Endocrine (2019) 63:62–69
https://doi.org/10.1007/s12020-018-1719-9

ORIGINAL ARTICLE

Maternal low-protein diet during lactation combined with early overfeeding impair male offspring's long-term glucose homeostasis

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Received: 8 May 2018 / Accepted: 10 August 2018 / Published online: 20 August 2018

Abstract

Purpose The early-life nutritional environment affects long-term glucose homeostasis, we investigated the effects of maternal low-protein diet combined with postnatal early overfeeding on the male offspring's glucose homeostasis in adulthood.

Methods Only male rats were used, and their delivery was considered postnatal-day 0 (PN0). Wistar rats' dams were divided into control (NP) or low-protein diet (LP). LP dams remained on the diet until PN14, after which all animals were supplied with the control diet. At PN2, litters were adjusted to 9 (control-NL) or 3 (postnatal-overfeeding-PO) pups, resulting in four experimental groups: NP-NL, NP-PO, LP-NL, and LP-PO. Litters were weaned on PN21. At PN80, a batch of animals from all experimental groups underwent surgery for cannula implantation, followed by intravenous glucose tolerance test (ivGTT), but the insulinogenic index (ISI) was calculated. At PN81, animals were euthanized and tissues were collected.

Results LP-diet and early postnatal-overfeeding were effective in promoting the expected biometric outcomes at PN21 and PN81, but the LP-PO animals present a biometric profile similar to the control (NP-NL) group. Postnatal-overfeeding increased fasting glycemia in LP-PO animals ($p < 0.01$). In the ivGTT, postnatal-overfeeding elevated the glycemia ($p < 0.0001$), exacerbated in LP-PO animals ($p < 0.0001$). Insulinemia was reduced by both, maternal LP-diet and postnatal-overfeeding, with a higher degree of reduction in LP-PO animals ($p < 0.0001$). Maternal LP-diet and postnatal-overfeeding reduced the ISI ($p < 0.0001$). Factors interaction lead the LP-PO to a lower ISI compared to all other groups ($p < 0.0001$).

Conclusions The combination of low-protein diet in breastfeeding dams with postnatal overfeeding disturbed the offspring's glucose metabolism in adulthood.

Keywords DOHaD · Maternal diet · Early postnatal overfeeding · Metabolic programming · Glucose homeostasis

Introduction

The current prevalence of non-communicable diseases, such as diabetes and obesity, have an overwhelming impact on the health of the population and on the demands on Public Health Systems across the globe. The Developmental Origins of Health and Disease (DOHaD) concept helps us to better understand the current situation, as epidemiological and experimental evidences point to the connection between individuals' early-life environment and their metabolic health in later life [1]. The postulation of the "thrifty phenotype" hypothesis by Hales and Barker in the early 90s is a milestone for the DOHaD concept that caught the attention of the scientific community by showing the link between the

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fetal and infant growth and later life impaired glucose tolerance in adulthood [2].

The postnatal early life plays a critical role for the determination of the metabolic health later in life, and the nutrition of mothers and their offspring during the lactation period has long-term effects on the latter's metabolism. Perinatal and infancy undernutrition is still a major public health concern, mainly for developing countries, where many of the newborns and infants are exposed to famine and malnutrition that leads to health risks, such as brain damage and insulin resistance, and other illnesses not all completely understood, and in extreme cases, to precocious death [3]. Additionally, experimental research has shown that undernutrition during the suckling phase impairs glucose homeostasis and autonomic nervous system function in the adult life [4]. Malnutrition during the early stages of lactation is reported to cause hypoinsulinemia and β-cell malfunction, and the administration of a poor protein diet to lactating dams during the first two-thirds of the lactation is an alternative experimental model to study early-life undernourishment during lactation and its lasting metabolic outcomes [5, 6].

On the other hand, obesity rates have increased in different population cohorts, including women of fertile age and children [7]. Early-life overnutrition, in turn, is associated with later hyperphagia, increased body weight (bw) and fat accumulation, and basal hyperinsulinemia [8]. Rodents' litter size reduction is a well-established experimental model to study early-life rapid weight gain by overnutrition and its metabolic consequences, which has been shown to induce animals, in later life, to overweight, hyperinsulinemia, and metabolic syndrome [9].

Although not all completely understood, it has been demonstrated that the mechanisms underlying how the early nutrition impacts long-term metabolism, especially the glucose homeostasis, are potentially based on closely related, nevertheless distinct, developmental pathways. The evidences suggests that gestational and early postnatal undernutrition will have greater implications for the offspring's metabolism, mainly when they face an energy-rich environment in later life. The mismatched conditions to the poor nutritional environment in early life create a situation in which the organism, metabolically "programmed" to cope with low food availability, fails to properly adapt to the energy-rich environment. On the contrary, the perinatal overnutrition developmental pathway seems to be related to alterations in the adipogenesis and/or eating behavior and appetite control, which creates the conditions for later chronic eating, obesity, and its pathophysiological consequences [10].

Notwithstanding the knowledge of the mechanisms that operates in early-life metabolic programming, there is still a need to better understand the possible consequences of perinatal nutritional challenges in different food environments. Epidemiological studies have shown high incidence levels of poor-quality diet in the very early food environment. Moreover, a significant association exists between food insecurity without hunger and altered bw in young children, even in developed countries [11, 12], which corroborated by the socioeconomic disparities in food quality driven by the elevated costs of healthier diets [13], contributes to an elevated consumption of a poor-quality diet in early life, especially for low-income families. Therefore, the elucidation of the consequences of different patterns of early-life nutrition on long-term health remains a major target for scientific research.

As demonstrated by studies that compared humans who were breastfed at infancy vs. those who were formula-fed, the long-term effects may depend on the quality and quantity of the neonatal nutrition [1]. The paradoxical relationship between food quality and quantity during early life, which seems to be often overlooked in experimental studies, may have lasting implications for the individuals' future metabolical health. Although both experimental models, perinatal undernutrition and early overnutrition, have been important to elucidate the metabolic programming mechanisms and their long-term consequences, they seem not to properly address the metabolic response from an early environment nutritional "quality vs. quantity" perspective.

We hypothesize that a poor-quality diet offered in large quantity during the suckling period may have a lasting impact on the individuals' glucose homeostasis. In this study, we combined a maternal low-protein diet with early postnatal overfeeding to investigate the effects of a poor-quality–large-quantity diet during the lactation period on the male offspring's later-life glucose homeostasis.

Methods

All the experimental protocols conducted in the present study comply with the Brazilian Association for Animal Experimentation guidelines and with Brazilian Federal Law. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Animals and diet

Wistar rats were obtained from the Central Animals Facility —Maringá State University, and housed and maintained in a sectorial laboratory animals house under standard conditions of light (12-h dark/12-h light cycle) and temperature (22° ± 2° C), with ad libitum access to standard chow and water. After an adaptation period of 5 days, the animals were placed in cages to mate, in a proportion of 3 females to 1 male, for 10 days. The breeding period was followed by

Table 1 Composition of the control and low-protein diets

Diet components	Control diet		Low-protein diet	
	g kg^{-1}	kJ kg^{-1}	g kg^{-1}	kJ kg^{-1}
Sucrose	127.2	2.129	200.0	3.347
Cornstarch	527.5	8.828	642.5	10.753
Casein (88% protein)	233.3	3.905	45.5	0.761
Mix of mineral salts[a]	32.0	–	32.0	–
Mix of vitamins[a]	16.0	–	16.0	–
Soybean oil	48.0	1.807	48.0	1.807
Fish oil	16.0	0.602	16.0	0.602
Total	1000.0	17.272	1000.0	17.272

[a]The mineral salts and vitamins mixture that was used in the manufactured diet followed the AIN-93 recommendation

The dietary component values are presented as g kg^{-1} of diet and the energy in kJ kg^{-1}

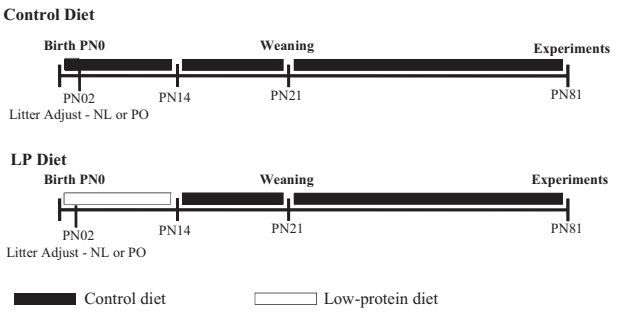

Fig. 1 Experimental model timeline: Delivery was considered PN0, when the diet—NP (normal protein—control) or LP (low-protein) were introduced. At PN2, litters from both diet dams were adjusted to either nine pups for normal litters (NL) or three pups for postnatal-overfeeding (PO) groups. The LP group of dams was maintained on this diet for the first 2 weeks (14 days) of the lactation period, and then switched to standard chow in the last week of lactation. The four groups' litters were weaned on PN21 and maintained on commercial chow (Nuvital®, Curitiba/PR, Brazil) until PN81, when the experiments were conducted

pregnant females' isolation until natural delivery, which was considered the postnatal-day 0 (PN0). On PN0, the dams were randomly assigned into two groups, the first group was supplied with a control balanced diet (normal protein—NP—23.3% of protein), while the second group was supplied with a low-protein (LP—4.5% of protein) isocaloric diet (Table 1). The other components of the normal and low-protein diets, such as the mixtures of vitamins and salts, followed the recommendations of the AIN-93 for laboratory rodents [14, 15]. The LP group of dams was maintained on this diet for the first 2 weeks (14 days) of the lactation period, and then switched to standard chow in the last week of lactation [5]. At PN2, litter sizes were adjusted to either nine pups for normal litters (NL) or three pups for postnatal-overfeeding (PO) groups (Fig. 1). Therefore, the experiments consisted of four groups: normal protein diet—normal litter (NP-NL), normal protein diet—postnatal overfeeding (NP-PO), low-protein diet—normal litter (LP-NL), and low-protein diet—postnatal overfeeding (LP-PO). The total number of dams used in this study were six dams for NP-NL and LP-NL; and eight dams for the NP-PO and LP-PO groups. Only male pups were used and cross-fostering (between same diet dams) was made to complete the number of male pups for the normal litters on PN2 when needed.

Food intake

The dams' food intake was monitored and estimated during the lactation period (PN0–PN21). The four groups' litters were weaned on PN21 by placing three animals per cage and maintaining them on commercial chow (Nuvital®, Curitiba/PR, Brazil). Spillage was not measured and an estimate of food intake (Fi) per rat per day was calculated by determining the difference between the amount of chow remaining (Df) and the total amount of food that was previously placed in the cage (Di). The food consumption was assessed weekly. Food intake (Fi) values were calculated as the difference between the amount of food remaining (final diet, Df) and the total food available (initial diet, Di), divided by the number of days and the number of rats in the cages: (Fi $(g) = $ Df–Di/7/1 for the lactation period; and, Fi $(g) = $ Df–Di/7/3 for the rats after weaning). The area under the curve (AUC) for the food consumption vs. time was calculated for the entire observation period: PN0–PN21 for the dams and PN21–PN80 for the offspring.

Biometric measurements

To assess the effects of the LP diet and litter size manipulation during the suckling phase, maternal food intake was estimated (as described) and bw was monitored during the lactation phase; data are presented as the AUC values for the period PN0 to PN21. In addition, the bw of the litters was also assessed at weaning (PN21). At PN81, after an overnight fast, a batch of animals from all groups ($n = 17$ from 6 litters for NP-NL; $n = 10$ from 4 litters for NP-PO, $n = 10$ from 4 litters for LP-NL; and $n = 11$ from 4 litters for LP-PO group), which had not undergone surgery, were weighed and euthanized via quick decapitation. Fat from the periepididymal pads was dissected and weighed, the value was correlated with the bw of each rat, and was calculated as g/100 kg bw.

Fasting glycemia and fasting insulinemia assessments, intravenous glucose tolerance test (ivGTT), and insulinogenic index (ISI) calculation

On PN80, a batch of animals from all groups ($n = 12$ animals per group, from 4 different litters) were anesthetized

Table 2 The effects of low-protein diet and litter size reduction in the dams estimated food intake (AUC) and body weight evolution (AUC) during the lactation period (PN0–PN21); and the effect of maternal (low-protein) diet and postnatal early overfeeding in the litters' body weight at weaning (PN21); estimated food intake from PN21 to PN80; body weight and periepididymal fat accumulation at PN81

	Dams AUC-Fi (PN0–PN21)	Dams AUC-BW (PN0–PN21)	Litters' body weight PN21 (g)	AUC food intake (21–81 days old)	Final body weight (g)	Periepididymal fat pad (g/100 g of bw)
NP-NL^{A}	5723 ± 117^{BCD}	6074 ± 132^{BCD}	42.4 ± 2.7^{BC}	$51{,}135 \pm 1087^{BC}$	328.02 ± 8.47^{BC}	0.64 ± 0.02^{BC}
NP-PO^{B}	3522 ± 113^{ACD}	5426 ± 127^{ACD}	65.8 ± 4.89^{ACD}	$59{,}514 \pm 1006^{ACD}$	362.90 ± 7.13^{ACD}	0.92 ± 0.06^{ACD}
LP-NL^{C}	2310 ± 216^{AB}	4518 ± 112^{AB}	19.6 ± 2.1^{ABD}	$43{,}274 \pm 3121^{AB}$	279.50 ± 3.42^{AB}	0.49 ± 0.02^{ABD}
LP-PO^{D}	2351 ± 129^{AB}	4626 ± 108^{AB}	35.1 ± 3.11^{BC}	$49{,}248 \pm 1707^{B}$	304.60 ± 4.46^{B}	0.64 ± 0.04^{BC}
Maternal diet	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.01$	$p < 0.001$	$p < 0.0001$
Litter size	$p < 0.0001$	$p < 0.05$	$p < 0.0001$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$
Interaction	$p < 0.0001$	$p < 0.01$	ns	ns	ns	ns

The values are presented as the mean ± SEM. The *n* of dams are NP-NL = 6, NP-PO = 8, LP-NL = 6, and LP-PO = 8; the offsprings' *n* are NP-NL = 17 (6 litters); NP-PO = 10 (6 litters); LP-NL = 10 (4 litters); and LP-PO = 11 (4 litters). Maternal diet and litter size (postnatal early overfeeding) were considered as the source of variation factors, *p*-value (<0.05) indicates significant effect and ns indicates not significant effect. Letters (A, B, C, and D) indicate the post-test results when the difference among groups was significant ($p < 0.05$)

using a ketamine and xylazine (3 and 0.6 ml/kg of bw) solution, prior to the surgical implantation of a silicone cannula into the right jugular vein, attached to the dorsal region of the neck. The animals were given a day for recovery from surgery. At PN81, after an overnight fast, the animals received a glucose infusion (1 g/kg of bw) through the cannula, followed by blood samples collected via a cannula. The first sample was collected immediately before the glucose infusion (time 0′), and was used for fasting glucose and fasting insulin assessment. Glucose infusion was then followed by blood samples collection at 5, 15, 30, and 60 min after infusion. The samples were centrifuged and the plasma was collected. Blood glucose concentration was determined using the glucose oxidase method [16] with a commercial kit (Gold Analisa®, Belo Horizonte, Brazil). To determine the insulin levels, the radioimmunoassay method was used coupled to a gamma counter (Wizard^{2} Automatic Gamma Counter-2470, PerkinElmer, Shelton, CT, USA) as previously described [14]. The intra-assay and inter-assay coefficients of variation for insulin were 4.25 and 7.30, respectively, and the limit of detection was 1.03 pmol l^{-1}. The ISI (defined as the increment of the insulin (λI)/increment of glucose (λG) in the IVGTT) was calculated by dividing the increase of plasma insulin concentration (λ Insulin) after glucose infusion relative to the fasting plasma insulin concentration (time 0′) by the corresponding increase of blood glucose concentration (λ Glucose) during the glucose tolerance tests [17, 18].

Statistical analysis

The results are given as mean ± SEM. The data were subjected to the D'Agostino-Pearson normality test and then analyzed by two-way ANOVA (source of variation factors: maternal diet and litter size) followed by Tukey's multiple comparisons post test. Mean differences were considered statistically significant at $p < 0.05$. Data analyses were performed using GraphPad Prism v6.01 (GraphPad Software Inc., San Diego, CA, USA).

Results

Both the LP diet and litter size reduction decreased food intake (LP diet $p < 0.0001$; litter size $p < 0.0001$) and bw development (LP diet $p < 0.0001$; litter size $p < 0.05$) of dams during the suckling phase (Table 2). As the bw at weaning (PN21, Table 2) shows the maternal LP diet during lactation reduced the litters' bw ($p < 0.0001$), while reduction in the litter size increased bw ($p < 0.0001$). The post test shows no significant difference between the control (NP-NL) and the LP-PO rats' bw at weaning. As indicated by the AUC for the food intake after weaning (Table 2), the

Table 3 The effects of the maternal (low-protein) diet and early postnatal overfeeding (by litter size) in the glycemic homeostasis of male adult rats (PN81)

	Fasting glycemia (mmol/L)	Fasting insulinemia (pmol/L)	λ Glycemia (mM)	λ Insulinemia (pmolM)	Insulinogenic index (λI/λG)
NP-NL^{A}	6.438 ± 0.3211	831.500 ± 68.65^{BC}	277.50 ± 14.20^{BCD}	105,850 ± 4537^{BCD}	400.90 ± 8.54^{BCD}
NP-PO^{B}	6.427 ± 0.3017	1148.333 ± 31.6^{ACD}	413.00 ± 22.70^{AD}	47,039 ± 2506^{ACD}	130.60 ± 9.07^{ACD}
LP-NL^{C}	5.505 ± 0.2380^{D}	591.333 ± 70.78^{AB}	393.80 ± 27.74^{AD}	65,639 ± 4158^{ABD}	182.40 ± 5.35^{ABD}
LP-PO^{D}	7.373 ± 0.2828 ^{C}	740.667 ± 51.13^{B}	558.50 ± 26.94^{ABC}	28,723 ± 2642^{ABC}	56.14 ± 5.42^{ABC}
Maternal diet	ns	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Litter size	$p < 0.01$	$p < 0.001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Interaction	$p < 0.01$	ns	ns	$p < 0.05$	$p < 0.0001$

The values are presented as the mean ± SEM. $n = 12$ rats per group from four different litters. Maternal diet and litter size were considered as the source of variation factors, p-value (<0.05) indicates significant effect, and ns indicates not significant effect. Letters (A, B, C, and D) indicate the post-test results when the difference among groups was significant ($p < 0.05$)

maternal low-protein diet significantly reduced the offspring's food consumption ($p < 0.01$), while the postnatal overfeeding increased the litters' food intake ($p < 0.001$), but no significant interaction was observed. Bw at PN81 was reduced by the low-protein diet ($p < 0.001$) and was increased by postnatal overfeeding ($p < 0.0001$). Periepididymal fat accumulation at PN81 showed a similar pattern, i.e., it was reduced by the low-protein diet ($p < 0.0001$) and increased by postnatal overfeeding ($p < 0.0001$). The biometric characteristics of the NP-NL control group and the LP-PO rats were not significantly different on adult life (Table 2).

Table 3 shows that although fasting glycemia at PN81 was not significantly affected by the maternal low-protein diet, postnatal overfeeding had a significant effect ($p < 0.01$). Only the LP-PO animals showed elevated glycemia ($p < 0.01$), reflecting a significant interaction between maternal diet and litter size ($p < 0.01$). Fasting insulinemia was reduced by maternal low-protein diet ($p < 0.0001$) and increased by postnatal overfeeding ($p < 0.001$), but there was no significant interaction between maternal diet and litter size. The ISI was reduced by both the maternal low-protein diet ($p < 0.0001$) and postnatal overfeeding ($p < 0.0001$), with a strong interaction among factors ($p < 0.0001$), which resulted in a lower ISI in LP-PO animals compared with that in all the other groups (Table 3).

Figure 2a shows the results of the ivGTT test, which consist of the glycemia pattern within 60 min of glucose infusion (time 0′). The maternal low-protein diet affected the AUC of glycemia ($p < 0.0001$) in a group-dependent way. Early overfeeding increased the AUC of glycemia ($p < 0.0001$), which was exacerbated in LP-PO animals ($p < 0.0001$), reflecting a significant interaction between maternal diet and litter size factors ($p < 0.0001$) (Fig. 2b).

Figure 3a shows the insulinemia behavior during the ivGTT test. The maternal low-protein diet reduced the AUC of insulinemia ($p < 0.0001$). Early overfeeding also reduced the AUC of insulinemia ($p < 0.0001$). This pattern was more evident in LP-PO animals ($p < 0.0001$), reflecting a significant interaction between maternal diet and litter size factors ($p < 0.0001$) (Fig. 3b).

Discussion

This study shows that different nutritional disturbances during the suckling phase, whether or not they are combined, may affect the long-term outcomes of the offspring's glucose metabolism, depending on the diet composition (quality) and availability (quantity). Moreover, this, to our knowledge, is the first study to show that the combination of a maternal low-protein diet with increased milk availability, which resembles a poor diet provided at a large quantity during lactation, severely impairs the offspring's glucose homeostasis in adult life, even though it did not necessarily result in an obese profile.

Maternal bw and food consumption during the lactation, as with the litters' bw at weaning (Table 2) shows that both regimens, LP maternal diet and postnatal early overfeeding, were effective in promoting the expected biometric effects. The rats from undernourished dams presented lower bw at weaning, while the postnatal overfed animals were obese at the same age, as previously observed [19, 20]. Interestingly, although the combination of LP diet and litter size reduction affected LP-PO dams' food intake and bw changes, the LP-PO offspring exhibited a bw, similar to the control (NP-NL) group.

The bw patterns observed at PN21 were maintained in adult life (PN81, Table 2). The offspring of malnourished breastfeeding mothers, when not in a mismatched environment, has been reported to feed less, weigh less, and accumulate less fat [21]. An opposite phenotype has been observed in early postnatal-overfeeding rats, which are commonly hyperphagic, overweight, and accumulate more fat than those reared on normal litters [19]. Accordingly, in our study, we observed that each of these early-life

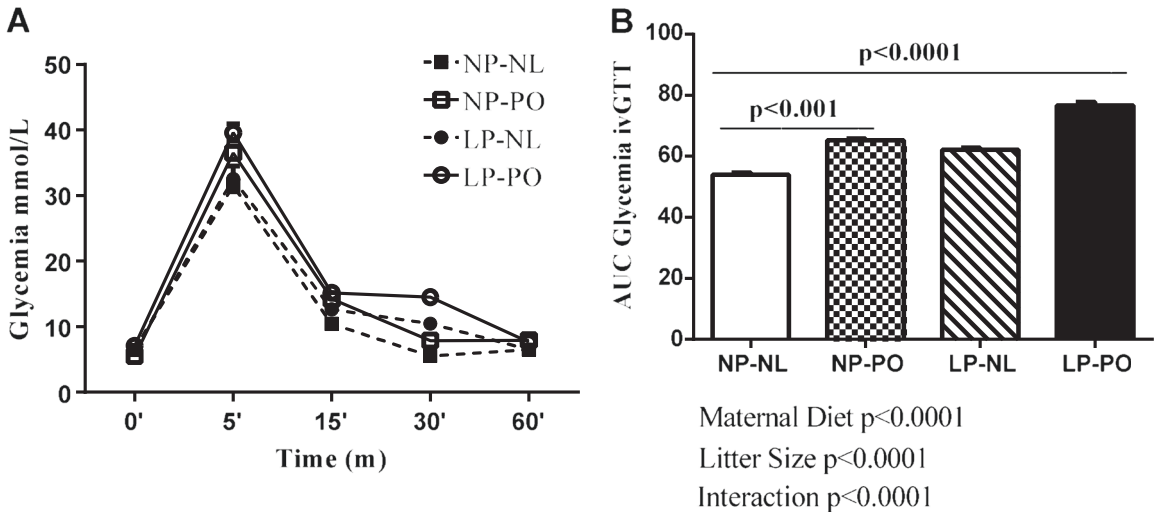

Fig. 2 **a** Blood glucose levels during the ivGTT at PN81. Data are presented as the mean ± SEM for the glycemia assessment ($n = 12$ per group, from four different litters). **b** Area under the curve for glycemia in ivGTT: source of variations—maternal diet and litter size; post-test differences among groups are presented as the level of significance (when $p < 0.05$) inside the figure

nutritional treatments, i.e., maternal low-protein diet and postnatal early overfeeding, were able to influence the food consumption on offspring throughout life, affecting bw and fat accumulation of young adult rats.

It is worth noting that the combination of treatments led to animals with a biometric profile similar to the control group in adult life, as the estimated food consumption or the biometric measures of the LP-PO group did not differ with the control group (NP-NL). Interestingly, although obesity is strongly predictive for cardiovascular risk in men with metabolic syndrome, undetected type 2 diabetes is more prevalent among those men whose metabolic syndrome was not associated with obesity [22]. This indicates that although obesity is often related to the metabolic programming of impairment for glucose homeostasis, there are metabolic effects, especially those related to glucose homeostasis, that occur even without obesity.

Offspring from prenatally undernourished dams and those that are exposed to postnatal early overfeeding by reduced litter size have been reported to be hyperphagic when compared with postnatally overfed rats from control dams [23], showing that the control of food consumption may be affected by both, prenatal and postnatal environments, mainly under mismatched conditions. In the present study, a large quantity of the low-protein food offered during the lactation period did not result in hyperphagia in the LP-PO group. Considering this result, we suggest that food quality may also play a role in the development of the mechanisms that control food satiety, aside from early food intake. A possible mechanism underlying the long-term control of food intake observed in the LP-PO group may include the increased availability of milk with a lower leptin concentration. An elegant study using malnourished dams in a cross-fostering model showed that maternal low-protein diet decreased milk leptin concentration, which was associated with the offspring's reduced plasma leptin levels throughout lactation [24]. Leptin is an adipokine known for its role in the regulation of food intake, among other processes during development, and its presence in breastmilk affects infant food intake and developmental neuroregulatory appetite systems [25].

Reusens and Remacle have pointed out that although there is a diversity of clues regarding the role of the perinatal environment in programming the endocrine pancreas, there is no unifying hypothesis that can explain why it is likely that different pathways can lead to the same later effects, i.e., impaired beta-cell function and diabetes [26]. In this study, although the postnatal overfeeding affected the fasting glycemia (Table 3), we did not observe differences in this parameter, except between the LP-NL and LP-PO groups, while basal insulinemia was elevated in PO offspring when compared with their respective controls. Ravelli and colleagues have shown that bottle-feeding babies are more susceptible to impaired glucose metabolism than those exclusively breastfed in the first week of life, suggesting that the quality of infant feeding is a determinant to glucose homeostasis in later life [27].

Undernourished offspring secretes lower levels of insulin that does not correspond to the glucose levels; nevertheless, these animals show high tissue insulin-sensitivity and they are able to maintain the glycemia homeostasis [4]. Notwithstanding, the present results for glycemia during the ivGTT show that the combination of low-quality food at high quantities during lactation exacerbated glucose intolerance in the LP-PO group. Notably, the LP-PO animals, when faced with a glucose challenge, presented with higher

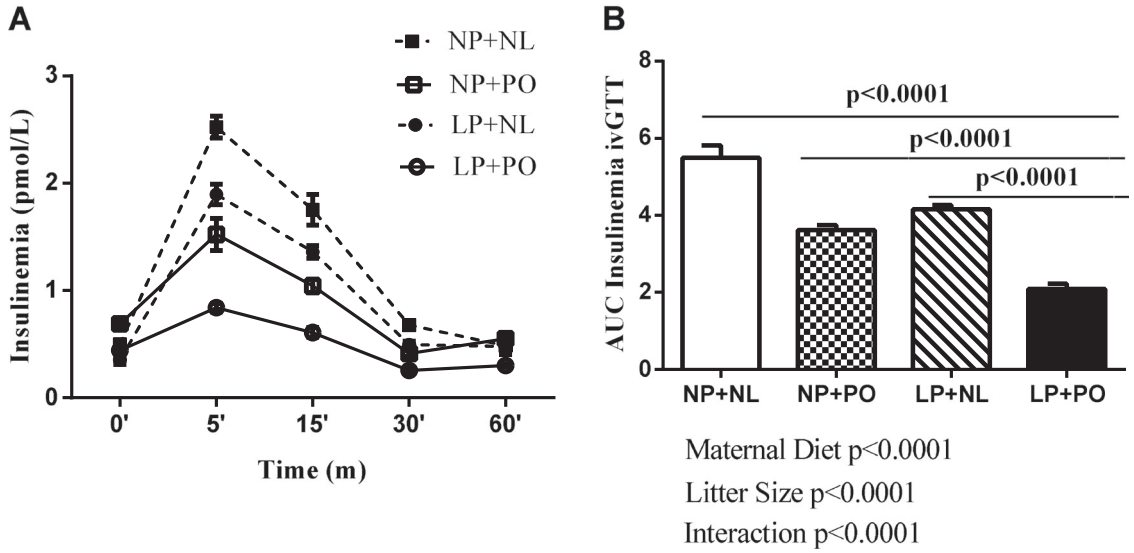

Fig. 3 **a** Blood insulin levels during the ivGTT at PN81. Data are presented as the mean ± SEM for the insulinemia assessment ($n = 12$ per group, from four different litters). **b** Area under the curve for insulinemia in ivGTT: source of variations—maternal diet and litter size; post-test differences among groups are presented as the level of significance (when $p < 0.05$) inside the figure

values for glycemia, accompanied by significantly lower insulin secretion compared with any other group. The ISI is a reliable index for beta-cell function; it is able to distinguish among subjects with different degrees of glucose intolerance [18]. In our study, both the maternal LP diet and postnatal overfeeding led to a reduced ISI value. However, as indicated by the ANOVA, the interaction (Table 3) between poor food quality with high availability in the diet leads to lower ISI values in LP-PO animals.

The current study shows that breastfeeding from an undernourished dam in larger quantities has deleterious effects later in life for the offspring's glucose metabolism. Although there is a lack of data regarding the quality of breastmilk, the literature shows that changes in milk composition may be a possible mechanism to explain the results observed in this study, as the protein content in the maternal diet has a potential to modify the developing regulatory systems of the offspring [28]. A recent study shows that a maternal low-protein diet is related to altered plasma amino acid levels in weaning rats [29]. Moreover, milk from undernourished dams is associated with a decrease in free amino acids with a lasting effect on the offspring's low insulin secretion [30]. Additionally, it has been shown that exclusively breastfed infants may develop excessive weight gain that appears to depend on inter-individual variations in the composition of the mother's milk [31], which, in light of the present results, reinforce the importance of maternal diet during the suckling period on the offspring's long-term metabolism.

In conclusion, we have shown that although the combination of maternal low-protein diet with postnatal overfeeding during lactation did not lead to an obese phenotype, glucose homeostasis in young adult offspring became severely impaired. Further studies will be needed to better understand the mechanisms underlying the role of quality vs. quantity of food consumption in early life on metabolic programming. Nevertheless, the disrupted glucose metabolism that was programmed by low-quality and high-quantity milk feeding during lactation may play an important role in metabolic disorders observed in different adult cohorts.

Funding The present study was financially supported by the Brazilian Federal Research Agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. A. Plagemann, T. Harder, K. Schellong, S. Schulz, J.H. Stupin, Early postnatal life as a critical time window for determination of long-term metabolic health. Best Pract. Res. Clin. Endocrinol. Metab. **26**(5), 641–653 (2012)
2. C.N. Hales, D.J. Barker, Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia **35**(7), 595–601 (1992)
3. Z. Abebe, D. Zelalem Anlay, B. Biadgo, A. Kebede, T. Melku, B. Enawgaw, M. Melku, High prevalence of undernutrition among

children in Gondar town, northwest Ethiopia: a community-based cross-sectional study. Int. J. Pediatr. **2017**, 5367070 (2017)
4. J.C. de Oliveira, S. Grassiolli, C. Gravena, P.C. de Mathias, Early postnatal low-protein nutrition, metabolic programming and the autonomic nervous system in adult life. Nutr. Metab. **9**(1), 80 (2012)
5. F.B. Barbosa, A.R. Medina, S.L. Balbo, P.C. de Freitas Mathias, Low protein diets administered to lactating rats affect in a time-dependent manner the development of young. Res. Commun. Mol. Pathol. Pharmacol. **106**(1-2), 63–76 (1999)
6. A.S. Moura, A.R. Carpinelli, F.B. Barbosa, C. Gravena, P.C. Mathias, Undernutrition during early lactation as an alternative model to study the onset of diabetes mellitus type II. Res. Commun. Mol. Pathol. Pharmacol. **92**(1), 73–84 (1996)
7. R.K. Masters, E.N. Reither, D.A. Powers, Y.C. Yang, A.E. Burger, B.G. Link, The impact of obesity on US mortality levels: the importance of age and cohort factors in population estimates. Am. J. Public Health **103**(10), 1895–1901 (2013)
8. A. Plagemann, K. Roepke, T. Harder, M. Brunn, A. Harder, M. Wittrock-Staar, T. Ziska, K. Schellong, E. Rodekamp, K. Melchior et al. Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J. Perinat. Med. **38** (4), 393–400 (2010)
9. S. Boullu-Ciocca, A. Dutour, V. Guillaume, V. Achard, C. Oliver, M. Grino, Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. Diabetes **54**(1), 197–203 (2005)
10. P.D. Gluckman, M.A. Hanson, Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int. J. Obes. **32**(Suppl 7), S62–S71 (2008)
11. E. Metallinos-Katsaras, A. Must, K. Gorman, A longitudinal study of food insecurity on obesity in preschool children. J. Acad. Nutr. Diet. **112**(12), 1949–1958 (2012)
12. A.L. Thompson, M.E. Bentley, The critical period of infant feeding for the development of early disparities in obesity. Soc. Sci. Med. **97**, 288–296 (2013)
13. N. Darmon, A. Drewnowski, Contribution of food prices and diet cost to socioeconomic disparities in diet quality and health: a systematic review and analysis. Nutr. Rev. **73**(10), 643–660 (2015)
14. A. Malta, E.G. de Moura, T.A. Ribeiro, L.P. Tofolo, L. Abdennebi-Najar, D. Vieau, L.F. Barella, P.C. de Freitas Mathias, P.C. Lisboa, J.C. de Oliveira, Protein-energy malnutrition at mid-adulthood does not imprint long-term metabolic consequences in male rats. Eur. J. Nutr. **55**(4), 1423–1433 (2016)
15. P.G. Reeves, F.H. Nielsen, G.C. Fahey Jr, 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. **123**(11), 1939–1951 (1993)
16. P. Trinder, Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol. **22**(2), 158–161 (1969)
17. Y. Oritani, T. Okitsu, E. Nishimura, M. Sai, T. Ito, S. Takeuchi, Enhanced glucose tolerance by intravascularly administered piceatannol in freely moving healthy rats. Biochem. Biophys. Res. Commun. **470**(3), 753–758 (2016)
18. A. Tura, A. Kautzky-Willer, G. Pacini, Insulinogenic indices from insulin and C-peptide: comparison of beta-cell function from OGTT and IVGTT. Diabetes Res. Clin. Pract. **72**(3), 298–301 (2006)
19. D.L. de Almeida, G.S. Fabricio, A.B. Trombini, A. Pavanello, L. P. Tofolo, T.A. da Silva Ribeiro, P.C. de Freitas Mathias, K. Palma-Rigo, Early overfeed-induced obesity leads to brown adipose tissue hypoactivity in rats. Cell. Physiol. Biochem. **32**(6), 1621–1630 (2013)
20. I.P. Martins, J.C. de Oliveira, A. Pavanello, C.C.I. Matiusso, C. Previate, L.P. Tofolo, T.A. Ribeiro, C.C. da Silva Franco, R.A. Miranda, K.V. Prates et al. Protein-restriction diet during the suckling phase programs rat metabolism against obesity and insulin resistance exacerbation induced by a high-fat diet in adulthood. J. Nutr. Biochem. **57**, 153–161 (2018)
21. F.B. Barbosa, K. Capito, H. Kofod, P. Thams, Pancreatic islet insulin secretion and metabolism in adult rats malnourished during neonatal life. Br. J. Nutr. **87**(2), 147–155 (2002)
22. P. Pajunen, A. Kotronen, E. Korpi-Hyovalti, S. Keinanen-Kiukaanniemi, H. Oksa, L. Niskanen, T. Saaristo, J.T. Saltevo, J. Sundvall, M. Vanhala et al. Metabolically healthy and unhealthy obesity phenotypes in the general population: the FIN-D2D Survey. BMC Public Health **11**, 754 (2011)
23. M.H. Vickers, B.H. Breier, D. McCarthy, P.D. Gluckman, Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Am. J. Physiol. Regul. Integr. Comp. Physiol. **285**(1), R271–R273 (2003)
24. J.S. Wattez, F. Delahaye, L.F. Barella, A. Dickes-Coopman, V. Montel, C. Breton, P. Mathias, B. Foligne, J. Lesage, D. Vieau, Short- and long-term effects of maternal perinatal undernutrition are lowered by cross-fostering during lactation in the male rat. J. Dev. Orig. Health Dis. **5**(2), 109–120 (2014)
25. X. Casabiell, V. Pineiro, M.A. Tome, R. Peino, C. Dieguez, F.F. Casanueva, Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. J. Clin. Endocrinol. Metab. **82**(12), 4270–4273 (1997)
26. B. Reusens, C. Remacle, Programming of the endocrine pancreas by the early nutritional environment. Int. J. Biochem. Cell Biol. **38** (5-6), 913–922 (2006)
27. A.C. Ravelli, J.H. van der Meulen, C. Osmond, D.J. Barker, O.P. Bleker, Infant feeding and adult glucose tolerance, lipid profile, blood pressure, and obesity. Arch. Dis. Child. **82**(3), 248–252 (2000)
28. A. Jahan-Mihan, J. Rodriguez, C. Christie, M. Sadeghi, T. Zerbe, The role of maternal dietary proteins in development of metabolic syndrome in offspring. Nutrients **7**(11), 9185–9217 (2015)
29. A. Kabasakal Cetin, H. Dasgin, A. Gulec, I. Onbasilar, A. Akyol, Maternal low quality protein diet alters plasma amino acid concentrations of weaning rats. Nutrients **7**(12), 9847–9859 (2015)
30. A. Martin Agnoux, J.P. Antignac, C.Y. Boquien, A. David, E. Desnots, V. Ferchaud-Roucher, D. Darmaun, P. Parnet, M.C. Alexandre-Gouabau, Perinatal protein restriction affects milk free amino acid and fatty acid profile in lactating rats: potential role on pup growth and metabolic status. J. Nutr. Biochem. **26**(7), 784–795 (2015)
31. C. Saure, M. Armeno, C. Barcala, V. Giudici, C.S. Mazza, Excessive weight gain in exclusively breast-fed infants. J. Pediatr. Endocrinol. Metab. **30**(7), 719–724 (2017)