METABOLISM CLINICAL AND EXPERIMENTAL 61 (2012) 812–822

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Metabolism

www.metabolismjournal.com

Early nutritional changes induce sexually dimorphic long-term effects on body weight gain and the response to sucrose intake in adult rats

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ARTICLE INFO

Article history:
Received 24 August 2011
Accepted 5 November 2011

ABSTRACT

Long-term metabolic effects induced by early nutritional changes are suspected to differ between males and females, but few studies have analyzed both sexes simultaneously. We analyzed the consequences of neonatal nutritional changes on body weight (BW) and the adult response to a sucrose-enriched diet in both male and female rats. Litter size was manipulated at birth to induce over- and undernutrition (4 pups: L4; 12 pups: L12; 20 pups: L20). From 50 to 65 days of age, half of each group received a 33% sucrose solution instead of water. Serum leptin, insulin, and ghrelin levels were analyzed at day 65. At weaning, rats from L4 weighed more and those from L20 weighed less than controls (L12). Body weight was greater in L4 rats throughout the study and increased further compared with controls in adult life. L20 males ate less and gained less weight throughout the study, but L20 females had a significant catch-up in BW. Sucrose intake increased total energy consumption in all groups, but not BW gain, with L4 males and L4 and L20 females reducing weight gain. Yet, sucrose intake increased serum leptin levels, with this increase being significant in L4 and L20 males. Our results suggest that females are more capable than males of recuperating and maintaining a normal BW after reduced neonatal nutrition. Furthermore, increased sucrose intake does not increase BW, but could alter body composition as reflected by leptin levels, with the percentage of calories consumed in the form of sucrose being affected by sex and neonatal nutrition.

Author contributions: EF-M: study design, animal manipulation and control, hormone analyses, data collection and analysis, and writing of manuscript. MG: hormone analyses and data interpretation. CG-C: animal manipulation and control, and data interpretation. MAS-G: animal manipulation and control. LMF: study design. MT-S: study design and data interpretation. JA: study design, data interpretation, and writing of manuscript. JAC: study design, statistical analysis, data interpretation, and writing of manuscript.

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doi:10.1016/j.metabol.2011.11.003

1. Introduction

The rising incidence of obesity continues to be a major medical concern in most developed countries, and it is now clear that diverse factors are involved in this phenomenon. Changes in lifestyle and diet in recent decades certainly play an important role in the obesity epidemic, with the effects of these changes being multiple. It is now clear that both the prenatal and neonatal environments can have long-lasting influences on growth and development, as well as the later response to metabolic challenges [1-26]. Thus, these early developmental influences in combination with a poor diet or excess caloric intake and insufficient physical activity most likely contribute extensively to the increase in obesity and its secondary complications.

Endocrine functions, including metabolism, in the adult animal can be conditioned by developmental changes in nutrition, hormonal imbalances, and increased stress, with the exact timing of these changes also influencing the outcomes [9-12]. Moreover, males and females do not respond equally to many of these early changes [5,9,11,14,15-22]. However, the large majority of studies in the literature do not comparatively evaluate responses in both sexes, with most studies being performed only in males. Hence, it is not clear whether metabolic outcome in females is equally influenced by many of these early environmental changes.

Reduction in litter size is a relatively noninvasive experimental manipulation that results in increased consumption due to increased maternal milk availability. This induces increased weight gain and food intake in animals not only during nursing but also thereafter, with this being associated with hyperleptinemia and hyperinsulinemia [6,8,23,24]. Likewise, an increase in litter size can induce neonatal undernutrition, which also has long-lasting metabolic effects [10-12,25,26]. However, whether postweaning weight gain and metabolism are equally affected in males and females by changes in litter size is not clear.

The type of energy consumed differently influences weight gain, body composition, and metabolic imbalances [11,27-30]. As increased dietary fat intake is believed to be an important factor in the poor metabolic status of a large percentage of the population, the effect of high-fat diets on weight gain and metabolism has been an active area of investigation [2,5,8,11,13]. Increased sugar consumption is also thought to be associated with the current rise in obesity and its secondary complications [27-33]. However, studies reporting the outcomes of increased sugar intake on weight gain are conflicting, indicating that weight may be unaffected, increased, or even decreased [34]. These differences could be due to the different types of sugar used, the duration of the dietary intervention, the age at which the diet is introduced, or even the sex of the animals.

In the studies reported here, our aims were to first assess whether postnatal weight gain in male and female rats is similarly affected by modifications in litter size at birth and then to determine if adult weight continues to be affected equally in the 2 sexes. Furthermore, the subsequent response to increased sucrose intake in terms of body weight (BW) and levels of the metabolic hormones leptin, insulin, and ghrelin, and its interaction with sex and early nutrition, was analyzed at adulthood.

2. Materials and methods

2.1. Animals

All experiments were designed according to the European Union laws for animal care, and the study was approved by the local institutional ethical committee (Universidad Complutense de Madrid, Nº de Registro en la Dirección General de Agricultura y Alimentación, Consejería de Economía y Empleo de la Comunidad Autónoma de Madrid, EX 08-UCS.).

Adult Wistar rats were purchased from Harlan Interfauna Ibérica (Barcelona, Spain) and allowed to acclimate for 2 weeks before mating. One male was placed in a cage with 3 females (n = 15) for 2 days. Rats were maintained at a constant temperature (21°C ± 1°C) and humidity (50% ± 1%) in a 12-hour light-dark cycle (lights on at 7:30 AM) and given free access to rat chow (Panlab, Barcelona, Spain) and water.

2.2. Litter organization

Only mothers that gave birth to between 8 and 12 pups were used for the study (mean litter size: 9.5 ± 1.2 pups per litter). All litters were arranged on the day of birth, postnatal day (PND) 0. A total of 2 litters of 20 pups (L20), 3 litters of 12 pups (L12), and 8 litters of 4 pups (L4) with equal numbers of males and females in each litter were used. Cross-fostering was used, and the litters were arranged such that the mean birth weights did not differ between groups (males: L4: 6.04 ± 0.15 g, L12: 5.98 ± 0.09 g, L20: 6.16 ± 0.08 g; females: L4: 5.91 ± 0.15 g, L12: 5.78 ± 0.10 g, L20: 5.88 ± 0.09 g).

On PND21, pups were weaned by removing them from their mothers. They were then placed 2 per cage according to sex and litter size. All rats were allowed free access to food and water until PND50. This resulted in the following number of animals in each group before the diet change was introduced (males: L4: 16 rats, L12: 18 rats, L20: 20 rats; females: L4: 16 rats, L12: 18 rats, L20: 20 rats).

2.3. Diet change

On PND50, before introduction of sucrose into the diet, glycemia was measured with a glucometer in all rats via a venous tail puncture (Optium Plus, Abbot Diabetes Care, Witney Oxon, UK). Water was substituted for a solution of 33% sucrose in tap water in half of each experimental group, with the remaining half continuing to consume normal tap water. All rats were allowed to eat and drink ad libitum until being killed at 65 days of age. This experimental paradigm resulted in rats receiving sucrose for a period of 15 days (PND50-PND65). This period corresponds to early adulthood and was chosen to avoid pubertal hormonal changes, as well as influences of old age, on metabolism and growth. This resulted in the experimental groups shown in Table 1.

Throughout the study, rats were weighed weekly; and 24-hour food intake was determined once a week until PND50.

Table 1 – Description of the experimental groups used in the study and the total number of animals in each group

Litter size	Males		Females	
	Control (water)	Sucrose	Control (water)	Sucrose
4 pups per dam	L4 (n = 8)	L4S (n = 8)	L4 (n = 8)	L4S (n = 8)
12 pups per dam	L12 (n = 9)	L12S (n = 9)	L12 (n = 9)	L12S (n = 9)
20 pups per dam	L20 (n = 10)	L12S (n = 10)	L12 (n = 10)	L12S (n = 10)

On PND50, when the sucrose treatment was begun, mean food and liquid intake was assessed daily. Intake was measured by placing a specific quantity of food in the cage or liquid in the drinking bottle and measuring the remaining amount 24 hours later. The mean intake per cage was divided by the number of rats per cage, and this was used as an "n" of 1 for statistical analysis. Intake is expressed as mean grams of food per rat per day or mean milliliters of liquid per rat per day.

All rats were killed by decapitation between 9:00 AM and 11:00 AM under nonfasting conditions. Trunk blood was collected in cooled tubes and then centrifuged at 5000 rpm for 5 minutes at 4°C. Serum was stored at –80°C until hormone levels were measured.

2.4. *Determination of serum levels of total and acylated ghrelin by radioimmunoassay*

Total and acylated ghrelin was measured by radioimmunoassay following the manufacturer's instructions (Linco Research, St Charles, MO).

The sensitivity of the method was 93 pg/mL for both assays, and the intra- and interassay coefficients of variation were 6.4% and 16.3% for total ghrelin and 7.4% and 13.4% for acylated ghrelin, respectively.

2.5. *Determination of serum leptin and insulin levels by enzyme-linked immunosorbent assay*

Serum levels of these hormones were measured by enzyme-linked immunosorbent assay following the manufacturer's instructions (Linco Research). The sensitivity of the method for leptin and insulin was 0.04 and 0.2 ng/mL, respectively. Absorbance in each well was measured by using a Tecan Infinite M200 (Grödig, Austria), and serum leptin and insulin concentrations were calculated from the standard curve. All samples were run in duplicate and within the same assay for all analyses. The intraassay variation was 2.2% for leptin and 1.9% for insulin, and the interassay variation was 3.4% for leptin and 7.6% for insulin.

2.6. *Statistical analysis*

The program STATVIEW, version 5.0 (SAS Institute, Cary, NC), was used for data analysis. All data are presented as mean ± SEM. Before the introduction of sucrose into the diet on PND50, a 2-way analysis of variance (ANOVA) was used to analyze the effect and interaction of sex and litter size on the measured variables. Afterward, 3-way ANOVAs were used to determine the effect and interaction of litter size, sex, and sucrose on the variables analyzed. To analyze weight changes and food intake over time, ANOVAs with repeated measures were used. When significant effects were found by 3-way or 2-way analyses, a 1-way ANOVA followed by the Scheffe F test was used to determine where specific differences existed among the experimental groups. The results were considered statistically significant at $P < .05$. Only physiologically significant differences are represented in the figures.

3. Results

3.1. *Effect of litter size and sex on food intake and weight gain before addition of sucrose to the diet*

On PND21, when pups were removed from their mothers, there was an effect of litter size ($F_{2,86}$: 334.635; $P < .0001$) on BW, with no effect of sex. At this time, both males and females from L4 weighed more than those from L12 or L20; and those from L12 weighed more than those from L20 (Fig. 1A; 1-way ANOVA: $F_{5,94}$: 134.522; $P < .0001$).

From PND21 to PND50, before the change in diet, there was an effect of both sex ($F_{1,88}$: 42.242; $P < .0001$) and litter size ($F_{2,88}$: 5.411; $P < .005$) on mean daily chow intake. Males consumed significantly more than females regardless of litter size. Males from L4 and L12 consumed significantly more than those from L20. In females, there was no significant difference in food intake regardless of litter size (Fig. 1B; 1-way ANOVA: $F_{5,94}$: 10.789; $P < .0001$).

There was a significant difference in weight gain between males and females from PND21 to PND50, regardless of litter size and with males from L4 and L12 gaining significantly more weight than those from L20, with no difference between the female groups (Fig. 1C; 1-way ANOVA: $F_{5,92}$: 20.137; $P < .0001$).

At PND50, before receiving the diet change, there was an effect of sex ($F_{1,85}$: 80.643; $P < .0001$) and litter size ($F_{2,85}$: 16.121; $P < .0001$) on BW. Males weighed more than females from litters of the same size. However, weights were indistinguishable between males from large litters (20 pups) and females from small litters (4 pups). Males from L4 continued to weigh significantly more than those from L12 and L20; and L12, more than L20. Females from L4 continued to weigh more than those from L12 and L20, with no difference between L12 and L20 (Fig. 1D; 1-way ANOVA: $F_{11,87}$: 28.844; $P < .0001$).

3.2. *Effect of litter size and sex on glycemia before addition of sucrose to the diet*

Before the introduction of sucrose into the diet, there was an effect of sex on blood glucose levels, with males having higher levels than females ($F_{1,88}$: 11.067; $P < .002$). In rats from L4, males had higher glucose levels than females (1-way ANOVA: $F_{5,94}$: 2.692; $P < .02$; males: L4: 95.5 ± 3.4 mg/dL, L12: 95.9 ± 2.3 mg/dL, L20: 98.5 ± 2.9 mg/dL; females: L4: 87.6 ± 2.5 mg/dL, L12: 89.7 ± 1.8 mg/dL, L20: 92.2 ± 2.3 mg/dL).

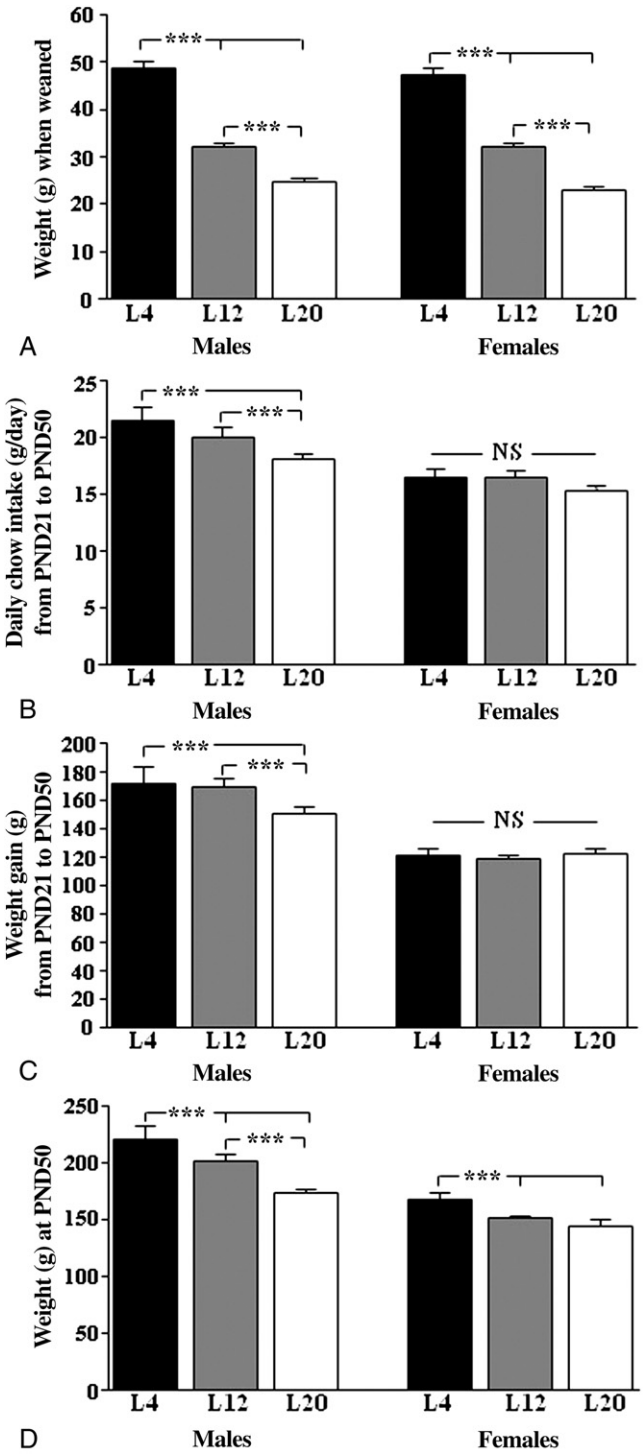

Fig. 1 – Mean BW at weaning (A), daily rat chow intake from weaning to 8 weeks of age (B), weight gain between weaning and 8 weeks of age (C), and weight at 8 weeks of age of male and female rats from litters of 4 pups (L4, n = 16 males, 16 females), 12 pups (L12, n = 12 males, 12 females), or 20 pups (L20, n = 20 males, 20 females). ***ANOVA P < .0001. NS indicates not significant.

3.3. *Effect of litter size, sex, and sucrose intake on food and liquid intake*

The mean daily intake of rat chow during the 2 weeks of diet change was affected by sex ($F_{1,88}$: 146.349; $P < .0001$), litter size ($F_{2,88}$: 3.6303; $P < .04$), and sucrose intake ($F_{1,88}$: 499.584; $P < .0001$), with an interaction between sex and sucrose intake ($F_{1,88}$: 33.796; $P < .03$). Males consumed more than females regardless of their litter size. In addition, males drinking sucrose also consumed more than females drinking sucrose. All rats drinking sucrose consumed significantly less rat chow than their controls regardless of litter size or sex. Males from L4 and L12 eating only chow continued to eat more than those from L20, whereas in females, litter size had no effect on food intake (Fig. 2A; 1-way ANOVA: $F_{11,88}$: 59,640; $P < .0001$).

The mean daily liquid intake during the 2 weeks of diet change was affected by litter size ($F_{2,88}$: 11.606; $P < .0001$) and sucrose availability ($F_{1,88}$: 22.654; $P < .0001$). Control males from L4 and L12 drank significantly more water than those from L20, whereas in control females, litter size had no effect. L4 males receiving sucrose drank significantly more than L12 and L20 males, whereas in females, both L4 and L12 rats drank more than L20 rats. All rats that were given sucrose water drank less than those drinking tap water, with this being significant in L12 males and L20 females (Fig. 2B; 1-way ANOVA: $F_{11,88}$: 5.347; $P < .0001$).

Kilocalories consumed from sucrose were affected by litter size ($F_{2,50}$: 8.494; $P < .0006$). There was no difference between the sexes in the total consumption of sucrose. L4 males consumed more kilocalories from sucrose than those from L12 or L20. Females from L4 and L12 ingested more kilocalories from sucrose than those from L20 (Fig. 2C; 1-way ANOVA: $F_{5,52}$: 3.647; $P < .007$).

There was an effect of sex ($F_{1,88}$: 90.929; $P < .0001$), litter size ($F_{2,88}$: 9.443; $P < .0002$), and sucrose intake ($F_{1,88}$: 90.429; $P < .0001$) on total kilocalories consumed during the 2 weeks of diet change. Males consumed more calories than females of the same litter size regardless of whether they ingested sucrose or not. In both sexes, all rats receiving sucrose ingested more calories than their controls. Control males from L4 consumed more calories than those from L20, as did L4 males receiving sucrose compared with L20 receiving sucrose. In females, there was no effect of litter size on rats eating only chow. However, females from L4 and L12 drinking sucrose ingested more calories than those from L20 drinking sucrose (Fig. 3A; 1-way ANOVA: $F_{11,88}$: 18.525 $P < .0001$).

The percentage of total kilocalories consumed due to sucrose was affected by both sex ($F_{1,52}$: 33.580; $P < .0001$) and litter size ($F_{2,52}$: 7.178; $P < .002$). Sucrose intake comprised a higher percentage of total kilocalorie intake in females compared with males of the same litter size (1-way ANOVA: $F_{5,50}$: 10.219; $P < .0001$; males: L4S: 52.0% ± 2.9%, L12S: 46.5% ± 0.4%, L20S: 47.2% ± 1.6%; females: L4S: 61.2% ± 2.9%, L12S: 57.6% ± 1.3%, L20S: 52.3% ± 1.2%).

3.4. *Effect of litter size, sex, and sucrose intake on weight gain*

There was an effect of sex ($F_{1,85}$: 75.754; $P < .0001$), litter size ($F_{1,85}$: 15.090; $P < .0001$), and sucrose intake ($F_{1,85}$: 3.821; $P < .05$),

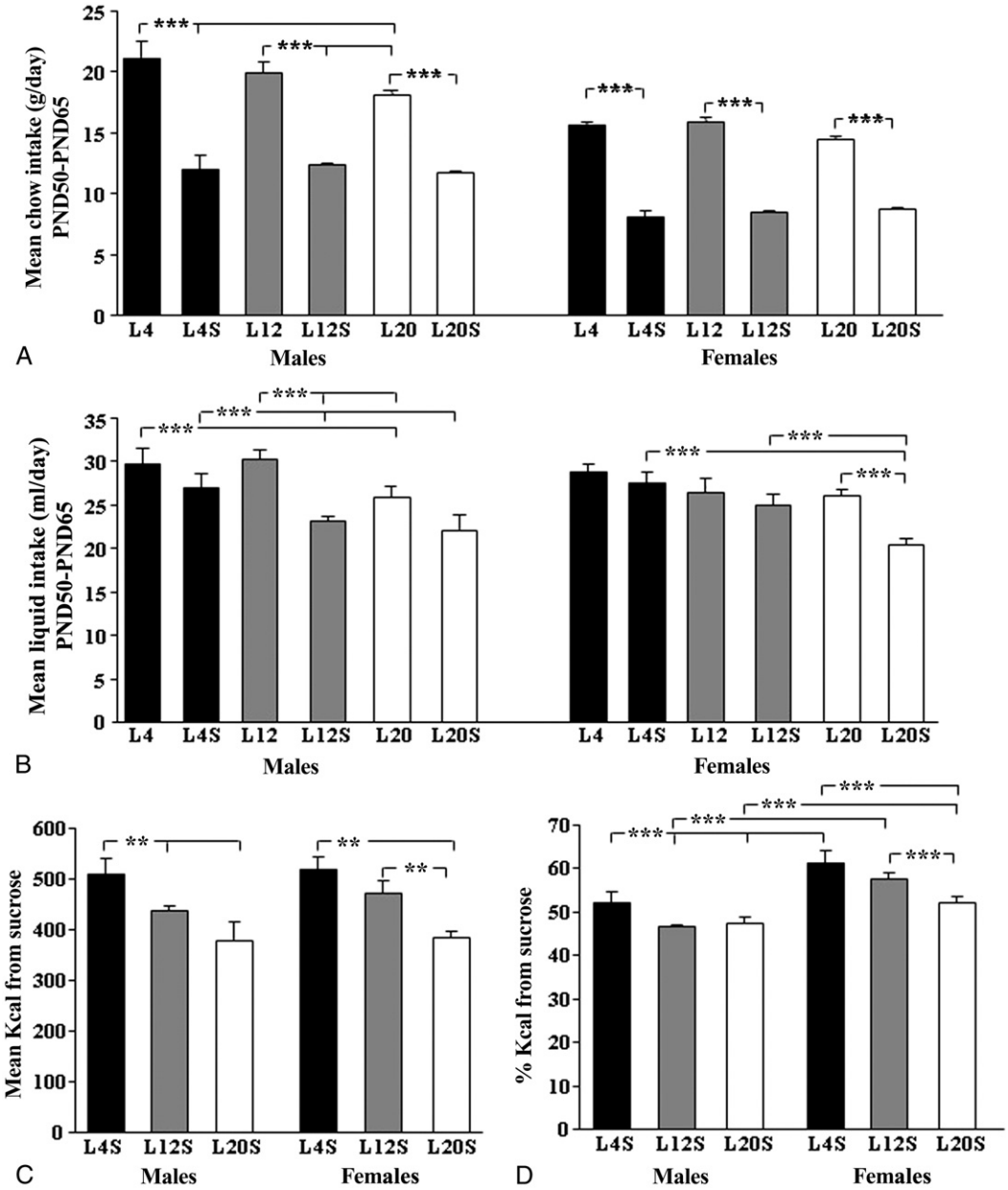

Fig. 2 – Mean chow intake (A), liquid intake (B), kilocalories from sucrose (C), and percentage kilocalories from sucrose (D) in male and female rats from litters of 4, 12, or 20 pups receiving either normal tap water or 33% sucrose (S) in their drinking water. L4 = litter of 4 on a normal diet (n = 8 males and 8 females), L4S = litter of 4 on a sucrose-enriched diet (n = 8 males and 8 females), L12 = litter of 12 on a normal diet (n = 6 males and 6 females), L12S = litter of 12 on a sucrose-enriched diet (n = 6 males and 6 females), L20 = litter of 20 on a normal diet (n = 10 males and 10 females), L20S = litter of 20 on a sucrose-enriched diet (n = 10 males and 10 female). ***ANOVA $P < .0001$, **$P < .005$.

with an interaction between sex and litter size ($F_{2,85}$: 10.597; $P < .0001$) and litter size and sucrose ($F_{1,85}$: 6.358; $P < .003$), on weight gain during the 2 weeks of diet change. Control L4 males gained more weight than L12 or L20 males. Likewise, L4 males receiving sucrose gained more weight than those from L12 or L20 drinking sucrose. Weight gain between L12 and L20 males was not significantly different during this period, and sucrose intake had no effect. Control L12 females gained less weight than those from L4 and L20. Sucrose intake resulted in less weight gain in females from L4 and L20, with no effect in those from L12. Males gained more weight than females from the corresponding group except in rats from L20, where weight gain was indistinguishable between males and females (Fig. 3B; 1-way ANOVA: $F_{11,85}$: 13.142; $P < .0001$).

Final BW was affected by sex ($F_{1,87}$: 278.133; $P < .0001$), litter size ($F_{2,87}$: 21.383; $P < .0002$), and sucrose intake ($F_{1,87}$: 12.096; $P < .002$), with an interaction between sex and litter size ($F_{2,87}$: 8.322; $P < .0005$). Males weighed more than females regardless of litter size or sucrose intake. In females on a normal diet, L4

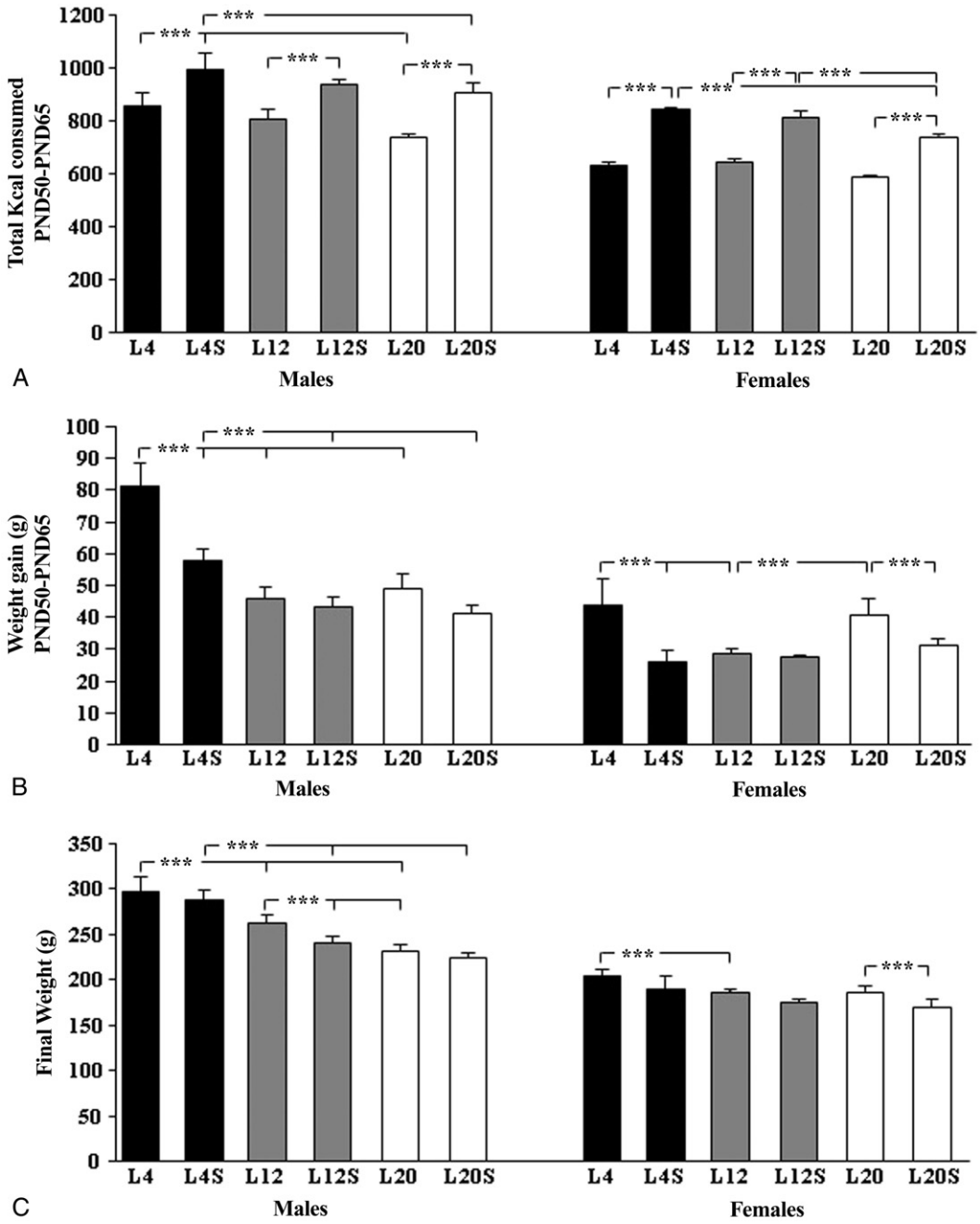

Fig. 3 – Total kilocalories consumed (A), weight gained (B), and final weight (C) in male and female rats from litters of 4, 12, or 20 pups receiving either normal tap water or 33% sucrose in their drinking water (S). ***ANOVA $P < .0001$.

rats weighed more than L12 rats. Sucrose intake significantly reduced final BW in rats from L20. Males from small litters weighed significantly more than those from normal-sized litters regardless of sucrose intake. In contrast, only male rats from L12 ingesting sucrose had a significant decline in final BW compared with their controls (Fig. 3C; 1-way ANOVA: $F_{11,87}$: 30.642; $P < .0001$).

3.5. *Effect of litter size, sex, and sucrose intake on glycemia and serum hormone levels*

Both sucrose ingestion ($F_{1,87}$: 6.210; $P < .02$) and sex($F_{1,87}$: 4,323; $P < .05$) had an effect on glycemia (Table 2). In males, there was an effect of sucrose intake on glucose levels ($F_{1,43}$: 6.771; $P < .02$), with sucrose increasing glycemia in all litter sizes; but this was only significantly in those from L20. There was no effect of either sucrose intake or litter size in females.

There was an effect of sex ($F_{1,64}$: 11.203; $P < .002$) on insulin levels, with no effect of litter size or sucrose intake (Table 2). Male rats had higher insulin levels compared with females regardless of the litter size or sucrose intake.

Serum leptin levels were affected by sex ($F_{1,62}$: 19.832; $P < .0001$), litter size ($F_{2,62}$: 3.393; $P < .05$), and sucrose intake ($F_{1,62}$: 13.076; $P < .0006$). Sucrose intake increased circulating leptin levels, with this difference being significant in L4 and L20 males (Fig. 4A; 1-way ANOVA: $F_{11,62}$: 4.703; $P < .0001$).

Table 2 – Circulating hormone levels in adult male and female rats that were raised in litters of 4 (L4), 12 (L12), or 20 (L20) pups and during 2 weeks before killing received either tap water (control) or 33% sucrose in their water

	Males						Females						P value
	L4		L12		L20		L4		L12		L20		
	Control	Sucrose	Control	Sucrose	Control	Sucrose	Control	Sucrose	Control	Sucrose	Control	Sucrose	
Glucose (mg/dL)	90.0 ± 4.2	102.1 ± 2.8	89.0 ± 5.9	91.9 ± 4.6	83.4 ± 4.1	102.0 ± 3.1[b]	91.0 ± 4.6	92.8 ± 1.8	89.1 ± 2.6	94.5 ± 2.6	87.1 ± 2.9	86.3 ± 3.8	Sucrose effect $P < .02$
Insulin (ng/mL)	1.5 ± 0.6	1.6 ± 0.6	1.2 ± 0.3	0.7 ± 0.2	0.8 ± 0.2	1.8 ± 0.6	0.4 ± 0.1[a]	0.7 ± 0.2	0.5 ± 0.1[a]	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.3[a]	ANOVA $P < .05$
Total ghrelin (pg/mL)	1324.1 ± 306.5	1549.5 ± 281.2	1523.8 ± 226.0	1846.9 ± 364.0	1589.9 ± 680.5	2478.7 ± 323.0	1223.9 ± 336.0	1638.4 ± 352.3	2223.2 ± 400.5	1798.5 ± 496.8	1662.8 ± 372.0	1838.5 ± 380.8	NS
Acylated ghrelin (pg/mL)	549.5 ± 65.5	515 ± 98.2	471.2 ± 53.5	676.4 ± 158.8	554.9 ± 166.0	530.2 ± 72.3	965.6 ± 403.9	432.7 ± 87.5	492.6 ± 79.0	320.9 ± 50.4	412.3 ± 65.9	399.0 ± 33.0	NS

NS indicates no statistically significant differences.

[a] Statistically different compared with control rats of the opposite sex from the same experimental group (litter size and sucrose intake).

[b] Statistically different compared with control rats of the same sex and litter size.

There was no effect of sex, sucrose, or litter size on either total or acylated ghrelin levels (Table 2).

4. Discussion

Here we show that male and female rats are equally affected by a decrease in litter size during nursing, with both sexes having significantly increased BW when they were weaned on PND21. The weight gain response to a normal rat chow diet was also similar between the sexes throughout early postnatal life. The differences in BW observed between L4 and L12 at PND21 were maintained until PND50 (approximately 15 g in females and 16 g in males), with no effect of neonatal overnutrition (ie, being raised in small litters) on further increased weight gain or food intake during this period in either sex. However, there was a delayed effect of neonatal overnutrition, as rats from both sexes increased their weight gain between PND50 and PND65 compared with those from normal-sized litters, with no effect on food intake. As food intake was not altered, this postpubertal difference in weight gain could be due to a change in metabolic efficiency.

Increased food intake during nursing due to a reduction in litter size has previously been shown to result in increased BW at the moment of weaning [6,8,23,24], and the data reported here not only confirm these previous reports but extend them to include both sexes and indicate that weight gain is not equally affected at all postnatal ages. Some developmental effects on weight gain have been previously reported to occur or become more apparent later in adult life or when a metabolic stress or challenge is introduced [2,5,8,26,35]. Hence, it is clear that when determining the effect of neonatal nutrition on adult metabolism, absolute weight gain at different periods of development should be taken into consideration. Moreover, how pubertal hormonal changes might be involved in this process deserves further investigation.

Unlike overnutrition, neonatal undernutrition had sexually dimorphic effects on long-term weight gain and food intake. An increase in litter size reduced BW in both sexes during nursing, with this difference being significant at PND21. Whereas female rats normalized their BWs by PND65, males continued to eat less and gain less weight throughout the study. Between PND21 and PND50, there was no effect of neonatal undernutrition on food intake or weight gain in females. However, L20 females gained significantly more weight than their controls between 8 and 10 weeks of age, resulting in no difference in BW at PND65. Significant compensatory growth or catch-up growth is a frequent phenomenon of intrauterine growth retardation or early undernutrition [26]; and intrauterine growth–retarded female rats have been reported to display a more complete catch-up compared with males [36], as observed here in undernourished females. Low for gestational age newborn girls are also reported to normalize body mass index more rapidly than boys [37], suggesting that males may be more susceptible to the long-term effects of early undernutrition on weight gain. As BW influences reproductive capacity in females [38], it remains to be demonstrated whether this sex has developed a specific defense mechanism to maintain reproductive

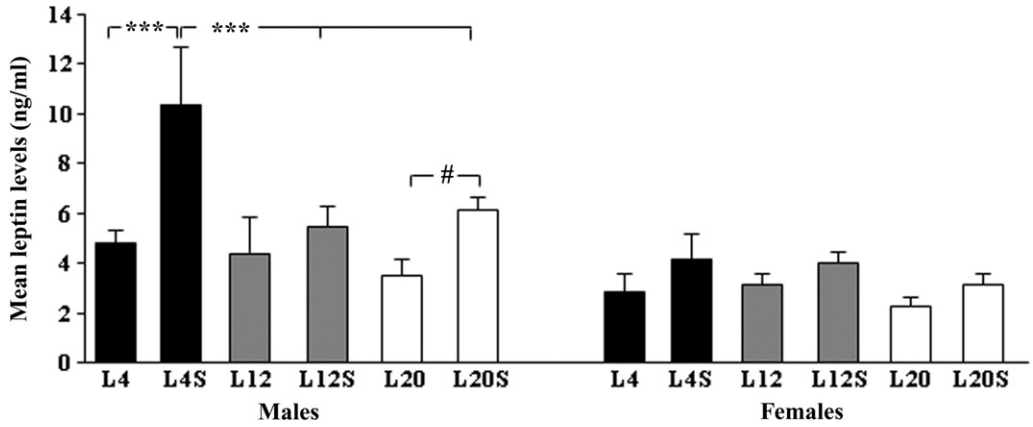

Fig. 4 – Mean serum leptin levels in male and female rats from litters of 4, 12, or 20 pups receiving either normal tap water or 33% sucrose in their drinking water (S). ***ANOVA $P < .0001$.

capabilities. Indeed, in situations of food restriction, female rats are more capable of preserving lean body mass and surviving than males [39].

In this study, we did not analyze body composition. Postpubertal males have more body fat than females; and this is associated with higher circulating leptin levels [40-42], as observed here. Previous studies have shown that neonatal overfeeding increases fat mass and decreases lean mass [16,17], whereas neonatal undernutrition decreases fat mass in adulthood [16], suggesting that the changes in BW observed here are most likely due to similar modifications in body composition. In parallel to changes in body fat, neonatal overnutrition increases and undernutrition decreases serum leptin levels in adult offspring [16]. We found no significant effect of litter size on circulating leptin levels. This discrepancy with other studies could be due to differences in experimental design, such as the age at which the animals were killed, but also to the fact that, in our study, the rats were not fasted.

We found no effect of litter size on any of the metabolic hormones analyzed, which, as stated above, could be due to these hormone levels being measured in nonfasting animals. Rats were not fasted to maintain the more acute influence of sucrose consumption, and this could also explain the large variance in some of the hormonal results. Although female rats are reported to have increased fasting insulin levels compared with males [43], we found them to have significantly lower nonfasting insulin levels. Male and female rats are also reported to have different insulin sensitivities, and this is suggested to be involved in differences in the response to carbohydrate intake and development of diabetes [43,44]. Whether this can explain the discrepancy from previous reports remains to be determined.

During the 2 weeks of a sucrose-enriched diet, all rats regardless of sex or neonatal nutrition decreased their rat chow intake as previously reported [45], but consumed significantly more energy. Maternal nutrition is reported to modulate food preferences of the offspring [13]; and male rats with neonatal overnutrition consumed more sucrose than controls, both in absolute quantity and in percentage of total kilocalorie intake, whereas in neonatally overnourished females, these parameters were unaffected. Although neonatally undernourished rats of both sexes consumed significantly fewer kilocalories in the form of sucrose compared with their controls, in males, the percentage of kilocalories due to sucrose was not different from controls, whereas in females, it was significantly reduced.

Hedonic behavior is affected by distinct factors including gonadal hormone status and sex [46-48], with females reported to have a higher preference for sweet foods [46-49]. In agreement with these previous reports, although females consumed fewer total calories than males from the same-sized litters, they consumed a higher percentage of their calories in the form of sucrose. Some authors report that males may actually have a higher sucrose preference than females [37]; however, as indicated by the results presented here, it is possible that factors such as early nutritional status must also be taken into consideration because it could mask the effect of sex.

Sucrose consumption increased total kilocalorie intake, but no increase in weight gain was observed. Rats on the sucrose-enriched diet gained either a similar amount or less weight than their controls. In male and female rats from normal-sized litters, sucrose intake did not affect weight gain, which is in agreement with that reported previously for male rats consuming sucrose for 3 weeks [46,48-50]. In contrast, both male and female rats from large litters that consumed sucrose gained less weight than their controls. It is interesting to note that L20 females had a significant reduction in weight gain with sucrose intake, whereas L20 males did not. As rapid catch-up growth is suggested to increase the possibility of later metabolic imbalances [26], the catch-up growth exhibited by females with neonatal undernutrition could have increased their susceptibility to this later metabolic challenge. The differential effects of sucrose on weight gain cannot be explained by the amount of kilocalories or percentage of kilocalories from sucrose in the different experimental groups. Maternal diet, fetal growth, and postnatal catch-up growth have all been associated with changes in insulin sensitivity, with some of these outcomes being sexually dimorphic [51-53]. Thus, it is possible that neonatal over- and undernutrition induce metabolic changes that

affect carbohydrate metabolism and adipose accrual, with at least some of these effects being different between the sexes.

Two weeks of increased sucrose intake had an overall effect on nonfasting glucose levels, but there were no effects of sucrose or litter size on insulin or total or acylated ghrelin levels. Again, the fact that the animals were not fasted should be taken into consideration. Sucrose intake increased nonfasting glucose levels in L4 and L20 males, with this reaching statistical significance only in neonatally undernourished males. Likewise, circulating leptin levels were increased in rats consuming sucrose, with this difference being statistically significant in both L4 and L20 males. Sucrose intake most likely induced fat accumulation, as reported previously [50,54,55] and as suggested by increased leptin levels; and it appears that male rats exposed to either over- or undernutrition during lactation are more susceptible to the metabolic changes induced by a sucrose-enriched diet. A direct correlation between body fat and circulating leptin levels is reported in both male and female rats, with postpubertal males having higher leptin levels than females in correlation with their higher fat mass [40-42]. However, males have a higher relative increase in leptin concentrations in relation to body fat content than do females, especially at higher percentages of body fat [40], which could at least partially explain the significant increase seen here in males and not in females. The distinct body fat deposits differentially express proteins involved in metabolic control and respond differently to metabolic changes [56,57], with visceral adipose tissue reported to contribute more to obesity-induced health problems [58,59]. Visceral fat mass is greater in adult males than females [41,56], and subcutaneous fat mass is greater in females both pre- and postpubertally [41,60]. Moreover, adipose tissue from the same depots differs between males and females, including expression of metabolic enzymes, receptors, and cytokines, with differential responses to a change in diet [56]. Thus, it is not unexpected that adipose proliferation, hypertrophy, or cytokine production might differ between the sexes in response to specific stimuli.

Sex steroids directly influence adipocyte metabolism [61]. However, the metabolic differences observed between males and females cannot be fully explained by the postpubertal hormonal differences [56,62]. The prenatal sex steroid environment has long been known to induce sexually dimorphic behaviors and functions in rodents [63], and modifications in sex steroid levels during early neonatal life affect both longitudinal growth and body composition [64-66]. Thus, the sex-related differences in body composition and responses to nutritional manipulations are most likely a result of innate genetic differences between the sexes that interact with both the neonatal and postpubertal hormonal environments, which are also influenced by the sex of the animal.

In conclusion, the results reported here clearly indicate that for the correct interpretation of data in studies of metabolic function, numerous factors should be taken into consideration including sex, age, and the early nutritional status of the subject when comparing or analyzing metabolic outcomes in response to any physiological or experimental challenge. Indeed, we have found that males and females have different long-term responses to changes in neonatal nutrition, with females being less susceptible to the effects of early undernutrition. Furthermore, although the weight gain response to short-term sucrose intake was similar in control males and females, the effect of early nutritional status on the response to sucrose intake was sexually dimorphic, with changes in circulating levels of metabolic hormones such as leptin more evident in males previously exposed to either under- or overnutrition. Interpretation of the results presented here is limited by the lack of data regarding changes in body composition. Indeed, further studies are necessary to thoroughly analyze the changes in body composition in both sexes throughout development in response to modifications in early nutrition and the mechanisms underlying these changes. However, it is clear that further emphasis must be placed on nutrition during both pregnancy and lactation in attempting to curtail obesity in future generations. The differential responses of males and females to metabolic manipulations also emphasize the need for studies to be performed in both sexes, including clinical assays to determine the effectiveness of obesity treatments.

Funding

Funding for this study was provided by grants from Fondo de Investigación Sanitaria (PI070182; PI0100747), Ministerio de Ciencia e Innovación (BFU2008-02950 C03-3), CIBER de Fisiopatología de Obesidad y Nutricion Instituto de Salud Carlos III, Fundación MutuaMadrileña AP2561/2008, and Fundación de Endocrinología y Nutrición. JAC is supported by the biomedical investigation program of the Consejería de Sanidad y Consumo de la Comunidad de Madrid.

Acknowledgment

The authors would like to thank Francisca Díaz and Sandra Canelles for their excellent technical support.

Conflict of Interest

The authors have nothing to declare.

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