The CB_1 Receptor Antagonist SR141716A Reverses Adult Male Mice Overweight and Metabolic Alterations Induced by Early Stress

Carina A. Valenzuela[1], Valeska A. Castillo[1], Carolina A. Aguirre[1], Ana M. Ronco[1] and Miguel N. Llanos[1]

Perinatal stress may cause metabolic and hormonal disruptions during adulthood. The aim of this study was to evaluate the effects of early postnatal nociceptive stimulation (NS) on body weight and other metabolic parameters during adulthood and to determine whether CB_1 endocannabinoid receptors (CB_1Rs) may be involved in these effects. Male mice were subjected to NS during lactation with a daily subcutaneous injection of saline solution. Subsequently, both control and NS-mice were treated from day 40 to 130, with an oral dose (1 µg/g body weight) of SR141716A, a specific CB_1R antagonist/inverse agonist. Mice body weight and food intake was periodically evaluated. Adult animals were then killed to evaluate epididymal fat pads and metabolic parameters. NS did not influence food intake in adult animals, but caused significant increases in body weight, epididymal fat pads, and circulating levels of leptin, corticosterone, and triglycerides (TGs). Chronic treatment with SR141716A normalized these parameters, with the exception of corticosterone levels. This treatment also reduced plasma levels of glucose, insulin, and total cholesterol in both adult control and NS-mice. In addition, fatty acid (FA) amide hydrolase (FAAH) activity (the enzyme able to hydrolyze endocannabinoids) from liver and epididymal fat of adult NS-mice was decreased by 40–50% in comparison to activities found in same tissues of control mice. Results suggest that overactive liver and epididymal fat CB_1R due to early NS may be involved in late metabolic alterations, which are sensitive to chronic treatment with SR141716A.

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INTRODUCTION

Studies in animal models as well as available human epidemiological data indicate that adult phenotype and predisposition to develop some diseases may not only lie on genetic factors (1). Long-term adverse health consequences due to inappropriate environmental perinatal conditions may become evident as a result of the metabolic plasticity present in mammalian organisms (2). It has been proposed that fetal programming has contributed to the current striking prevalence of the metabolic syndrome and associate pathologies in adults (1). Early epidemiological studies first showed an inverse relationship between body weight at birth and risk to develop certain chronic pathologies in adulthood (3,4). Subsequent experimental studies using animal models provided further support to those epidemiological data (5,6). Fetal/neonatal programming was then defined as the "induction, suppression, or permanent deficient development of somatic structures by an early stimulus or insult occurred in a critical period of life, resulting in long-term functional consequences" (7). Because obesity is one of the health risk factors that is considered programmable in early life, several studies have evaluated the effects of early stimuli associated to body weight programming. For example, it has been observed that stress-sensitive pregnant rats subjected to a stressful stimulus during the prenatal period deliver pups with long-term effects in their body weight; thus, adult males become 15% heavier than their control counterparts (8). Furthermore, studies performed in genetically nonsusceptible mice models have shown that pups repeatedly subjected to a mild stress during lactation show overweight in adulthood, and have increased inflammatory markers associated to insulin resistance (9,10). Additional work has also demonstrated that these mice have alterations in the levels of glucose, insulin, lipids, leptin, corticosterone, and adrenocorticotrophic hormone (11).

It is known that chronic stress alters metabolism through diverse mechanisms, including hyperactivation of the hypothalamus–pituitary–adrenal (HPA) axis (12). It is well documented that the hypothalamus, among other functions, plays a key role in integrating biochemical and behavioral components involved in the regulation of food ingestion and body weight (13). Among these components, the endocannabinoid system (ECS) has a preponderant role controlling energy balance

[1]Laboratorio de Nutrición y Regulación Metabólica, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile. Correspondence: Miguel N. Llanos (mllanos@inta.cl) or (mllanos16@gmail.com)

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through central regulation of appetite and peripheral energy metabolism (14–16). Studies in both animals and humans have shown a modulating interaction between stress and some components of the ECS (17). Thus, several types of either acute or chronic stress stimuli will trigger endocannabinoids production (mainly anandamide and/or 2-arachydonoyl glycerol) and the subsequent activation of type 1 endocannabinoid receptors (CB_1R) in a time- and site-specific manner in the brain. This is considered to be one of the mechanisms that help the organism to recover from the consequences of stress (18). In this regard, it is possible that CB_1R overactivity in specific tissues could be involved in long-term adverse health effects, as a result of an episode of chronic stress in early life. With this in mind, this study evaluated the effects of early postnatal nociceptive stimulation (NS) on body weight and other metabolic and hormonal parameters during adulthood, and we also analyzed whether these effects implicate a role for CB_1R present in some tissues involved in energy homeostasis.

METHODS

All procedures performed in this study were first analyzed and approved by the Bioethics' Committee for Animal Experimentation of the Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile. Santiago, Chile.

Animals

Synchronously pregnant female CD-1 mice were kept in the animal house under normal conditions of humidity and temperature (22–24 °C), on a 12:12 h light–dark cycle (light on at 0700 hours and then off at 1900 hours). Animals had free access to purified tap water and food. A normal diet of 4 kcal/g, equivalent to 2.8 assimilated kcal/g (Champion, Santiago, Chile), was used during the study. The animal food was composed of cereals, vegetable protein (soy), animal protein (fish and meat), wheat bran, forage, and a mixture of vitamins and minerals. Further analysis indicated that 100 g of animal food had: protein 21 g; total fat 4.5 g; ash 6.4 g; crude fiber 3.8 g; non-nitrogenous energy 55.8 g, and humidity 8.5 g. Experiments were performed in winter to avoid seasonal interference in animal physiology (19).

Perinatal procedures

Procedures were carried out as previously described by Loizzo *et al.* (9,11). From day 16th of pregnancy, female mice were daily examined at 0900 and 1900 hours for the presence of pups. After 12–16 h since the detection of pups, 6–8 litters of homogeneous size (12–14 subjects) were put together and males separated from females. Afterward, six male pups showing homogeneous weights were randomly selected and assigned to a substitute mother so that pups received random cross-lactation. Animals were then randomly assigned to one of the following groups:

1. Control mice: pups were removed daily for a short time from the home cage using a vinyl gloved hand to measure body weight.
2. Stressed, nociceptive-stimulated mice (NS-mice): during the whole lactation (21 days), pups were removed daily from the home cage and each pup was gently picked up using a vinyl gloved hand, weighed, and injected subcutaneously in the back with sterile saline solution (1 µl/g body weight) with a microsyringe (26-gauge needle).

Procedures were always performed by the same experimenter and total time was 8–10 min/cage. Twice a week animals were transferred to clean cages, with fresh clean bedding material.

Procedures and treatments after lactation

At 21 days of age, animals were separated from their mothers and groups of three animals were placed in new cages to avoid isolation-induced stress. Some groups of mice were killed at this age to evaluate levels of circulating corticosterone. Remaining groups were weighed every 10 days until 130 days old.

From day 40 to 130, both control and NS-animals were randomly assigned to receive daily either an oral dose of SR141716A, a CB_1R antagonist (rimonabant, 1 µg/g body weight; Sanofi-Aventis, Paris, France) suspended in no more than 20 µl double-distilled water or only 20 µl of double-distilled water. SR141716A in suspension was kept at −20 °C and strongly agitated just before administration.

At day 130, males were killed according to the guidelines for rodents euthanasia provided by the American Medical Veterinary Association (20).

Food intake measurements

Food intake was measured once a week by subtracting lost food inside the cage due to spilling out. Accumulated food intake amount per cage (three animals) was calculated during the whole experimental period (day 40–130).

Blood collection

Animals aged 21 and 130 days old were anesthetized and killed as stated above (no fasting conditions for measurement of corticosterone; and 6-h fasting for other measurements). After rapid decapitation, trunk blood samples (0.3–1 ml) were collected in tubes containing EDTA at 0–4 °C. Blood samples were centrifuged at 3,500*g* for 12 min at 0–4 °C. After centrifugation plasma was carefully aspirated, transferred to plastic cryotubes, and maintained at −80 °C until analysis.

Plasma glucose, lipids, and hormonal levels

Plasma levels of glucose, triglycerides (TGs), and cholesterol were assessed in triplicate using a commercial enzymatic kit (DIALAB, Neudorf, Austria). Corticosterone levels were determined in duplicate using a commercial ELISA Kit according to the manufacturer instructions (cat. no. 500651; Cayman Chemical, Ann Arbor, MI). Plasma leptin levels were assessed in duplicate using a commercial colorimetric sandwich ELISA Kit (cat. no. MOB00; R&D Systems, Minneapolis, MN). A commercial ELISA kit was also used to measure plasma insulin concentrations from duplicate samples (cat. No. EZMADP-60K; Linco Research, St Charles, MO).

Intraperitoneal glucose tolerance test

Glucose tolerance tests were performed in control and NS-mice. Animals were first fasted during 6 h and subsequently injected with a glucose solution (1.5 mg/g body weight intraperitoneally). Tail blood was collected at 0, 15, 30, 60, 90, and 120 min relative to glucose injection. Blood glucose levels were determined with an Accu-Check Performa glucometer (Roche Diagnostics, Mannheim, Germany).

FAAH activity determinations

Procedures were mainly performed as previously described by Maccarrone *et al.* (21). Fatty acid (FA) amido hydrolase (FAAH) activity was determined in liver and epididymal fat from control and NS-mice after surgical removal. Tissues were homogenized with a Heidolph homogenizer DIAX 600 in ice-cold Tris–EDTA buffer (50 mmol/l Tris–HCl, pH 7.4, 1 mmol/l EDTA). The homogenates were sequentially centrifuged at 800*g* for 5 min and 11,000*g* for 20 min at 4 °C. The final pellets were resuspended in Tris–EDTA buffer, quickly frozen in liquid nitrogen, and stored at −80 °C until use.

The assay of FAAH was performed by measuring the release of ^{3}[H]-arachidonic acid (AA) from ^{3}[H]-anandamide (Arachidonyl 5,6,8,9,11,12,14,15-^{3}[H]); 215 C_i/mmol; NEN Radiochemicals, Boston, MA) using thin-layer chromatography to separate hydrolysis products.

Reaction was initiated by the addition of tissue homogenate (30–40 µg protein) and after 15-min incubation at 37 °C was stopped with 1 ml of ice-cold methanol/chloroform mixture (2:1 vol/vol). Specific FAAH activity was expressed as pico moles of released AA/mg protein/min.

Statistical analysis

Results are expressed as mean ± s.e.m. To compare body weight, amount of epididymal fat, hormonal and lipid plasma concentrations, and accumulated total food intake of different experimental groups, the nonparametric Kruskal–Wallis test was applied. When significant differences were found among groups ($P < 0.05$), paired comparisons using the nonparametric Mann–Whitney U test were done. This last test was also utilized to compare GTT and FAAH activity results. Shapiro–Wilk's W and Levene tests were previously done to evaluate normal distribution of data and homogeneity of variances.

RESULTS

Body weight and epididymal fat

Figure 1a shows the increase in body weight during lactation in both control and NS-mice. No significant differences in weight gain were observed between both groups throughout lactation. However, during adulthood early NS-mice had body weights different to control, non-NS animals. From 50 to 130 days of age, NS-mice had a significantly 7% higher body weight than control animals (Figure 1b).

Daily SR141716A-treatment significantly decreased the body weight of NS-mice compared to vehicle-treated NS-mice. This effect was observed from day 60 to 130 (Figure 2). When control, non-NS mice were treated with SR141716A, a nonsignificant trend toward a decrease in body weight was also observed (for instance, 130-day-old control mice had 49.9 ± 5.5 g and SR-treated control mice had 47.3 ± 3.2 g; mean ± s.d., $n = 18$/group). In addition, when comparing the mean adult weight of SR141716A-treated NS-mice with that of SR141716A-treated control mice, no significant differences were observed (49.7 ± 4.5 g vs. 47.3 ± 3.2 g, respectively; mean ± s.d., $n = 18$/group).

Adult NS-mice had a significant higher amount of epididymal fat than control animals. Chronic SR141716A-treatment resulted in a significant decrease in epididymal fat in both groups (Figure 3).

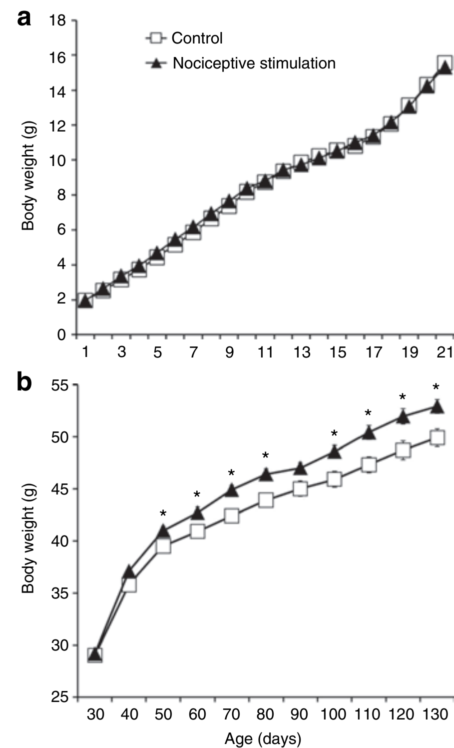

Figure 1 Time course of body weight of nociceptive-stimulated and control mice (**a**) during lactation, and (**b**) at different ages from day 30 to 130. $*P < 0.05$ Mann–Whitney U test (mean ± s.e.m.; $n = 30$/group). All error bars are included, but some of them are masked by the symbols size.

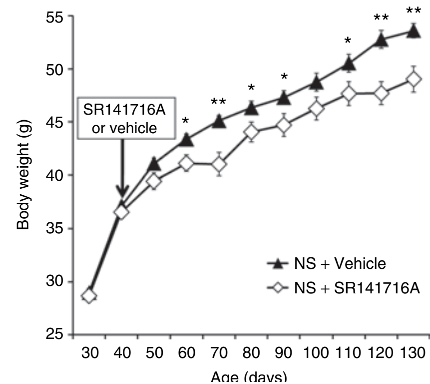

Figure 2 Body weight of nociceptive-stimulated mice under chronic SR141716A-treatment from day 40 to 130. $*P < 0.05$; $**P < 0.01$ Mann–Whitney U test (mean ± s.e.m.; $n = 18$/group). Some error bars are masked by the symbols size. NS, nociceptive stimulation.

Hormonal levels

Corticosterone. Plasma levels of immunoreactive corticosterone (ir-CTT) in both NS and control mice were measured to evaluate the magnitude of stress. Mean concentration of ir-CTT in 21-day-old animals was similar (about 55–60 ng/ml) in both groups. Nevertheless, at 130 days of age ir-CTT levels decreased to about 15 and 40 ng/ml in control and NS-mice, respectively. Therefore, adult animals subjected to a nociceptive stress during lactation had significantly higher levels of ir-CTT than control mice. SR141716A-treated control and NS-mice had similar plasma ir-CTT concentrations (Table 1).

Leptin. Adult plasma leptin levels were significantly higher in NS-mice than in controls, a result in accordance with the observed higher amounts of epididymal fat in NS-animals. In SR141716A-treated animals, leptin levels showed a marked decrease both in control and NS-mice (Table 1).

Insulin. Adult insulin plasma levels were similar in control and NS-mice. These values were significantly decreased when animals from both groups were treated with SR141716A (Table 1).

Plasma biochemical markers

Lipids and glucose. With regard to glucose levels, although there is a trend to higher values in NS-mice, this difference is not significant when compared with control mice. In SR141716A-treated control and NS-mice, there is a similarly significant decrease in the value of glycemia (Table 1).

Table 1 Effect of SR141716A on plasma levels of hormones and biochemical markers in 130-day-old control and nociceptive-stimulated (NS) mice

Plasma level of	Control+vehicle	NS+vehicle	Control+SR141716A	NS+SR141716A
Corticosterone (ng/ml)	15.6 ± 5.6[a]	41.3 ± 5.6[b]	44.2 ± 12[b]	36.7 ± 15.7[b]
Leptin (ng/ml)	11.1 ± 2.2[a]	19.2 ± 2.1[b]	1.78 ± 1.2[c]	6.97 ± 1.5[a]
Insulin (ng/ml)	0.83 ± 0.12[a]	1.12 ± 0.09[a]	0.21 ± 0.0003[b]	0.24 ± 0.024[b]
Glucose (mg/dl)	168.9 ± 12.7[a]	187.7 ± 20.6[a]	96.1 ± 3.01[b]	95.31 ± 6.8[b]
Triglycerides (mg/dl)	89.6 ± 9.9[a]	115.78 ± 10.3[b]	97.2 ± 16.9[a]	87.6 ± 2.8[a]
Cholesterol (mg/dl)	106.5 ± 2.6[a]	103.77 ± 4.8[a]	85.9 ± 1.8[b]	74 ± 7.1[b]
LDL (mg/dl)	45.5 ± 4.3[a]	31.95 ± 6.8[b]	8.7 ± 2.7[c]	ND
HDL (mg/dl)	43.4 ± 2.7[a]	48.56 ± 3.2[a]	55.7 ± 0.4[b]	55.6 ± 1.7[b]

Each data corresponds to the mean ± s.e.m. (n = 7/group). Different letters indicate significantly different values (P < 0.05, Mann–Whitney U test).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; ND, non detectable (LDL levels below detection limit).

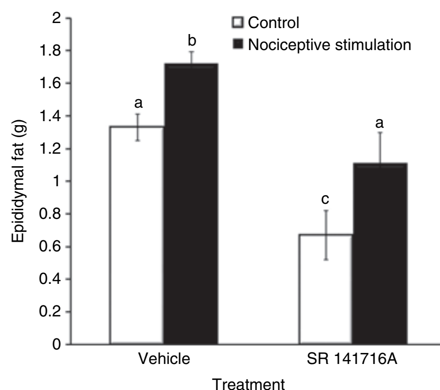

Figure 3 Effect of chronic SR141716A-treatment on the amount of epididymal fat pads of adult control and nociceptive-stimulated mice. Different letters indicate significantly different values (P < 0.05; Mann–Whitney U test; mean ± s.e.m.; n = 12/group).

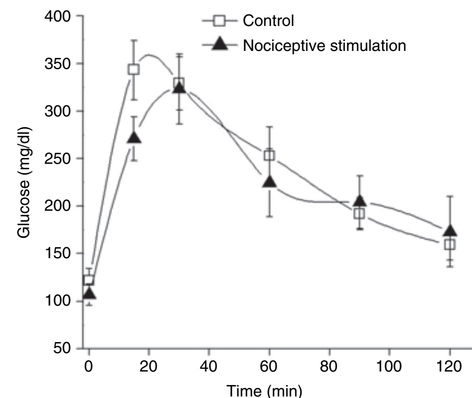

Figure 4 Time course of blood glucose concentrations during intraperitoneal glucose tolerance test (1.5 mg/g body weight) in adult control and nociceptive-stimulated mice. Values obtained from each group (mean ± s.em.; n = 6/group) are not significantly different.

TG plasma levels of NS-mice were 30% higher than levels present in control animals. When NS-mice were treated with SR141716A, TG levels diminished to levels similar to those of control mice. Antagonist treatment did not significantly affect the TG levels of control animals (Table 1).

Cholesterol levels were similar in control and NS-mice. SR141716A-treatment in control mice decreased cholesterols level by 23%; however, in NS-mice a 40% reduction was found.

Adult, 130-day-old NS-mice had low-density lipoprotein-cholesterol levels 30% lower than control mice. As for total cholesterol, both SR141716A-treated groups had a significant decrease in their respective low-density lipoprotein-cholesterol levels. On the other hand, high-density lipoprotein-cholesterol levels were similar in control and NS-mice, which were significantly increased at the same extent after SR141716A-treatment (Table 1).

Intraperitoneal glucose tolerance test

When the glucose tolerance test was performed in 130-day-old animals, no significant differences in blood glucose levels at any time point between control and NS-mice were found (Figure 4).

FAAH activity determinations

When FAAH activity was measured in liver from NS and control mice, it was found that NS-mice had a reduced activity in this tissue; indeed, it was a 62.4 ± 12.9% of the activity found in control liver. Similar results were obtained when FAAH activity was measured in epididymal fat from both groups. In this case, activity was reduced to a 53.8 ± 7.9% of the activity value found in epididymal fat obtained from control animals. In both groups, it may be noteworthy that liver FAAH activity was markedly higher than the activity present in epididymal fat (Table 2).

Food intake

When analyzing five cages (three animals per cage) from different groups to determine accumulated food intake during 12 weeks (day 40–130), we observed that control mice ate a similar amount of food than NS-mice (1,127 ± 40 g vs. 1,085 ± 60 g, respectively; mean ± s.d.). Same results were obtained when comparing SR141716A-treated control and NS-mice (1,057 ± 156 g vs. 1,062 ± 107 g, respectively; mean ± s.d.).

DISCUSSION

Results of this study demonstrate that genetically nonsusceptible mice subjected to early NS during lactation (NS-mice),

Table 2 Liver and epididymal fat FAAH activity from adult control and nociceptive-stimulated mice

	FAAH activity (pmol/mg protein/min)[a]		
Tissue	Control	Nociceptive stimulated	P^b
Liver	513 ± 90	320 ± 66	0.049
Epididymal fat	125.4 ± 15.6	67.5 ± 9.9	0.011

FAAH, fatty acid amide hydrolase.
[a]Values represent the mean ± s.e.m. (tissues were extracted from five 130-day-old animals per experimental group). [b]Mann–Whitney *U* test.

become overweight and acquire permanent metabolic perturbations in adulthood, such as increased levels of leptin, TGs, and ir-CTT. Most of these changes can be reversed by chronic treatment with SR141716A, a selective antagonist/reverse agonist of CB_1R. Moreover, NS-mice show increased epididymal fat accumulation and reduced FAAH activity (the enzyme able to hydrolyse endocannabinoids) in liver and epididymal fat.

With regard to the stress stimuli, it is important to note that at the end of lactation ir-CTT levels were similar in NS-mice and controls. This suggests that soft manipulation of control animals during lactation (cage changes, daily weight evaluation, and initial separation from their mothers) may have constituted stressful enough activities, and thus generating ir-CTT levels comparable to those levels induced by a daily subcutaneous injection of saline solution. Interestingly, in adulthood (130 days old) ir-CTT levels were significantly higher in NS-mice than in control mice, which might indicate that the early painful stimulation was able to program a permanent alteration in the regulation of HPA axis, leading to permanent elevated ir-CTT levels. It has been described that a chronic stressful stimulus increases endocannabinoid production and activation of CB_1R in a time and site-specific manner in the brain, as a homeostatic mechanism to avoid consequences of stress (17). This fact suggests the presence of an endocannabinoid tone able to exert CB_1R-mediated inhibitory actions on the HPA axis activation. In our study, SR141716A-treatment was not effective in reducing ir-CTT concentrations of adult NS-mice, indicating that blockade of CB_1R does not further alter glucocorticoids levels when the HPA axis has been previously activated. Furthermore, the antagonist induced a significant increase of ir-CTT levels in control mice, a finding which is also consistent with previous results (17). Therefore, when blocking CB_1R, endocannabinoids would be unable to exert an inhibitory action on the HPA axis and thus circulating corticosterone levels would increase. Interestingly, these ir-CTT levels present in SR141716A-treated control animals were not different from those found in SR141716A-treated NS-mice. This suggests that systemic corticosterone levels alone would not explain the weight gain and metabolic abnormalities observed in NS-mice; an involvement of CB_1R activity should be also necessary.

During lactation, NS and control mice displayed a similar body weight, indicating that body weight alterations in adulthood are not a consequence of differences in early feeding or consumption patterns. Moreover, food intake, either weekly or cumulative during 12 weeks, did not significantly differ between control and NS-mice. These results suggest that overweight generated by early NS might be due to alterations in the peripheral control of energy metabolism and not at the central regulatory level of appetite. In this sense, in addition to endocannabinoids actions on appetite (14,15), they also stimulate *de novo* FA synthesis in liver (16) and TG accumulation in adipose tissue (15). All these effects—absent in mice lacking CB_1R ($CB_1^{-/-}$)—could be inhibited by SR141716A in the wild-type mice (16,22–24). In fact, in this study, reduced activity of CB_1R due to chronic SR141716A-treatment could antagonize late effects of early NS on increased epididymal fat pads and total body weights. Early stress effects on adult body weight and epididymal fat pads were previously described by Loizzo *et al.* (11). These authors found larger fat pads in stressed mice than in control animals, concomitant to a high percentage of epididymal adipocytes with significantly increased volume, indicating highly efficient TG storage. Lower accumulation of epididymal fat in both SR141716A-treated control and NS-mice found in our study, may indicate that the blockade of CB_1R at the adipocyte level may be influencing TG storage (15,23–26). Increased epididymal fat mass observed in NS-mice was directly related to higher levels of plasma leptin found in these mice. However, after SR141716A-treatment, leptin concentrations were significantly decreased in NS and control mice presumably as a result of epididymal fat reduction.

In this study after 6 h of fasting, NS-mice did not show either elevated levels of plasma insulin or hyperglycemia. On the contrary, NS-mice had similar insulin and glucose levels to those found in control animals. In addition, when both groups of animals were subjected to a glucose tolerance test, no different results were obtained (**Figure 4**). A previous study concluded that painful manipulations administered early in life are able to modify pancreas programming leading to glucose intolerance along with elevated insulin levels in adult mice (11). Nevertheless, when comparing insulin and glucose plasma levels reported in the aforementioned study with results from recent studies (27,28), it may be concluded that the former values are lower than normal ranges found in CD-1 mice fed with different diets (plasma glucose 100–200 mg/dl; plasma insulin 0.5–3.5 ng/ml) (ref. 28). Therefore, previously reported statements about this matter (11), should be reviewed at the light of these results. Although glucose and insulin levels present in NS-animals are within normal ranges, administration of SR141716A produced a significant reduction of these values in both NS and control animals. Treatment with SR141716A may affect glucose homeostasis through its action at different levels (29), thus leading to augmented insulin sensitivity and decreased glucose circulating levels such as those found in this study.

Circulating TG levels in NS-mice were higher by 30% compared to levels of control mice. This may be an indication of augmented TG synthesis in hepatocytes, which is likely due to increased *de novo* synthesis of FAs. It has been shown that activation of CB_1R present in hepatocytes leads to *de novo* synthesis of FA *via* the induction of the expression of lipogenic

transcription factor steroid regulatory element–binding protein and its target enzymes, acetyl CoA carboxylase 1 and FA synthase (16). Preliminary data obtained in our laboratory (not shown) indicate no difference either in liver or epididymal fat CB_1R expression between control and NS-mice. However, in this study we have shown a significant decrease of FAAH activity in liver and epididymal fat of NS-mice. This situation could lead to a greater availability of endocannabinoids for a sustained CB_1R activity present in both tissues. Under these circumstances, *de novo* FA synthesis in liver cells may be increased as a result of these overactive CB_1R, which finally may result in elevated circulating levels of TG (as very-low-density lipoprotein). On the other hand, overactive CB_1R in epididymal fat may result in increased lipoprotein lipase activity (15) and subsequent availability of free FAs to be finally stored as TG in adipocytes. All these metabolic pathways may be blocked by targeting CB_1R with SR141716A. Interestingly, only NS-mice showed decreased levels of circulating TG after treatment with SR141716A, a result suggesting a greater susceptibility of this overactive liver CB_1R to the antagonist. In addition, SR141716A-treatment also resulted in a significant improvement in the lipid profile in both NS and control groups; this fact may indicate a CB_1R-mediated shift from carbohydrate to lipid oxidation as elegantly demonstrated in a previous report (30).

A key role for FAAH activity in energy homeostasis has been recently shown in FAAH-deficient ($FAAH^{-/-}$) mice. These $FAAH^{-/-}$ animals have increased body weight and total amount of adipose tissue, among other metabolic alterations (31). These findings are similar to those found here in NS-mice with reduced liver and epididymal fat FAAH activity. Furthermore, the importance of FAAH in overweight /obesity has been already reported in human subjects (32).

In conclusion, this study shows that aversive neonatal environmental factors play an important role in the development of a modified phenotype in adult mice. NS during lactation may modify HPA axis and peripheral ECS programming and regulation, evidenced in this study as a decreased FAAH activity in some tissues involved in energy homeostasis, which in turn could lead to hormonal and metabolic alterations during the adult life.

Although it is not possible to completely extrapolate animal model results to human subjects, it may be suggested that perinatal insults could generate an ECS-mediated abnormal programming leading to adult phenotypes more susceptible to physiopathological alterations. It is of further concern that this abnormal programming might be transmitted to subsequent generations *via* epigenetic mechanisms, which could be particularly relevant for those changes in population health (such as increasing obesity) which cannot be explained by genomic changes (2).

Finally, greater knowledge about the biochemical and molecular mechanisms related to the unhealthy consequences during adulthood of early aversive environmental conditions will provide new preventive and therapeutic strategies, in which ECS modulation could play a key role.

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DISCLOSURE

The authors declared no conflict of interest.

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