Short- and long-term effects of maternal nicotine exposure during lactation on body adiposity, lipid profile, and thyroid function of rat offspring

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Abstract

Epidemiological studies show a higher prevalence of obesity in children from smoking mothers and smoking may affect human thyroid function. To evaluate the mechanism of smoking as an imprinting factor for these dysfunctions, we evaluated the programing effects of maternal nicotine (NIC) exposure during lactation. Two days after birth, osmotic minipumps were implanted in lactating rats, divided into: NIC (6 mg/kg per day s.c.) for 14 days; Control – saline. All the significant data were $P < 0.05$ or less. Body weight was increased from 165 days old onwards in NIC offspring. Both during exposure (at 15 days old) and in adulthood (180 days old), NIC group showed higher total fat (27 and 33%). In addition, NIC offspring presented increased visceral fat and total body protein. Lipid profile was not changed in adulthood. Leptinemia was higher at 15 and 180 days old (36 and 113%), with no changes in food intake. Concerning the thyroid status, the 15-days-old NIC offspring showed lower serum-free tri–iodothyronine (FT_3) and thyroxine (FT_4) with higher TSH. The 180-days-old NIC offspring exhibited lower TSH, FT_3, and FT_4). In both periods, liver type 1 deiodinase was lower (26 and 55%). We evidenced that NIC imprints a neonatal thyroid dysfunction and programs for a higher adiposity, hyper-leptinemia, and secondary hypothyroidism in adulthood. Our study identifies lactation as a critical period to NIC programing for obesity, with hypothyroidism being a possible contributing factor.

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Introduction

Several epidemiological and animal studies have shown that malnutrition, hormones, and other stressful events during critical periods of early life permanently alter the function of the body's systems of the progeny. This association has been named programing, which is defined as the phenomenon that putatively underlies relationships among nutritional experiences of early life and adult diseases (Lucas 1994, Barker 2003, Moura & Passos 2005, de Moura et al. 2008).

Some environmental and dietary chemicals that can mimic or interfere with the normal action of hormones are referred to as 'endocrine disruptors'. Additional studies predict the existence of chemical 'obesogens', molecules that inappropriately regulate lipid metabolism and adipogenesis to promote obesity (Grun & Blumberg 2006, Tabb & Blumberg 2006).

During pregnancy, cigarette smoking causes low birth weight (Butler & Goldstein 1973, Navarro et al. 1989, DiFranza & Lew 1995), and epidemiological studies suggest that maternal smoking during pregnancy might be a risk factor for childhood obesity (Morley et al. 1995, Vik et al. 1996, Blake et al. 2000, von Kries et al. 2002, Toschke et al. 2002, Bergmann et al. 2003, Wideroe et al. 2003, Goldani et al. 2007); however, the mechanisms to explain the development of obesity are still unclear.

Newman et al. (1999) observed that rats exposed to nicotine (NIC), the main addictive compound of tobacco smoke, in utero, are heavier at 9 weeks old when compared with controls. Williams & Kanagasabai (1984) reported that fetal NIC exposure in rats increases body fat in the fetus on the 20th day of gestation (term on the 21st day), suggesting that fetal NIC exposure results in increased adiposity in the offspring.

Only few studies suggest that the first postnatal week is critical for NIC programing of body weight (BW) and body fat distribution. NIC exposure in rats, extending from the gestational period to the 10th day of lactation, increases BW in offspring at 35 days old. In male offspring, this effect is transient, but in females the higher BW persists until 90 days of age (Chen & Kelly 2005). Additionally, rats whose mothers were treated with NIC for 14 days before mating and during

pregnancy until weaning become heavier at 70 days old when compared with the control group. At 6 months of age, NIC exposure results in increased BW, fat pad weight, and perivascular adipose tissue in the offspring (Gao *et al.* 2005).

Increased body fat/weight is associated with enhanced levels of the adipocyte hormone, leptin (Friedman & Halaas 1998). However, the association between smoking and leptin levels is controversial. In tobacco smokers, both hyperleptinemia (Hodge *et al.* 1997, Eliasson & Smith 1999, Nicklas *et al.* 1999) and hypoleptinemia (Wei *et al.* 1997, Donahue *et al.* 1999) have been described.

Smoking can also affect the thyroid gland (Christensen *et al.* 1984, Ericson & Lindgrade 1991, Fisher *et al.* 1997, Utiger 1998). Although there is less data on the effect of tobacco compounds upon the thyroid, thiocyanate, but not NIC, is associated with hypothyroidism (Muller *et al.* 1995, Fukata *et al.* 1996). In addition, maternal smoking influences the thyroid function in infants (Meberg & Marstein 1986, Karakaya *et al.* 1987, Chanoine *et al.* 1991). Passive smoking from both parents affects thyroid function. Thyroglobulin and thiocyanate concentrations at birth and at 1 year of age in infants whose parents are smokers are greater than in infants with nonsmoking parents (Gasparoni *et al.* 1998). According to Lauberg *et al.* (2004), smoking mothers have lower iodide content in breast milk and their offspring have lower urinary iodide. This study suggests that NIC decreases maternal milk iodide transfer.

Our group has been working with several imprinting factors during lactation that are capable of programing the hormonal regulation and body composition (Passos *et al.* 2002, 2004, Dutra *et al.* 2003, Vicente *et al.* 2004, Toste *et al.* 2006*a,b*, Fagundes *et al.* 2007, de Moura *et al.* 2007). Particularly, maternal nutritional and hormonal changes during the lactation period in rats were shown to program the thyroid function in adult life (Passos *et al.* 2002, 2007, Dutra *et al.* 2003, Lins *et al.* 2005, Bonomo *et al.* 2008, Lisboa *et al.* 2008). Since thyroid dysfunction is associated with marked changes on both energy expenditure and BW (Pontikides & Krassas 2007), it is interesting to evaluate the thyroid status in the model of programing by maternal NIC exposure during lactation. In addition, it seems likely that thyroid hormones and leptin play mutual roles (Ahima *et al.* 1996, Escobar-Morreale *et al.* 1997, Ortiga-Carvalho *et al.* 2002, Rosenbaum *et al.* 2002, Oliveira *et al.* 2007, De Oliveira *et al.* 2007).

Despite experimental evidence of NIC programing during gestation and also when the exposure extends from pregnancy to lactation, to our knowledge, there are no studies focusing on the effects of NIC exposure exclusively during the early postnatal period. This may be of particular relevance since there is a high rate of smoking relapse among women who stop smoking during pregnancy (McBride & Pirie 1990). Thus, our aim was to evaluate the short- and long-term consequences of maternal NIC exposure, solely during lactation, on BW, body composition, serum leptin, and thyroid function of rat offspring at different ages. Since there is an association between

higher visceral fat mass (VFM) and other components of the metabolic syndrome, such as dyslipidemia, we also studied the lipid profile of the NIC-treated animals.

Materials and Methods

Wistar rats were kept in a temperature-controlled room (25 ± 1 °C) with artificial darkness–light cycles (lights on at 0700 h and lights off at 1900 h). Virgin female rats 3 months of age were caged with male rats in the proportion of 3:1. After mating, each female was placed in an individual cage with free access to water and food until delivery. The use of the animals according to our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/189/2007), which based its analysis on the principles described in the Guide for the Care and Use of Laboratory Animals (Bayne 1996).

Experimental model of maternal NIC exposure during lactation

Two days after birth, 12 lactating rats were randomly assigned to one of the following groups:

> NIC ($n=6$) – dams were lightly anesthetized with thiopental, a 3×6 cm area on the back was shaved, and an incision made to permit s.c. insertion of osmotic minipumps (Alzet, 2ML2, Los Angeles, CA, USA). Pumps were prepared with NIC-free base diluted in a saline solution (NaCl 0·9%) to deliver an initial dose rate of 6 mg/kg of NIC per day (during 14 days of lactation), as previously described (Abreu-Villaca *et al.* 2004*a,b*). At this dose rate, this paradigm produces plasma NIC levels similar to those in typical smokers – ∼25 ng/ml (Lichtensteiger *et al.* 1988). The incision was closed and the mothers were permitted to recover in their home cages. Control (C, $n=6$) – dams were implanted with osmotic minipumps containing only saline solution, released for the same period as that of minipumps with NIC.

Generally, pregnant rats produced 10–12 pups and, to avoid the influence of the litter size in the programing effect, we only used mothers whose litter size was 10 offspring. At birth, litter adjustment was performed and six male pups were kept per NIC or C mother to maximize the lactation performance.

During lactation, BW (mothers and pups) and relative food intake (g/100 g BW) of the mothers were daily monitored. From weaning (21 days of lactation) until 180 days, BW of the offspring was monitored every 4 days and relative food intake was monitored every 15 days.

We used two offspring from each mother at each age point (12 rats per group). The experiment was performed twice: at the first, offspring were killed at 15, 21, and 180 days old; and at the second, offspring were killed at 15, 90, and 180 days old. The killing occurred by decapitation to collect blood, VFM, and carcass.

Body composition

After the killing, VFM was quickly excised and weighed for evaluation of the central adiposity – mesenteric, epidydimal, and retroperitoneal (Toste *et al.* 2006*a*, Fagundes *et al.* 2007), and data were expressed as g/100 g BW.

Body composition (total fat and protein mass) was determined by carcass analysis (Toste *et al.* 2006*a*, Fagundes *et al.* 2007). NIC and C offspring were eviscerated; the carcasses were weighed, autoclaved for 1 h, and homogenized in distilled water (1:1). The homogenates were stored at 4 °C for analysis.

Homogenates (3 g) were used to determine fat content gravimetrically. Samples were hydrolyzed in a shaking water bath at 70 °C for 2 h with 30% KOH and ethanol. The total fatty acids and nonesterified cholesterol were removed with three successive washings with petroleum ether. After drying overnight in vacuum, all tubes were weighed and data were expressed as g fat/100 g carcass.

Protein content was determined in 1 g homogenates. Tubes were centrifuged at 2000 g for 10 min. The total protein concentrations were determined by the Lowry method (Lowry *et al.* 1951). Data were expressed as g protein/100 g carcass.

Lipid profile

Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) were analyzed in the adult offspring using Biosystem commercial test kits (Simões *et al.* 2007).

LDL-C and VLDL-C were obtained using Friedewald calculations:

1) LDL-C (mg/dl) = TC − (TG/5) − HDL-C.
2) VLDL-C (mg/dl) = TG/5.

Hormones determination by RIA

Blood samples were centrifuged (1500 g/20 min/4 °C) to obtain serum and were individually kept at −20 °C until assay. All measurements for each hormone were performed in one assay.

Leptin was measured by specific RIA kit (Linco Research, Inc., St. Charles, MO, USA), which measures both rat and mouse leptin with a range of detection from 0·5 to 50 ng/ml; the intra-assay variation was 2·9%.

Free thyroid hormones (free tri-iodothyronine (FT_3) and thyroxine (FT_4)) were determined by commercial RIA kit (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) with an assay sensitivity of 0·045 ng/dl (T_4) and 0·06 pg/ml (T_3). The intra-assay variation was 2·8% (T_4) and 3·6% (T_3).

TSH was measured by specific RIA, using a rat TSH kit supplied by the National Institute of Health (NIH, USA) and

expressed in terms of the reference preparation provided (RP-3). The intra-assay variation was 2·3%, with 0·18 ng/ml as the lower limit of detection.

Liver D1 activity determination

In order to confirm the thyroid status, liver D1 activity of 15 and 180 days old offspring was measured in the microsomes by the release of ^{125}I from ^{125}I-reverse T_3 (rT_3), as previously reported (Lisboa *et al.* 2003). Assay was performed in phosphate buffer containing 1 mM EDTA (pH 6·9) in the
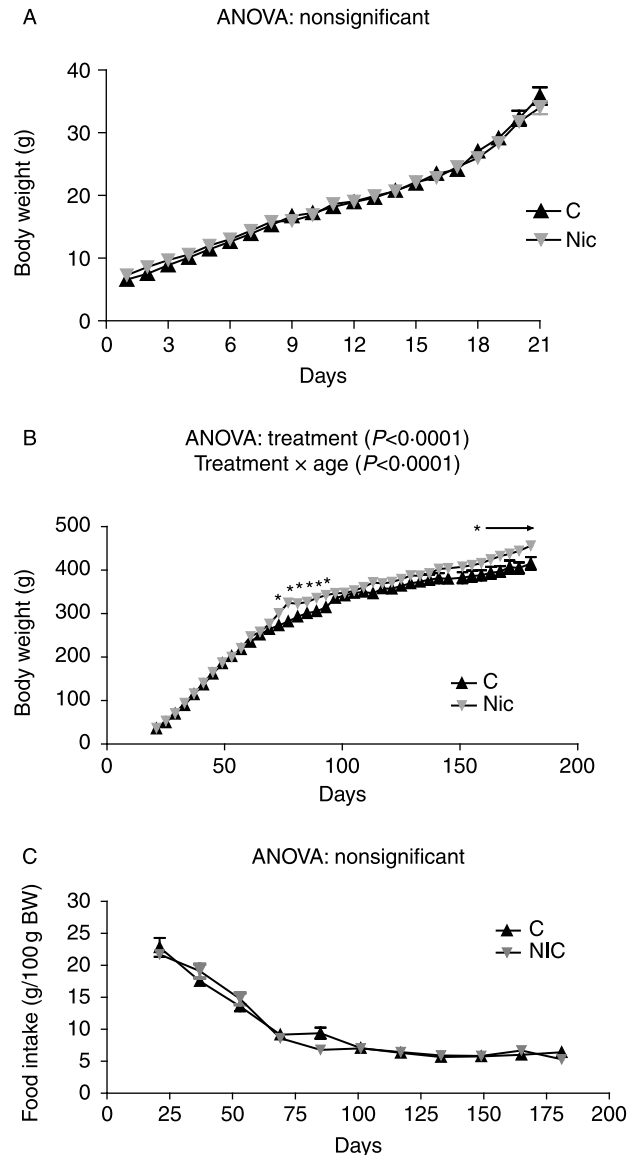

Figure 1 Body weight during lactation (A), after weaning (B), and food intake (C) of offspring whose mothers were nicotine (gray) or saline (black) exposed during lactation. Values represent mean ± S.E.M. of 12 rats per group. *$P < 0·05$ versus C.

presence of 1·5 µM rT$_3$ and 10 mM dithiothreitol. Free ^{125}I of enzymatic deiodination was eluted from Dowex 50 W-X2 columns (Bio-Rad, EUA) with 10% acetic acid. Deiodination percentual in the presence of the enzyme was around 10–20%. Amount of free ^{125}I in blank was <1–2% of the total radioactivity in the reaction mixture. Specific enzyme activity was expressed by nanomoles of rT$_3$ deiodinated/ h×mg of protein.

Statistical analysis

Results were reported as mean±S.E.M. The GraphPad Prism 4 and Statview 5·0 programs were used for statistical analyses and graphics. Initially, two-way ANOVA on each variable (BW and food intake evolutions, total body fat, protein content, leptin, FT$_3$, FT$_4$, TSH, and D1) were carried out. Treatment and age were used as between-subjects factors. Whenever this initial test indicated treatment effects that differed among the different ages, data were then re-examined separately using one-way ANOVAs; however, where treatment effects did not interact with age, only the main effect was recorded without testing of individual differences. TC, TG, HDL, LDL, and VLDL data were analyzed by Student unpaired *t*-test. Differences were considered significant at $P<0.05$.

Results

During NIC exposure, NIC mothers had no BW and food intake change compared with the C group. Maternal NIC exposure did not change BW gain of the offspring during lactation (Fig. 1A). However, after weaning, NIC offspring presented higher BW compared with C offspring (treatment: $F_{1,1475}=111.4$, $P<0.0001$), an effect that was dependent on the age (treatment×age: $F_{39,1475}=2.4$, $P<0.0001$). Accordingly, data were subdivided into separate ages for further analysis. After subdivision, we found higher BW for NIC offspring between 75 and 100 days of life (around 10%, $P<0.05$) as well as after 165 days old, reaching 10% ($P<0.05$) at 180 days old (Fig. 1B). We did not observe food intake alterations during the entire experimental period, as depicted in Fig. 1C.

Body composition of the offspring is shown in Fig. 2. NIC treatment affected body fat mass (treatment: $F_{1,56}=9.6$, $P<0.004$) and the effects were age dependent (treatment ×age: $F_{3,56}=3.4$, $P<0.03$): NIC offspring showed higher total fat mass on the 15th day of lactation (+27%: Fig. 2A, $P<0.003$), at 90 (+25%: Fig. 2A, $P<0.02$), and at 180 days old (+33%: Fig. 2A, $P<0.05$) days old. VFM (Fig. 2B) and total protein content (Fig. 2C) also differed between treatment groups (treatment: $F_{1,68}=7.8$, $P<0.006$ and treatment: $F_{1,56}=4.6$, $P<0.04$ respectively); however, there were no treatment×age interactions. Accordingly, across the four analyzed ages, NIC elicited increased values for VFM and total protein content when compared with C offspring.

Treatment caused hyperleptinemia (treatment: $F_{1,58}=7.6$, $P<0.008$) and the effects were age dependent (treatment

×age: $F_{3,58}=3.1$, $P<0.04$). Separate analyses for each age demonstrated a trend towards significance at 15 days (+36% − treatment: $F_{1,11}=4.4$, $P<0.06$) and a significant increase at 180 (+113% − treatment: $F_{1,18}=9.3$, $P<0.007$) days in offspring whose mothers were NIC exposed during lactation, as shown in Fig. 3. NIC offspring presented no change in lipid profile when they were 180 days old (Table 1).

Figure 4 shows the thyroid function of animals whose mothers were NIC or saline exposed. NIC treatment affected FT$_3$ (treatment: $F_{1,51}=9.7$, $P<0.004$), so that NIC offspring
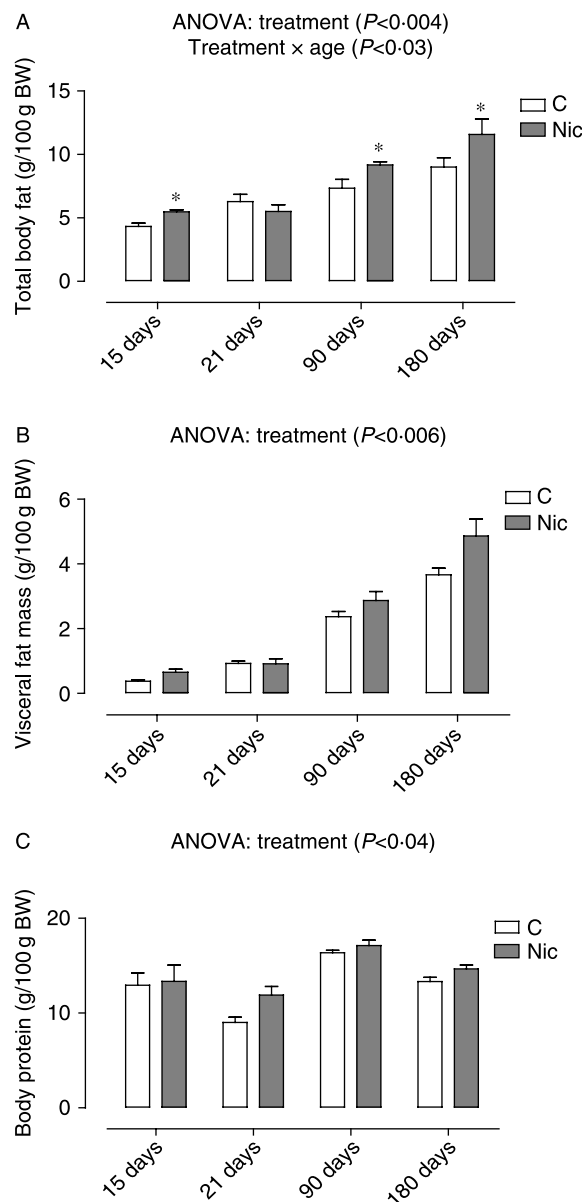

Figure 2 Body total fat mass (A), visceral fat mass (B), and body total protein mass (C) at 15, 21, 90, and 180-days-old offspring whose mothers were nicotine or saline exposed during lactation. Values represent mean±S.E.M. of 6–12 rats per group. *$P<0.05$ versus C.

ANOVA: treatment ($P<0.008$)
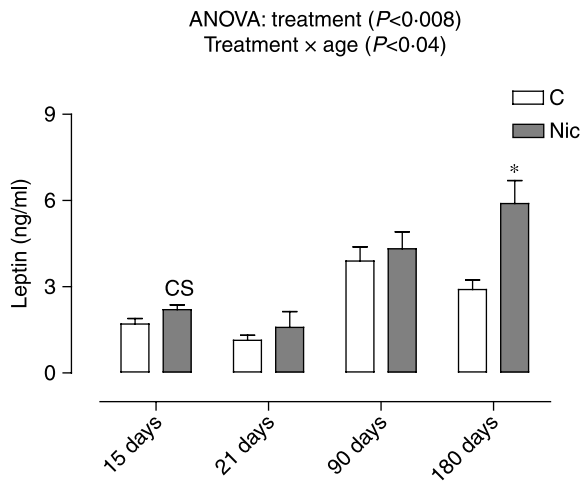Treatment × age ($P<0.04$)

Figure 3 Serum leptin at 15, 21, 90, and 180-days-old offspring whose mothers were nicotine or saline exposed during lactation. Values represent mean±S.E.M. of 6–12 rats per group; cs, close to significance.*$P<0.05$ versus C.

presented lower serum FT_3 when compared with C rats, an effect that was largely determined by differences between NIC and C offspring at 15, 21, and 180 days (Fig. 4A). As for FT_4 (treatment: $F_{1,62}=8.2$, $P<0.006$; treatment×age: $F_{3,62}=3.1$, $P<0.0001$), decreased values for NIC offspring reached significance in 15 days (-31% – treatment: $F_{1,18}=53.3$, $P<0.0001$) and 180 (-15% – treatment: $F_{1,14}=7.7$, $P<0.02$) days old offspring (Fig. 4B). NIC treatment also affected TSH (treatment: $F_{1,53}=7.7$, $P<0.008$; treatment ×age: $F_{3,53}=6.5$, $P<0.0008$). The 15-days-old NIC pups presented higher TSH ($F_{1,12}=5.3$, $P<0.04$). In contrast, at 21 ($F_{1,11}=5.7$, $P<0.04$), 90 ($F_{1,14}=9.4$, $P<0.009$), and 180 ($F_{1,16}=6.2$, $P<0.02$) days, lower TSH in NIC offspring reached significance (Fig. 4C). NIC offspring presented lower liver D1 activity (treatment: $F_{1,29}=31.4$, $P<0.0001$; treatment×age: $F_{1,29}=7.1$, $P<0.02$) on 15 days (-26% – treatment: $F_{1,13}=4.8$, $P<0.05$) and 180 days (-55% – treatment: $F_{1,29}=33.0$, $P<0.0001$; Fig. 4D).

Table 1 Lipid profile of adult rats whose mothers received nicotine during lactation. Values represent mean±S.E.M. of 6–12 rats per group.

	180 days-old offspring	
	Control	Nicotine
TC (mg/dl)	59·1±1·9	53·3±3·1
HDL-C (mg/dl)	12·7±0·8	13·8±1·1
LDL-C (mg/dl)	33·6±1·9	32·5±3·4
VLDL-C (mg/dl)	12·8±1·1	14·8±4·8
TG (mg/dl)	64·1±5·6	50·6±2·9

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoproteins; TG, triglycerides.

Discussion

Previous studies have demonstrated that there is a high prevalence of women who do not ever quit smoking during pregnancy or lactation (O'Campo *et al.* 1992). However, there is a high rate of smoking relapse among women who stopped smoking during pregnancy (McBride & Pirie 1990). Despite this fact, the majority of epidemiological studies on maternal smoking and experimental data on NIC exposure were observed during pregnancy or pregnancy and lactation.

In our study, for the first time, it was evidenced that in rats maternal NIC exposure, only during lactation, causes neonatal thyroid hypofunction and programs for overweight, hyperleptinemia, and lower function of the pituitary–thyroid axis later in the offspring life. In fact, lactation is a critical period of life, as in this phase important cognitive and neurological development occurs, which suggests that adverse environmental changes can cause physiological modifications that predispose the development of some diseases in adulthood (Mott *et al.* 1991, Symonds 2007).

In rodents, NIC exposure during pregnancy does not alter the BW gain of the mothers (Chen & Kelly 2005). However, experimental studies have documented an inverse relationship between cigarette smoking and BW, showing that cessation of NIC exposure is usually accompanied by weight gain (Levine *et al.* 1987). According to Li *et al.* (2000), adult rats exposed to NIC reduce BW and are hypophagic. Despite these previous data, in the present study, NIC exposure from the 2nd to the 14th day of lactation did not affect the mother's BW gain or food intake. Since during lactation many mechanisms are activated in order to supply the high energy requirement, including hyperphagia, basal metabolic rate reduction, and preferential nutrient flux for lactogenesis (Dewey 1998), it is possible that during this particular phase, these mentioned events are more important and counterbalance the well-known effects of NIC in nonlactating animals.

In previous experimental studies, pre- and postnatal NIC exposure failed to cause changes in BW during the exposure period (Chen & Kelly 2005, Gao *et al.* 2005). Interestingly, in our study, despite the fact that NIC exposure did not affect BW gain of NIC offspring during lactation, these rats showed greater total and VFM when they were 15 days old, when they were still being exposed to NIC.

Some studies have shown that there is an increased risk of obesity in children whose mothers smoked during pregnancy (Vik *et al.* 1996, von Kries *et al.* 2002). Our data showed maternal NIC exposure during lactation programs for higher VFM, total body fat, and protein content in adulthood. These alterations in body composition may be responsible for the higher BW of NIC offspring. These programed rats did not show food intake changes, which suggest the development of a hypometabolic status. Chen & Kelly (2005) showed that NIC treatment during pregnancy and for the first 10 days of life affected BW with sex-dependent changes. BW was significantly higher on 35, 63, and 90 days old, in female rats.
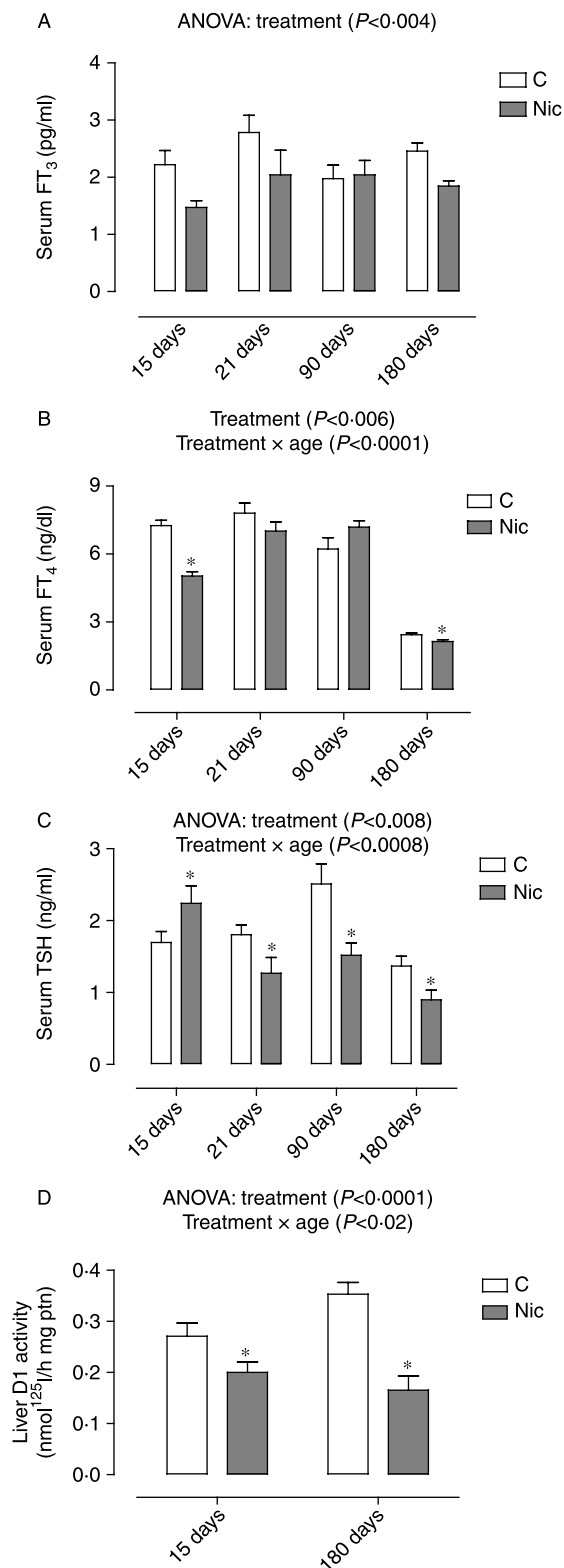
However, in male rats, BW was transiently higher only when they were 35 days old. Another study showed that values for BW, left ventricular weight, epididymal, mesenteric, and perirenal fat weight were significantly higher in 6-months-old offspring from NIC-treated mothers before mating, during pregnancy and lactation (Gao *et al*. 2005). However, there was no report of total fat mass due to NIC exposure only during pregnancy or lactation.

It was already reported that NIC treatment causes change in the lipid pattern in adult female rats (Abd el Mohsen *et al*. 1997). However, there is no data regarding the long-term effect of maternal NIC exposure during lactation on serum lipid profile. In our study, for the first time, we observed no change in TG, TC, LDL-C, HDL-C, and VLDL-C levels in adult NIC offspring, despite its overweight and higher adiposity.

We observed that NIC offspring did not present changes in BW until they were 75 days old. We detected a transient increase in BW gain between 75 and 100 days of life. After 165 days of life, again we observed overweight in NIC group, which persisted until they were 180 days old. This could be associated with critical phases in which rats complete their sexual maturation (75–100 days old) and thereafter start the ageing process (>150 days). Chen & Kelly (2005) injected lower NIC doses from pregnancy until lactation and perhaps this explains the transient effect in BW gain compared with our study that shows a more prolonged effect. Also, they did not evaluate older rats.

At 15 days of age, NIC rats showed a trend to higher serum leptin levels ($+36\%$, $P < 0.06$), which actually were significantly higher at 180 days old. This effect may be caused by higher adiposity since this hormone is mainly produced by adipose tissue (Ahima 2005). As already discussed, we did not detect any food intake change; therefore, it is possible that these animals presented hypothalamic leptin resistance to its anorexigenic effect. These data corroborate previous studies carried out by our group concerning other programing models (Passos *et al*. 2004, Bonomo *et al*. 2007), in which BW changes were not accompanied by food intake alterations.

Leptin and leptin receptors are both found in skeletal muscle (Wang *et al*. 1998, Steinberg & Dyck 2000, Maroni *et al*. 2003). Some studies have shown a direct effect of leptin on muscle, increasing glucose, and fatty acid metabolism (Wang *et al*. 1998, Steinberg & Dyck 2000, Ceddia *et al*. 2001, Maroni *et al*. 2003). Leptin stimulates GH release by the stimulation of GH-releasing hormone (Tannenbaum *et al*. 1998). It is known that GH increases amino acid uptake into muscles, increases protein synthesis, and decreases protein catabolism (Casanueva & Dieguez 1998). Thus, it is possible that, in the present study, the high leptin levels of NIC

Figure 4 Serum FT$_3$ (A), FT$_4$ (B), and TSH (C) at 15, 21, 90, and 180-days-old offspring whose mothers were nicotine or saline exposed during lactation. Liver D1 activity (D) at 15 and 180-days-old offspring. Values represent mean±s.e.m. of 6–12 rats per group. *$P < 0.05$ versus C.

offspring stimulate muscle protein synthesis through GH action, resulting in the higher amount of total body protein content.

Concerning thyroid status, as previously mentioned, we have detected that maternal NIC exposure only during lactation leads to lower thyroid hormone serum concentration in young and adult offspring. This lower serum hormone concentration seems to cause a hypofunction that was confirmed by the lower liver D1 activity on NIC offspring at 15 and 180 days old, since this enzyme activity and/or expression is considered a marker of thyroid status that is decreased in hypothyroidism and increased in hyperthyroidism (Bianco & Kim 2006). In rats, NIC exposure extending from the gestational period to the 10th day of lactation did not change serum thyroid hormones at 10-days-old offspring (Chen & Kelly 2005). However, these authors did not study the thyroid hormones profile in other periods of life. The higher total and central body fat mass of young and adult NIC offspring may be due, at least in part, to their possible hypothyroidism. An earlier adiposity has been reported in children with congenital hypothyroidism, suggesting that thyroid dysfunction during fetal and neonatal life affects body mass index during the first years of life (Livadas *et al.* 2007).

Some studies have shown changes in thyroglobulin, thyroid hormones, TSH, and goiter caused by tobacco (Christensen *et al.* 1984, Ericsson & Lindgrade 1991, Muller *et al.* 1995, Fisher *et al.* 1997, Utiger 1998). There are several mechanisms by which smoking can affect thyroid hormone levels. Tobacco smoke contains thiocyanate that has been shown to be a potential anti-thyroid factor (Meberg & Marstein 1986, Dai *et al.* 1996, Lauberg *et al.* 2004). Our findings showed that early NIC exposure causes a transient thyroid dysfunction during lactation. NIC pups presented lower thyroid hormones and higher TSH at 15 days old, suggesting a primary thyroid hypofunction in neonatal life. After NIC withdrawal, weaned pups presented normal serum T_4; however, TSH and T_3 levels were lower. In a similar way to the thiocyanate (Perron *et al.* 2001, Lauberg *et al.* 2004), it is possible that NIC inhibits the mammary sodium iodide symporter, reducing the supply of iodine to pups during lactation causing hypothyroidism, which was partially corrected by the absence of NIC, at weaning.

Neonatal NIC treatment programs for lower serum TSH in 90-days-old offspring. At 180-days-old, we found lower serum TSH and thyroid hormones concentrations. Both FT_3 and FT_4 are in the lower normal ranges, when we compared with a large sample of FT_3 and FT_4 of rat controls at the same age from different experiments of our laboratory (data not shown). However, for TSH, all of the NIC values were lower than the 10% lowest control values, suggesting a truly thyrotroph hypofunction. These findings suggest the development of extra-thyroidal dysfunction of adult NIC offspring confirmed by lower TSH levels since weaning. The mechanism for this event is still unclear, but it is possible that maternal NIC exposure caused a central hypothyroidism, induced by a hypothalamic–pituitary dysfunction,

with reduced thyrotropin-releasing hormone (TRH)–TSH production and/or release or TRH action on the pituitary.

In the present study, we can not explain whether the effect of programing by maternal NIC exposure is caused by a direct or indirect NIC action. There are, at least, three hypotheses to explain the NIC effects in our experimental model. First, NIC transfer through maternal milk (Dahlstrom *et al.* 1990, Narayanan *et al.* 2002) may change some factor(s) in offspring. Second, maternal alterations caused by NIC treatment, for example, hormonal changes, may be transferred through the milk to the pups. And finally, the programing by NIC exposure can result from both mothers and pups functional changes.

In conclusion, we have demonstrated that maternal NIC exposure, exclusively during lactation, programs for a higher BW gain and adiposity in adult life of the offspring as well as for hyperleptinemia. In addition, early NIC exposure possibly leads to a neonatal thyroid hypofunction and programs for central hypothyroidism, which may partially explain the overweight at adulthood. Then, NIC can be one of the tobacco compounds responsible for the thyroid dysfunctions, and maternal smoking may be considered an important risk factor for the development of thyroid diseases in offspring. Altogether, our present data evidence how the events caused by early NIC exposure only during the critical period of lactation are complex and capable of changing the future development of the offspring, possibly acting as an endocrine disruptor and an obesogen factor. Furthermore, the higher adiposity detected in our model may help to explain the prevalence of obesity in children exposed to cigarette smoke during the perinatal period.

Declaration of interest

Funding

Acknowledgements

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