threonine	Gestation	141.8±4.4[a]	135.3±3.3[a]	141.1±2.9[a]	311.7±10.1[b]	274.2±6.5[c]	290.7±7.7[c]	**<.0001**	**.0004**	**.01**
	Lactation	275.0±25.2[a]	228.4±4.0[b,c,d]	250.7±3.8[b,d]	243.0±5.2[a,b]	181.5±4.4[c]	223.4±2.9[d,c]	**.0008**	**<.0001**	.66
Tryptophan	Gestation	23.0±0.7[a]	17.7±0.4[a]	16.3±0.2[a]	50.7±1.6[b]	29.8±0.7[c]	33.7±0.9[d]	**<.0001**	**<.0001**	**<.0001**
	Lactation	44.7±4.1[a]	24.9±0.44[b,c]	29.0±0.4[b,c]	39.5±0.84[a]	19.8±0.5[c]	25.9±0.3[b,c]	**.004**	**<.0001**	.70
Valine	Gestation	171.4±5.3[a]	146.5±3.6[a]	132.5±2.7[a]	376.9±12.2[b]	296.7±7.0[c]	273.0±7.2[c]	**<.0001**	**<.0001**	**<.0001**
	Lactation	332.6±30.5[a]	247.2±4.4[b]	235.4±3.5[b]	293.8±6.3[a]	196.5±4.7[b]	209.8±2.7[b]	**.002**	**<.0001**	.59

Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. [a,b,c,d,e] Mean values with different superscript letters differed significantly from each other ($P<.05$). EAA, essential amino acid; G, gestation.
* Average daily intake corresponding to the mean daily intake throughout the period. Data were tested using statistical model 1.

Table 2B
PUFA intakes (mg/day) during gestation and lactation in each dam group

Gestation diet / Dams		NPM_G $n=3$	NPP_G $n=3$	NPT_G $n=4$	HPM_G $n=4$	HPP_G $n=3$	HPT_G $n=4$	*P* Quantity	*P* Source	*P* Quantity × source
Total PUFA	Gestation	645.9±19.8*[a]	644.5±15.7[a]	628.2±12.8[a]	565.5±19.3[b]	549.2±14.3[b]	214.2±4.6[c]	**<.0001**	**<.0001**	**<.0001**
	Lactation	1253±115[a]	1088±19[a,c]	1116±17[a,c]	1107±24[a,c]	865±21[b]	995±13[b,c]	**.0004**	**.001**	.50
PUFA n-6	Gestation	601.3±18.5[a]	588.9±14.3[a]	568.9±11.6[a]	523.7±17.9[b]	501.0±13.1[b]	191.9±4.1[c]	**<.0001**	**<.0001**	**<.0001**
	Lactation	1167±11[a]	994±18[a,c]	1011±15[a,c]	1031±22[a,c]	790±19[b]	901±12[b,c]	**.0004**	**<.0001**	.69
PUFA n-3	Gestation	54.6±1.7[a,d,c]	50.0±1.2[c]	58.1±1.2[d]	42.1±1.5 [b]	47.0±1.2[bc]	18.2±0.4[e]	**<.0001**	**<.0001**	**<.0001**
	Lactation	105.9+9.6[a]	84.4±1.5[b,c]	103.2±1.6[a]	93.5±2.0[a,b]	67.1±1.6[c]	92.0±1.2[a,b]	**.0004**	**.0004**	.50

Data were tested using statistical model 1. Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. [a,b,c,d,e] Mean values with different superscript letters differed significantly from each other (*P*<.05).
* Average daily intake corresponding to the mean daily intake throughout the period.

evolution of pup body weight during the postweaning period (from PND28) is presented in Fig. 3B. Throughout the postweaning period, the body weight of offspring was significantly lower in the turkey and pea diet groups than in the milk diet groups (source, *P*<.0001; source × time, *P*<.0001).

A significant interaction was revealed between protein quantity during gestation and time, which could be explained by the lower body weight in the HP**M**$_G$-NP**M**$_W$ than in the HP**M**$_G$-DSS group (quantity × time, *P*=.02). Animals in the HP**M**$_G$-DSS group had a significantly higher weight than the NP**M**$_G$-DSS group (quantity × source × time, *P*=.03). On PND70, final weight gain (Table 4) was significantly increased in all the milk diet groups when compared to the pea and turkey diet groups (source, *P*<.0001).

Cumulative energy (kcal) and final macronutrient (En%) intakes on PND70 are shown in Fig. 4A and B, respectively**.** The final food intakes in DSS groups, compared to the NP**M**$_G$-NP**M**$_W$ and HP**M**$_G$-NP**M**$_W$ control groups, were significantly increased by approximately 500 kcal in the turkey groups (NP**T**$_G$-DSS and HP**T**$_G$-DSS) and by up to 1000 kcal in the NP**M**$_G$-DSS and HP**M**$_G$-DSS groups (source, *P*=.03, postweaning, *P*<.0001). In DSS groups on PND70, compared to the NP**M**$_G$-NP**M**$_W$ and HP**M**$_G$-NP**M**$_W$ control groups, protein and lipid levels (% of total energy) significantly increased, whereas carbohydrate levels (% of total energy) significantly decreased (postweaning, *P*<.0001).

Intakes (% of total energy) of each protein source (milk, pea and turkey protein) during DSS (PND28–70) are reported in Table 5. The intake of milk protein was significantly higher in the HP**T**$_G$-DSS group compared to the NP**M**$_G$-DSS group (source, *P*=.03). Pea protein intake was similar in all the groups. Turkey protein intake was significantly lower in the NP**T**$_G$-DSS and HP**T**$_G$-DSS groups (turkey) than in other DSS groups (source, *P*<.0001).

3.3. Offspring: consuming an HP diet during gestation affected adiposity

The data on body composition are presented in Table 4. The results concerning total adipose tissue (% of body weight) are shown in Fig. 5A. The weights of total and subcutaneous tissues (% of body weight) in adult pups were significantly higher in all groups exposed to the HP diet during gestation, irrespective of the protein source. Total adipose tissue weight in the different groups was increased by (1) 18% in HP**M**$_G$-NP**M**$_W$ compared to NP**M**$_G$-NP**M**$_W$, (2) 16% in HP**M**$_G$-DSS compared to NP**M**$_G$-DSS, (3) 26% in HP**P**$_G$-DSS compared to NP**P**$_G$-DSS and (4) 17% in HP**T**$_G$-DSS compared to NP**T**$_G$-DSS.

The number of adipocytes, their average diameter and weight were significantly higher in the pea and turkey groups than in the milk groups (Table 4). The interaction between protein quantity and protein source found for the number of adipocytes could be explained by significantly higher values in the HP**T**$_G$-DSS group than in the NP**T**$_G$-DSS group (quantity × source, *P*=.0007). The distribution of adipocyte classes is presented in Fig. 5B. The relative abundance (%) of adipocytes shifted to smaller (0 to 40 μm) and fewer adipocytes in the medium range (40 to 80 μm) in the HP gestation group than in the NP group (quantity, *P*=.01). The relative abundance (%) of adipocytes in the "40–80 μm" class was higher in the pea and turkey diet groups than in the milk diet groups (source, *P*=.006), the opposite applying in the largest class (80 to 140 μm) (source, *P*=.0005). The interaction between protein quantity and the postweaning diet could be explained by a significantly higher abundance (%) of adipocytes in the "40–80 μm" class in the HP**M**$_G$-NP**M**$_W$ group than in the NP**M**$_G$-NP**M**$_W$ group (quantity × postweaning, *P*<.05) and conversely in the "80–140 μm" class (quantity × postweaning, *P*<.05). Adipocyte abundance (%) in the larger classes (>140 μm) was null in all groups on PND70.

3.4. Offspring: adults from HP gestation groups displayed higher levels of fasting leptin

3.4.1. Fasted state (Table 6)

Fasting plasma triglyceride (TG) levels were significantly higher in NP**M**$_G$-NP**M**$_W$ than in all other groups, explaining the significant effects of protein quantity during gestation of the postweaning diet and of the interaction between these factors (quantity, *P*=.04; postweaning, *P*=.006; quantity × postweaning, *P*=.007).

Glycemia was significantly lower in the turkey groups than in other protein source groups (source, *P*<.05), while plasma insulin levels remained unchanged.

Further, plasma leptin levels were significantly (1) higher in the HP than in the NP control gestation groups (quantity, *P*<.05) and (2)

Table 3
Birth and time of weaning data

Gestation diet / Dams	NPM_G $n=3$	NPP_G $n=3$	NPT_G $n=4$	HPM_G $n=4$	HPP_G $n=3$	HPT_G $n=4$	*P* Quantity	*P* Source	*P* Quantity × source
Average litter weight (g)	69.4±3.6	52.9±6.3	63.6±1.1	68.2±3.1	53.9±4.7	58.6±2.6	.49	**.003**	.69
Average litter size (number of pups)	11.7±0.9	9.0±1.0	11.5±0.5	12.0±0.4	10.3±0.9	10.5±0.6	.85	**.03**	.27
Average pup weight in the litter (g)	6.2±0.2	5.9±0.3	5.5±0.2	5.7±0.2	5.2±0.3	5.6±0.2	.08	.21	.24
Average litter weight at PND21 (g)	53.3±3.2[a]	34.1±0.6[c]	39.6±1.2[b,c]	45.2±3.8[a,b]	35.4±0.3[b,c]	39.6±1.0[b,c]	.24	**<.0001**	.12

Data were tested using statistical model 1. Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. [a,b,c] Mean values with different superscript letters differed significantly from each other (*P*<.05).

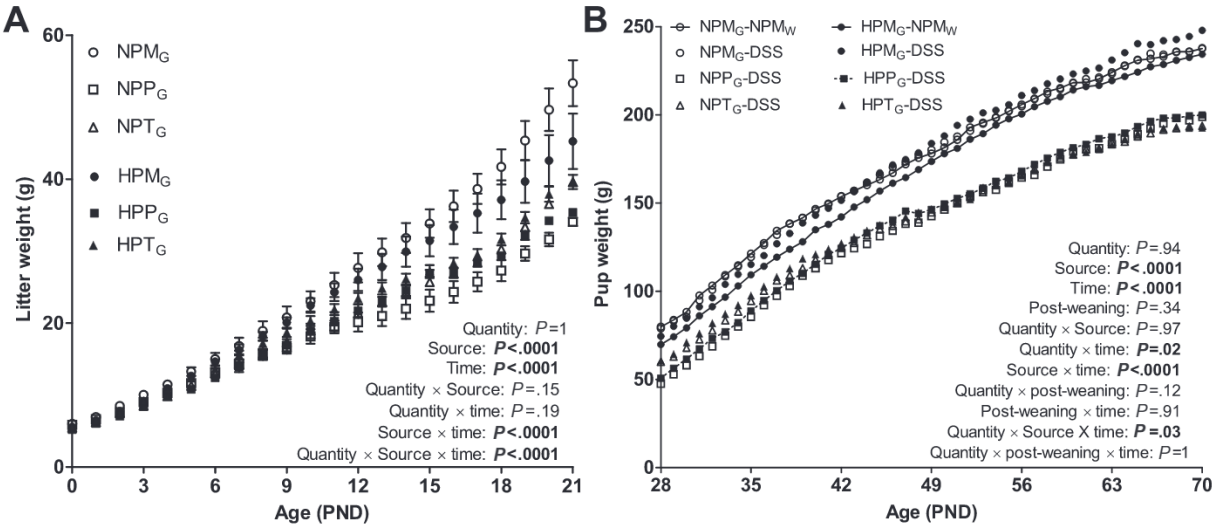

Fig. 3. Course of litter weights during lactation from PND0 to PND21 (A). Pup body weight during the postweaning period from PND28 to PND70 in each group (B). Data were tested using statistical models 1t and 2t. Quantity: protein quantity during gestation factor. Source: protein source during gestation and lactation factor. Data are means±S.E.M.

lower in the pea and turkey groups than in the milk postweaning groups (source, P=.004).

3.4.2. Fed state (Table 6)

Plasma TG levels in fed adult offspring were significantly higher in the DSS group than in the NP**M**$_W$ postweaning group (postweaning, P=.008).

Glycemia was significantly increased in the NP *versus* HP gestation groups and in the DSS *versus* NP**M**$_W$ groups (quantity, P=.04; postweaning, P=.02). Plasma insulin levels did not differ between the groups. Plasma leptin levels were significantly lower in the pea and turkey groups than in the milk groups (source, P=.0002).

3.5. Offspring: DSS induced a specific pattern of gene expression in the liver

TG levels in the liver (%) were significantly higher in the NP**M**$_W$ than the DSS postweaning groups (NP**M**$_G$-NP**M**$_W$, 12.1%±1.1%; HP**M**$_G$-NP**M**$_W$, 9.5%±1.2%; NP**M**$_G$-DSS, 7.0%±1.1%; HP**M**$_G$-DSS, 6.6%±1.1%; NP**P**$_G$-DSS, 6.0%±0.9%; HP**P**$_G$-DSS, 5.9%±0.5%; NP**T**$_G$-DSS, 5.3%±0.8%; HP**T**$_G$-DSS, 7.9%±0.7%; postweaning, P<.0001).

The results concerning liver gene expression are shown in Table 6. The expression of glucokinase (*Gck*) and pyruvate kinase (*Pklr*) decreased significantly in the DSS groups (postweaning, P≤.005). The expression of fatty acid synthase (*Fas*) (postweaning, P=.01), acetyl-CoA carboxylase (*Acc*) (postweaning, P<.05) and the sterol regulatory element-binding transcription factor 1 isoform c (*Screbf1c*) (postweaning, P=.005) also diminished significantly in the DSS groups, but no effect was found for that of stearoyl-CoA desaturase 1 (*Scd1*). The expression of phosphoenolpyruvate carboxykinase (*Pepck*) increased in the DSS groups (postweaning, P=.0005) and particularly in NP**M**$_G$-DSS and HP**M**$_G$-DSS (source, P=.03). That of the glucose-6-phosphatase catalytic subunit 1 (*G6pc1*) increased in the DSS groups (postweaning, P=.02) and notably in the NP gestation groups (quantity, P<.05). Insulin receptor (*Ir*) and insulin receptor substrate 1 (*Irs1*) expression in the liver did not differ between the diet groups (data not shown).

Table 4
Offspring body composition and characteristics after weaning

Group name Pups	NPM$_G$-NPM$_W$ n=8	NPM$_G$-DSS n=8	NPP$_G$-DSS n=8	NPT$_G$-DSS n=8	HPM$_G$-NPM$_W$ n=8	HPM$_G$-DSS n=8	HPP$_G$-DSS n=8	HPT$_G$-DSS n=8	*P* *Quantity*	*Source*	*Postweaning*	*Source × quantity*	*Quantity × postweaning*
Weight													
BW on PND28 (g)	79.9±3.6^{a}	79.0±3.6^{b}	47.5±1.8^{b,c}	60.0±1.6^{b,c}	69.8±4.2^{b,c}	74.5±2.8^{b,c}	50.7±2.0^{c}	60.6±1.9^{b,c}	.48	**<.0001**	.43	.37	.14
Final BW on PND70 (g)	237.6±6.7^{a,c}	237.9±11.2^{a,c}	198.8±4.7^{b,c}	193.8±6.2^{b}	234.3±5.9^{a,c}	247.9±4.9^{a}	199.9±2.9^{b,c}	192.7±8.0^{b}	.91	**<.0001**	.33	.76	.12
Final BW gain on PND70 (g)	157.7±4.7^{a,b,c}	158.9±9.8^{a,b,c}	151.2±3.5^{a,b,c}	133.8±4.3^{b,c}	164.6±3.8^{a,c}	173.4±4.6^{a}	149.2±2.9^{a,b,c}	132.1±6.9^{b}	.52	**<.0001**	.38	.26	.19
Adipose tissue													
VAT: final BW (%)	7.7±0.5	9.2±0.6	6.9±0.6	6.9±0.2	9.0±0.6	10.2±0.8	8.1±0.7	7.5±0.7	.15	**.003**	**.0007**	.91	.72
SAT: final BW (%)	5.2±0.3	6.3±0.5	4.8±0.4	5.0±0.3	6.4±0.6	7.1±0.4	6.5±0.6	6.1±0.4	**.006**	**.04**	**.002**	.87	.61
Adipocytes													
Average diameter (μm)	69.5±1.8^{a,b}	75.2±6.7^{a,b}	59.5±2.5^{a,b}	62.0±2.5^{a,b}	76.5±4.5^{a}	72.3±4.7^{a}	63.1±1.5^{a,b}	52.4±5.2^{b}	.78	**.0003**	.79	.31	.20
Average weight (μg)	0.16±0.01	0.23±0.06	0.10±0.1	0.12±0.01	0.23±0.04	0.20±0.04	0.12±0.01	0.08±0.03	1	**.0005**	.47	.64	.07
Number (x10^{8})	1.2±0.13^{a}	1.2±0.3^{a}	2.0±0.2^{a}	2.0±0.3^{a}	1.1±0.3^{a}	1.1±0.2^{a}	1.7±0.3^{a}	4.6±1.1^{b}	.06	**<.0001**	1	**.0007**	1

Data were tested using statistical model 2. Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. ^{a,b,c} Mean values with dissimilar superscript letters differed significantly between the groups (P<.05). BW, weight; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

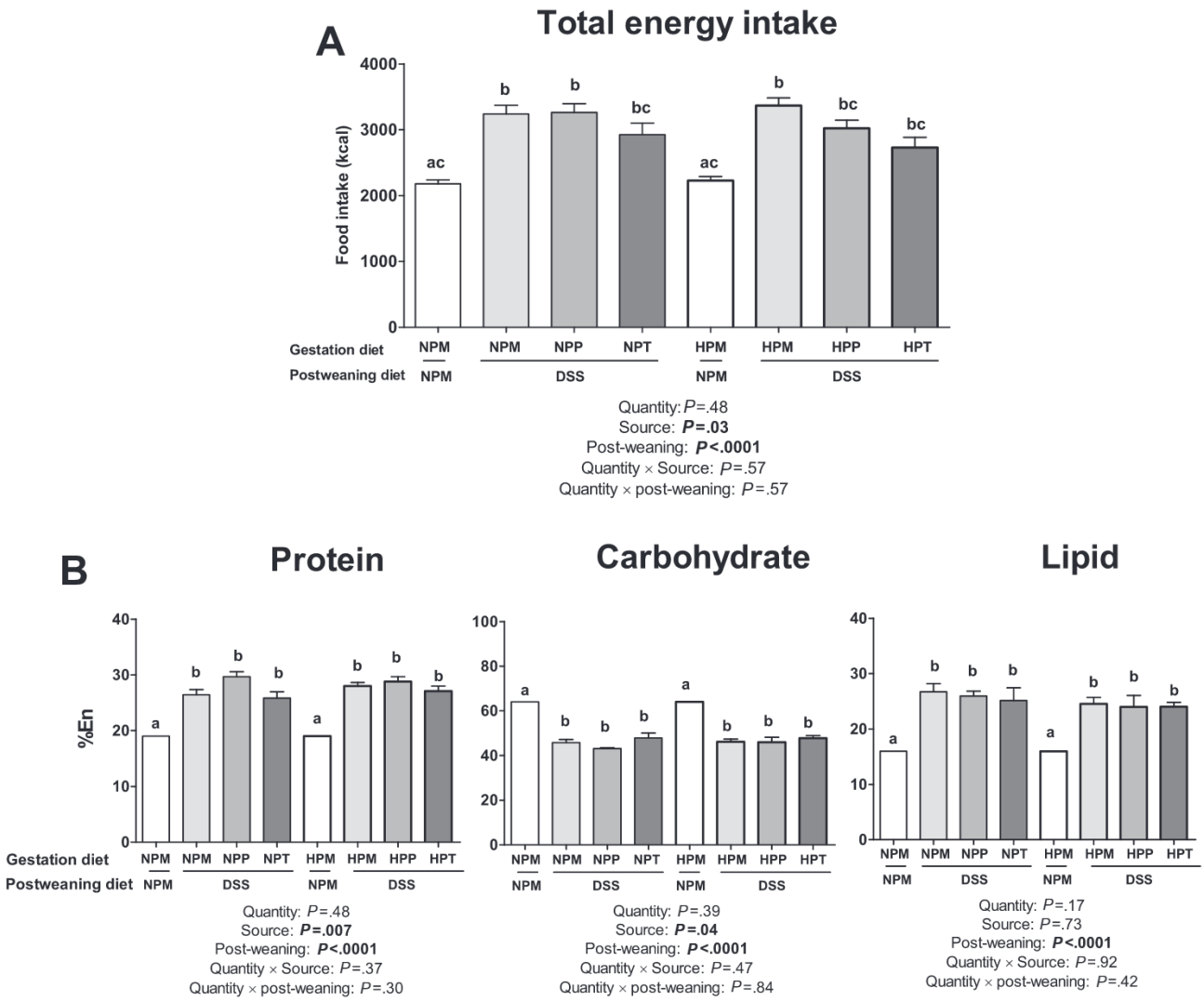

Fig. 4. Total food intake (kcal) on PND70 (A). Protein, carbohydrate and lipid intake (% of total energy intake) on PND70 (B). Data were tested using statistical model 2. Quantity: protein quantity during gestation factor. Source: protein source during gestation and lactation factor. Data are means±S.E.M. [a,b,c] Mean values with different superscript letters were significantly different ($P<.05$).

4. Discussion

The present study in rats used two different levels of protein (NP_G and HP_G) and three different protein sources (milk, pea and turkey proteins) to evaluate the potential impact of both the quantity and quality of protein given to dams during gestation and lactation on offspring health. For this purpose, phenotypic parameters and risk factors for metabolic disorders were determined in female offspring permitted to self-select their macronutrients (DSS) after weaning. The results showed that (1) exposure to an HP diet during gestation resulted in higher adiposity in female adult offspring and (2) exposure to different qualities of protein during gestation and lactation affected the weight gain of pups from birth to adulthood and their final adiposity.

In dams, groups receiving an HP diet during gestation *versus* an NP diet had higher protein but lower energy intake (mean of all HP_G *versus* NP_G). During lactation rather than gestation, food intake in dams was increased around twofold despite the fact that when all groups received an NP diet during lactation, groups that had been under an HP diet rather than an NP diet during gestation also had a lower energy intake. At birth, the litters from dams that had received pea proteins during gestation were smaller and lighter, but overall, all pups from the different dam diet groups had normal and similar birth weights, suggesting normal fetal growth trajectories in the different experimental groups [30]. However, after birth, the quality of protein given to the dams during pup's early life notably during lactation affected the weight gain of pups, resulting in a lower weight gain among pups from the turkey and pea dam groups compared to the milk dam groups. Notably, pup weight gain was markedly lower in the pea diet groups at the end of lactation.

Under the experimental design, pups were subjected to a DSS option after weaning, *versus* a control NP**M**$_W$ diet. Regardless of the maternal protein source, the former increased their protein intake by 26–30 En%, increased their lipid intake by 24–27 En% and decreased their carbohydrate intake by 43–48 En%, resulting in an increased total energy intake overall. These results on food intake obtained in 10-week-old pups under the DSS option agreed with previous observations made on 15-week-old weaned female pups also put on DSS [13,31]. In the present work, the DSS option indicated no preference by female offspring during the postweaning period for the protein source consumed by their mother and to which they had been exposed *in utero* (during gestation) and postnatally during lactation.

Table 5
Final protein choice on PND70 in response to DSS

Group name Pups	NPM_G-NPM_W *n*=8	NPM_G-DSS *n*=8	NPP_G-DSS *n*=8	NPT_G-DSS *n*=8	HPM_G-NPM_W *n*=8	HPM_G-DSS *n*=8	HPP_G-DSS *n*=8	HPT_G-DSS *n*=8	*P*				
									Quantity	*Source*	*Postweaning*	*Source × quantity*	*Quantity × postweaning*
Final milk protein intake* (% EI)	19**	7.3±0.6[a]	9.9±0.7[a,b]	10.1±0.7[a,b]	19**	9.7±0.9[a,b]	9.5±0.8[a,b]	10.9±0.8[b]	.14	**.03**	X	.17	X
Final pea protein intake* (% EI)	-	10.5±0.7	10.1±0.7	8.3±0.7	-	9.5±0.5	9.9±1.0	9.3±0.5	.93	.17	X	.40	X
Final turkey protein intake* (% EI)	-	8.7±0.4[a,b,c]	9.7±0.4[c]	7.4±0.3[a,b]	-	8.9±0.7[a,b,c]	9.4±0.6[a,c]	6.9±0.3[b]	.56	<**.0001**	X	.74	X

Data were tested using statistical model 3. Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. [a,b,c] Mean values with different superscript letters differed significantly between the groups (*P*<.05). EI, energy intake.
* Protein intakes were determined from the consumption of the food cups of **NPM** (milk protein diet), **HPP** (pea protein diet) or **HPT** (turkey protein diet).
** Quantity (%) of milk protein is indicated in the table but was not tested in the model because groups fed the **NPM** postweaning diet were not allowed to choose other protein-derived diets.

When protein quality in the maternal diet is adequate, food intake and protein levels must meet nutritional needs in humans [16] and rats [15]. But some compositional variations may occur [32] when the protein quality is poorer, as shown in the present study regarding the lower content of a large part of EAA in pea and turkey proteins compared to milk proteins. Indeed, probably because of their different amino acid compositions, the different protein sources directly affected both the EAA intake of dams (resulting in a lower EAA intake with pea and turkey compared to milk proteins during both gestation and lactation) and the lower methionine–cysteine intake with pea rather than milk and turkey proteins during gestation. Next, because of differences between the lipid compositions of the diets, a lower PUFA intake was seen in the HP**T**$_G$ group during gestation, and a lower intake of PUFA n-6 and n-3 was seen with the diets containing pea protein (and particularly in the HP**P**$_G$ group) during lactation. In lactating female mice, other studies had shown that a low milk protein diet (10 En%) compared to a normal- (20 En%) [33] or high-protein diet [18] induced a higher energy intake. This effect was also observed in response to a maternal diet of poor quality (low-protein quantity or quality diets) [34, 35]. It has also been shown previously that variations in the quantities of dietary fatty acids in the maternal diet during lactation modify milk fatty acid levels [16], which may impact the growth of pups [19, 20].

Interestingly, whatever the maternal protein source supplied during gestation and lactation, all adult female offspring from the HP gestation groups had higher total and subcutaneous adiposity, associated with smaller adipocytes in periovarian adipose tissue. These results agreed with the findings of studies in rats which revealed that an HP diet during gestation impacted offspring health by increasing their adiposity at weaning [11], at adult stage [9], during adulthood [10, 13, 36] and in response to a postweaning high-fat diet [10, 13].

Adipose tissue growth occurs through two mechanisms: hyperplasia (increase of cell number) and hypertrophy (increase of cell size). A study reported that cell size variation was linked to diet and cell number variation was linked to both genetics and diet [37]. In our work, measure of cell-size distribution showed that the higher adiposity in the HP gestation groups was associated with a higher amount of smaller adipocytes in periovarian adipose tissue. This increased small cell size rate could lead to a higher fat storage capacity and thus the adipose tissue expansion [38]. Interestingly, a human study showed that a higher proportion of small cells was associated with higher ratio of visceral to total fat [39]. Then, the number of adipocytes in our HP**T**$_G$-DSS group was markedly higher than in all the other groups. Fatty acids from the maternal diet are known to be transferred through the placenta, and an excess of saturated fats together with a deficiency of PUFA n-3 appears to be a promoter of adipocyte proliferation in offspring [40].

During the present study, gene expression in the livers of animals in the DSS groups revealed a reduction of glycolysis and lipogenesis from glucose and an increase in gluconeogenesis in the DSS groups (rise in *Pepck* and *G6pc1* expression) that could be associated with the lower intake of carbohydrates and higher intake of protein and fat. However, *G6pc1* expression increased less markedly in HP gestation groups, suggesting lower glycogen storage in the liver [41], in a way that was unrelated to changes in insulin signaling. In a previous study on the effects of an HP diet on liver metabolism, dysregulation of the insulin signaling pathways due to a reduction in insulin receptor and insulin receptor substrate 1 gene expression was shown on PND105 [13], whereas such a dysregulation was not observed during the present animal study on PND70.

5. Conclusion

In conclusion, this study shows that the quantity and quality of maternal protein ingested during the perinatal period affect weight gain and adiposity in female offspring during lactation and after

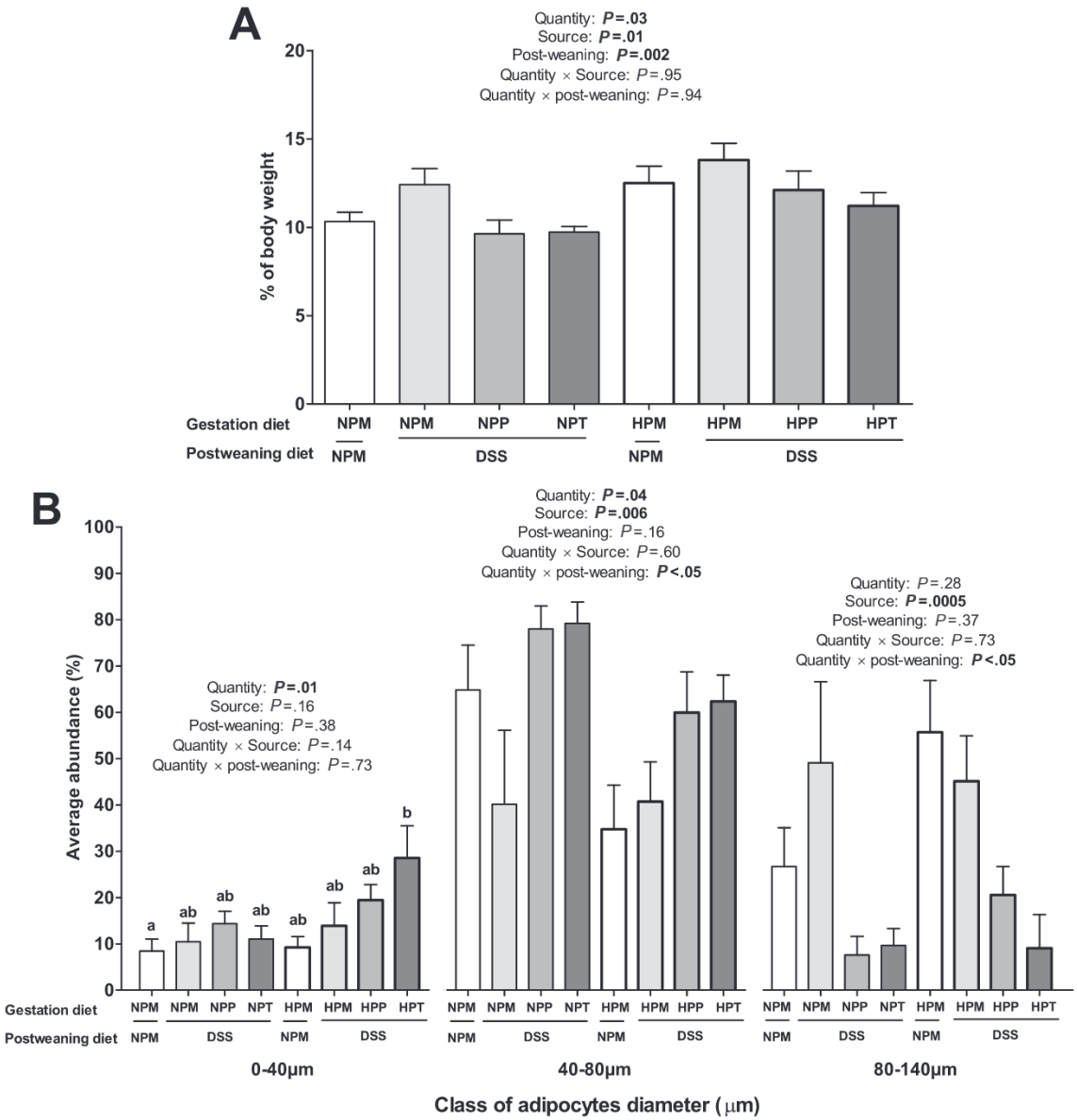

Fig. 5. Total adiposity (% of body weight) (A) and adipocyte characteristics (classes by diameter) on PND70 (B). Data were tested using statistical model 2. Quantity: protein quantity during gestation factor. Source: protein source during gestation and lactation factor. Data are means±S.E.M. [a,b] Mean values with different superscript letters differed significantly between the groups ($P<.05$).

weaning. The EAA profile of the protein source is presumed to play a crucial role in the growth of offspring [14]. After weaning, offspring from milk groups gained more weight than other groups, and the offspring from the pea groups appeared to recover growth better than those in the turkey groups. Our results suggested that maternal methionine–cysteine intake could be involved. A previous study notably showed that rats born from dams fed a low-protein diet that was supplemented with methyl donors (including methionine) had a lower weight gain when fed a hypercaloric diet compared with offspring born from the control group [42]. However, another work revealed that increasing the methionine supplement in the low-protein diet retards the growth of the fetus and thus affects the mature adult body weight [43]. Either way, it was clearly reported that both LP and HP diets, through the low or the excess of amino acid amount, can impair the development of the offspring [44]. Regardless of protein quality, the quantity of maternal protein affected the development of adiposity in adult female offspring. Also, a high maternal protein intake came with a low maternal carbohydrate intake which seems to have played a crucial role in the metabolic homeostasis demonstrated by the increased adiposity in the offspring. In previous studies on similar experimental models, the dysregulation of glucose homeostasis [12] and insulin signaling [13] were incriminated in the development of metabolic risk associated with excessive body weight. Early life, especially prenatal, exposure to maternal nutrition can induce persistent metabolic and physiological changes in fetus by modifications of epigenetic profiles, leading notably to chronic disorders such as obesity [45, 46]. Epigenetic mechanisms play a key role in regulating tissue specific gene expression [22, 47]. Several works studied the relationship between maternal protein diet and epigenetic modifications. These investigations allowed to identify DNA methylation or histone modification, targeting notably gene promoters, in different organs (liver, placenta, kidney and pancreas) [48]. Methyl donors, such as methionine, in the maternal protein diet can be incriminated in the DNA methylation changes [49]. Alteration

Table 6
Metabolite and hormone levels in fed and fasting plasma and gene expression in the liver on PND70

Group name		NPM_G-NPM_W	NPM_G-DSS	NPP_G-DSS	NPT_G-DSS	HPM_G-NPM_W	HPM_G-DSS	HPP_G-DSS	HPT_G-DSS	*P*				
Pups		*n*=8	*n*=8	*n*=8	*n*=8	*n*=8	*n*=8	*n*=8	*n*=8	*Quantity*	*Source*	*Postweaning*	*Source × quantity*	*Quantity × postweaning*
Metabolites in plasma														
Triglycerides	Fasted	1.64±0.11[a]	1.15±0.10[b]	1.11±0.04[b]	1.06±0.06[b]	1.12±0.06[b]	1.10±0.14[b]	1.07±0.10[b]	1.10±0.09[b]	**.04**	.84	**.006**	.87	**.007**
(mmol/L)	Fed	1.91±0.28	2.61±0.63	2.22±0.46	2.81±0.43	2.03±0.31	3.51±0.47	2.70±0.37	3.12±0.40	.20	.34	**.008**	.82	.36
Glucose	Fasted	85.5±2.3	89.4±5.2	83.0±4.1	75.6±4.7	89.9±3.8	88.0±4.3	83.6±3.2	81.4±2.5	.43	<**.05**	.84	.67	.48
(mg/dL)	Fed	160.5±4.4	170.4±6.4	166.3±5.0	172.9±5.1	149.4±7.3	165.9±6.3	157.9±7.0	159.2±4.8	**.04**	.58	**.02**	.74	.58
Insulin	Fasted	0.22±0.06	0.17±0.08	0.23±0.07	0.07±0.04	0.21±0.06	0.23±0.09	0.30±0.14	0.12±0.03	.51	.15	.83	.99	.74
(μg/L)	Fed	1.11±0.36	1.25±0.35	1.03±0.47	1.32±0.34	0.71±0.08	0.80±0.13	1.02±0.30	0.65±0.13	0.09	0.99	0.68	0.55	.95
Leptin	Fasted	5102.6±844.8	7020.0±1403.3	4430.0±765.2	3905.9±821.2	7285.4±1292.6	10,243.9±1993.2	6737.9±1887.1	4595.9±874.8	<**.05**	.004	<**.05**	.65	0.64
(pg/mL)	Fed	5766.4±1550.1	6125.0±620.8	2594.7±426.1	2975.5±223.2	5974.4±1359.5	6814.1±1139.7	3833.0±748.4	2893.8±728.6	.41	**.0002**	.57	.75	.86
*mRNA levels in the liver**														
Gck		1.00±0.17	0.64±0.09	0.65±0.08	0.55±0.09	0.96±0.18	0.55±0.15	0.68±0.11	0.68±0.05	.95	.85	**.0008**	.64	.64
Pklr		1.00±0.11[a]	0.76±0.15[a,b]	0.40±0.05[b]	0.48±0.04[b]	0.74±0.10[a]	0.54±0.08[b]	0.65±0.08[a,b]	0.51±0.05[a,b]	.60	.33	**.005**	.09	.62
Fas		1.00±0.31	0.57±0.15	0.36±0.12	0.36±0.08	0.67±0.21	0.31±0.043	0.46±0.07	0.41±0.12	.71	**.03**	**.0005**	.88	.56
Acc		1.00±0.16	0.83±0.18	0.51±0.06	0.57±0.05	0.78±0.11	0.52±0.06	0.72±0.09	0.59±0.07	.30	.63	<**.05**	**.04**	.72
Scref1c		1.00±0.13	0.75±0.076	0.67±0.11	0.65±0.06	1.03±0.19	0.67±0.07	0.61±0.09	0.63±0.09	.67	.76	**.005**	.95	.60
Scd1		1.00±0.33	0.92±0.46	0.21±0.05	1.78±0.80	2.94±1.3	1.49±0.79	3.046±1.9	0.59±0.29	.18	.89	.40	.13	.48
Pepck		1.00±0.15	1.93±0.37	1.15±0.23	1.05±0.19	0.86±0.16	2.12±0.59	1.54±0.42	1.05±0.26	.71	**.03**	**.0005**	.88	.56
G6pc1		1.00±0.30	2.00±0.55	1.99±0.39	1.57±0.33	0.89±0.27	1.31±0.28	1.32±0.27	1.00±0.08	<**.05**	.44	**.02**	.99	.42

Data were tested using statistical model 2. Quantity: protein quantity during gestation factor. Source: protein source during gestation factor. Data are means±S.E.M. [a,b] Mean values with different superscript letters differed significantly between the groups ($P<.05$). GCK, glucokinase; PKLR, liver pyruvate kinase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; SCD1, stearoyl-CoA desaturase 1; SREBF1C, sterol regulatory element-binding transcription factor 1 isoform c; PEPCK, phosphoenolpyruvate carboxykinase; G6PC1, glucose-6-phosphatase catalytic subunit 1.

* Gene expression is presented as an arbitrary unit using the NP**M**$_G$-NP**M**$_W$ group as the reference sample (gene expression=1).

of these epigenetic processes can be incriminated in the sensitivity of adiposity development in the HP offspring in our study. Then, the present work did not investigate gender-specific impact of maternal protein quantity and quality ingested during the perinatal period. Focus on female offspring after a maternal dietary intervention from early gestation revealed programming effects. However, focus on male offspring could lead to different consequences that can be explained by gender-dependent response to the window of maternal dietary intervention [28].

Acknowledgments

We would like to thank Morgane Dufay, Joséphine de Beauregard and Armelle Garcia for their technical assistance.

Funding

INRA's DID'IT metaprogram and the ALIAS project managed by INRA and AgroParisTech provided support for the PhD grant and partial financial support for the experimental work. Danone Nutricia Research (DNR) offered partial support for the experimental work.

Declarations

All authors read and approved the final manuscript. Andrea Kodde, Bert J.M. van de Heijning and Eline M. van der Beek are employees of DNR.

Authors' contributions

G.C., C.C., N.D., A.K., B.v.d.H., D.T. and A.-M.D. designed the research; G.C., C.C., C.D. and A.-M.D. conducted experiment and data collection; G.C. and P.B. performed statistical analysis of data; G.C., C.C., F.B., E.M.v. d.B., A.K., B.v.d.H., D.T. and A.-M.D. wrote the paper; G.C. and A.-M.D. had primary responsibility for the final content, and all the authors read and approved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnutbio.2019.108333.

References

[1] Barker DJ. The fetal and infant origins of adult disease. BMJ 1990;301:1111.
[2] Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71:1344s–52s.
[3] Muhlhausler BS, Adam CL, Findlay PA, Duffield JA, McMillen IC. Increased maternal nutrition alters development of the appetite-regulating network in the brain. FASEB J 2006;20:1257–9. https://doi.org/10.1096/fj.05-5241fje.
[4] Remacle C, Bieswal F, Bol V, Reusens B. Developmental programming of adult obesity and cardiovascular disease in rodents by maternal nutrition imbalance. Am J Clin Nutr 2011;94:1846S–52S. https://doi.org/10.3945/ajcn.110.001651.
[5] Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? Programming the metabolic syndrome by maternal dietary imbalance. J Physiol 2004;561:355–77. https://doi.org/10.1113/jphysiol.2004.072009.
[6] Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. American Journal of Physiology-Endocrinology And Metabolism 2000;279:E83–7.
[7] Bellinger L, Lilley C, Langley-Evans SC. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. Br J Nutr 2004;92:513. https://doi.org/10.1079/BJN20041224.
[8] Zambrano E, Bautista CJ, Deás M, Martínez-Samayoa PM, González-Zamorano M, Ledesma H, et al. A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat: maternal low protein alters offspring growth and metabolism. J Physiol 2006;571:221–30. https://doi.org/10.1113/jphysiol.2005.100313.
[9] Daenzer M, Ortmann S, Klaus S, Metges CC. Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. J Nutr 2002;132:142–4.
[10] Hallam MC. Reimer RA. A maternal high-protein diet predisposes female offspring to increased fat mass in adulthood whereas a prebiotic fibre diet decreases fat mass in rats. Br J Nutr 2013;110:1732–41. https://doi.org/10.1017/S0007114513000998.
[11] Vanselow J, Kucia M, Langhammer M, Koczan D, Metges CC. Maternal high-protein diet during pregnancy, but not during suckling, induced altered expression of an increasing number of hepatic genes in adult mouse offspring. Eur J Nutr 2015. https://doi.org/10.1007/s00394-015-0906-1.
[12] Desclee de Maredsous C, Oozeer R, Barbillon P, Mary-Huard T, Delteil C, Blachier F, et al. High-protein exposure during gestation or lactation or after weaning has a period-specific signature on rat pup weight, adiposity, food intake, and glucose homeostasis up to 6 weeks of age. Journal of Nutrition 2016;146:21–9. https://doi.org/10.3945/jn.115.216465.
[13] Carlin G, Chaumontet C, Blachier F, Barbillon P, Darcel N, Blais A, et al. Maternal high-protein diet during pregnancy modifies rat offspring body weight and insulin signalling but not macronutrient preference in adulthood. Nutrients 2019;11:17. https://doi.org/10.3390/nu11010096.
[14] Jahan-Mihan A, Rodriguez J, Christie C, Sadeghi M, Zerbe T. The role of maternal dietary proteins in development of metabolic syndrome in offspring. Nutrients 2015;7:9185–217. https://doi.org/10.3390/nu7115460.
[15] Keen CL, Lönnerdal B, Clegg M, Hurley LS. Developmental changes in composition of rat milk: trace elements, minerals, protein. Carbohydrate and Fat The Journal of Nutrition 1981;111:226–36. https://doi.org/10.1093/jn/111.2.226.
[16] Ballard O, Morrow AL. Human milk composition. Pediatr Clin North Am 2013;60:49–74. https://doi.org/10.1016/j.pcl.2012.10.002.
[17] Innis SM. Impact of maternal diet on human milk composition and neurological development of infants. Am J Clin Nutr 2014;99:734S–41S. https://doi.org/10.3945/ajcn.113.072595.
[18] Pine AP, Jessop NS, Oldham JD. Maternal protein reserves and their influence on lactational performance in rats 3. The effects of dietary protein restriction and stage of lactation on milk composition. Br J Nutr 1994;72:815. https://doi.org/10.1079/BJN19940087.
[19] Oosting A, Verkade HJ, Kegler D, van de Heijning BJM, van der Beek EM. Rapid and selective manipulation of milk fatty acid composition in mice through the maternal diet during lactation. Journal of Nutritional Science 2015;4. https://doi.org/10.1017/jns.2015.13.
[20] Bautista CJ, Montaño S, Ramirez V, Morales A, Nathanielsz PW, Bobadilla NA, et al. Changes in milk composition in obese rats consuming a high-fat diet. Br J Nutr 2016;115:538–46. https://doi.org/10.1017/S0007114515004547.
[21] Millward DJ, Layman DK, Tomé D, Schaafsma G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am J Clin Nutr 2008;87:1576S–81S. https://doi.org/10.1093/ajcn/87.5.1576S.
[22] Wu G, Bazer FW, Cudd TA, Meininger CJ, Maternal Nutrition Spencer TE, Development Fetal. Recent advances in nutritional. Sciences 2004;9:2169–72.
[23] Rees WD, Hay SM, Brown DS, Antipatis C, Palmer RM. Maternal protein deficiency causes hypermethylation of DNA in the livers of rat fetuses. J Nutr 2000;130:1821–6. https://doi.org/10.1093/jn/130.7.1821.
[24] Richter CK, Skulas-Ray AC, Champagne CM, Plant Protein Kris-Etherton PM. Animal proteins: do they differentially affect cardiovascular disease risk? Advances in Nutrition 2015;6:712–28. https://doi.org/10.3945/an.115.009654.
[25] Jahan-mihan A, Smith CE, Hamedani A, Anderson GH. Soy protein-based compared with casein-based diets fed during pregnancy and lactation increase food intake and characteristics of metabolic syndrome less in female than male rat offspring. Nutr Res 2011;31:644–51. https://doi.org/10.1016/j.nutres.2011.08.003.
[26] Maslova E, Rytter D, Bech BH, Henriksen TB, Rasmussen MA, Olsen SF, et al. Maternal protein intake during pregnancy and offspring overweight 20 y later. American Journal of Clinical Nutrition 2014;100:1139–48. https://doi.org/10.3945/ajcn.113.082222.
[27] Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nutr 1993;123:1939–51. https://doi.org/10.1093/jn/123.11.1939.
[28] Kalisch-Smith JI, Simmons DG, Dickinson H, Review Moritz KM. Sexual dimorphism in the formation, function and adaptation of the placenta. Placenta 2016;54:10–6. https://doi.org/10.1016/j.placenta.2016.12.008.
[29] Foucault A-S, Mathé V, Lafont R. Even P, Dioh W, Veillet S, et al. Quinoa extract enriched in 20-hydroxyecdysone protects mice from diet-induced obesity and modulates adipokines expression. Obesity 2012;20:270–7. https://doi.org/10.1038/oby.2011.257.
[30] Sandovici I, Hoelle K, Angiolini E, Constância M. Placental adaptations to the maternal–fetal environment: implications for fetal growth and developmental programming. Reprod Biomed Online 2012;25:68–89. https://doi.org/10.1016/j.rbmo.2012.03.017.
[31] Jean C, Fromentin G, Tomé D, Larue-Achagiotis C. Wistar rats allowed to self-select macronutrients from weaning to maturity choose a high-protein, high-lipid diet. Physiol Behav 2002;76:65–73.
[32] Davis TA, Nguyen HV, Garcia-Bravo R, Fiorotto ML, Jackson EM, Lewis DS, et al. Amino acid composition of human milk is not unique. J Nutr 1994;124:1126–32. https://doi.org/10.1093/jn/124.7.1126.
[33] Derrickson EM, Lowas SR. The effects of dietary protein levels on milk protein levels and postnatal growth in laboratory mice (Mus musculus). J Mammal 2007;88:1475–81. https://doi.org/10.1644/06-MAMM-A-253R2.1.

[34] Sampson DA, Hunsaker HA, Jansen GR. Dietary protein quality, protein quantity and food intake: effects on lactation and on protein synthesis and tissue composition in mammary tissue and liver in rats. J Nutr 1986;116:365–75. https://doi.org/10.1093/jn/116.3.365.
[35] Moretto VL, Ballen MO, Gonçalves TSS, Kawashita NH, Stoppiglia LF, Veloso RV, et al. Low-Protein diet during lactation and maternal metabolism in rats. ISRN Obstetrics and Gynecology 2011;2011:1–7. https://doi.org/10.5402/2011/876502.
[36] Thone-Reineke C. High-protein nutrition during pregnancy and lactation programs blood pressure, food efficiency, and body weight of the offspring in a sex-dependent manner. AJP: regulatory, integrative and comparative. Phys Ther 2006;291:R1025–30. https://doi.org/10.1152/ajpregu.00898.2005.
[37] Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, et al. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLoS Comput Biol 2009;5:11.
[38] Jo J, Shreif Z, Periwal V. Quantitative dynamics of adipose cells. Adipocyte 2012;1: 80–8. https://doi.org/10.4161/adip.19705.
[39] Kursawe R, Eszlinger M, Narayan D, Liu T, Bazuine M, Cali AMG, et al. Cellularity and adipogenic profile of the abdominal subcutaneous adipose tissue from obese adolescents: association with insulin resistance and hepatic steatosis. Diabetes 2010;59:2288–96. https://doi.org/10.2337/db10-0113.
[40] Kabaran S, Besler HT. Do fatty acids affect fetal programming? J Health Popul Nutr 2015;33. https://doi.org/10.1186/s41043-015-0018-9.
[41] Rui L. Energy metabolism in the liver. In: Terjung R, editor. Comprehensive physiology. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2014. p. 177–97. https://doi.org/10.1002/cphy.c130024.
[42] Giudicelli F, Brabant A-L, Grit I, Parnet P, Amarger V. Excess of methyl donor in the perinatal period reduces postnatal leptin secretion in rat and interacts with the effect of protein content in diet. PLoS ONE 2013;8:e68268. https://doi.org/10.1371/journal.pone.0068268.
[43] Rees WD, Hay SM, Cruickshank M. An imbalance in the methionine content of the maternal diet reduces postnatal growth in the rat. Metabolism 2006;55:763–70. https://doi.org/10.1016/j.metabol.2006.01.012.
[44] Herring CM, Bazer FW, Johnson GA. Wu G. Impacts of maternal dietary protein intake on fetal survival, growth, and development. Exp Biol Med 2018;243: 525–33. https://doi.org/10.1177/1535370218758275.
[45] Li Y. Epigenetic mechanisms link maternal diets and gut microbiome to obesity in the offspring. Front Genet 2018;9. https://doi.org/10.3389/fgene.2018.00342.
[46] Li S, Chen M, Li Y, Tollefsbol TO. Prenatal epigenetics diets play protective roles against environmental pollution. Clin Epigenetics 2019;11. https://doi.org/10.1186/s13148-019-0659-4.
[47] Symonds ME, Sebert SP, Hyatt MA, Budge H. Nutritional programming of the metabolic syndrome. Nat Rev Endocrinol 2009;5:604–10. https://doi.org/10.1038/nrendo.2009.195.
[48] Lee H-S. Impact of maternal diet on the epigenome during in utero life and the developmental programming of diseases in childhood and adulthood. Nutrients 2015;7:9492–507. https://doi.org/10.3390/nu7115467.
[49] McGee M, Bainbridge S. Fontaine-Bisson B. A crucial role for maternal dietary methyl donor intake in epigenetic programming and fetal growth outcomes. Nutr Rev 2018;76:469–78. https://doi.org/10.1093/nutrit/nuy006.