High Dietary Sodium Intake Increases White Adipose Tissue Mass and Plasma Leptin in Rats

Miriam H. Fonseca-Alaniz, Luciana C. Brito, Cristina N. Borges-Silva, Julie Takada, Sandra Andreotti, and Fabio B. Lima

Abstract

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Objective: Salt restriction has been reported to increase white adipose tissue (WAT) mass in rodents. The objective of this study was to investigate the effect of different sodium content diets on the lipogenic and lipolytic activities of WAT.

Research Methods and Procedures: Male Wistar rats were fed on normal-sodium (NS; 0.5% Na^+), high-sodium (HS; 3.12% Na^+), or low-sodium (LS; 0.06% Na^+) diets for 3, 6, and 9 weeks after weaning. Blood pressure (BP) was measured using a computerized tail-cuff system. At the end of each period, rats were killed and blood samples were collected for leptin determinations. The WAT from abdominal and inguinal subcutaneous (SC), periepididymal (PE) and retroperitoneal (RP) depots was weighed and processed for adipocyte isolation, rate measurement of lipolysis and D-[U-^{14}C]-glucose incorporation into lipids, glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme activity evaluation, and determination of G6PDH and leptin mRNA expression.

Results: After 6 weeks, HS diet significantly increased BP; SC, PE, and RP WAT masses; PE adipocyte size; plasma leptin concentration; G6PDH activity in SC WAT; and PE depots and malic activity only in SC WAT. The leptin levels correlated positively with WAT masses and adipocyte size. An increase in the basal and isoproterenol-stimulated lipolysis and in the ability to incorporate glucose into lipids was observed in isolated adipocytes from HS rats.

Discussion: HS diet induced higher adiposity characterized by high plasma leptin concentration and adipocyte hypertrophy, probably due to an increased lipogenic capacity of WAT.

Key words: adipocytes, lipolysis, lipogenesis, leptin, blood pressure

Introduction

Obesity causes or exacerbates many health problems including respiratory complications, coronary heart disease, type 2 diabetes mellitus, osteoarthritis, and hypertension (1). Data from the Third National Health and Nutrition Examination Survey showed a sharp increase in the prevalence of high blood pressure (BP)[1], with an increasing BMI —causing an excessive accumulation of white adipose tissue (WAT)—a significant risk factor for the development of hypertension (2).

WAT growth involves the storage of triacylglycerol (TAG) in lipid droplets inside the adipocytes as a result of fatty acid (FA) esterification with glycerol-3-phosphate. There are two sources of FA: 1) uptake from plasma lipoproteins (chylomicrons and very-low-density lipoproteins) and 2) intra-cytoplasmatic de novo FA synthesis from acetylcoenzyme A. The latter process is assumed to be the

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[1] Nonstandard abbreviations: BP, blood pressure; WAT, white adipose tissue; TAG, triacylglycerol; FA, fatty acid; SNS, sympathetic nervous system; NS, normal-sodium; LS, low-sodium; HS, high-sodium; HR, heart rate; SC, subcutaneous; PE, periepididymal; RP, retroperitoneal; G6PDH, glucose-6-phosphate dehydrogenase; RT-PCR; reverse transcriptase-polymerase chain reaction; SE, standard error.

real lipogenesis. It occurs in association with positive energy balance and depends on the supply of lipogenic substrates and co-factors (such as the reduced form of nicotinamide adenine dinucleotide phosphate), as well as on metabolically-related enzyme activation (3).

Lipid mobilization from adipocytes, known as lipolysis, consists of TAG hydrolysis and free FA and glycerol release. This process is triggered by lipolytic hormones, mainly catecholamines, acting through β-adrenoceptor activation and represents an important mechanism for controlling WAT mass (4).

Adipocytes also act as endocrine cells and secrete a wide variety of hormones, cytokines, and other bioactive compounds such as leptin, tumor necrosis factor-α, interleukin-6, angiotensinogen, plasminogen activator inhibitor-1, adiponectin, adipsin, and resistin, among others (5). The *ob* gene product, known as leptin, is an adipocyte-derived hormone that regulates appetite and energy expenditure, and its gene expression and plasma concentration reflect body fat content (5–7). Leptin has been implicated in the generation of obesity-induced hypertension due to stimulation of hypothalamic regions that are part of the central sympathetic nervous system (SNS) (8).

Dietary sodium chronic restriction, an important nonpharmacological approach for the prevention and treatment of hypertension (9–11), has been related to increased WAT mass in rats (12–14). If this increase in adiposity observed in rodents also applies to human beings, it could mitigate the beneficial value of this therapeutic approach in reducing BP in hypertensive subjects. Moreover, if this increased adiposity is associated with high leptin plasma concentration, the potential injury can be even higher.

There is no information regarding the effect of this intervention on WAT lipogenic and lipolytic fluxes. As both processes contribute to the size of adipose depots, we aimed to investigate the mechanism by which the amount of sodium intake influences lipolysis and the activity of some enzymes involved in lipid synthesis. The effect of salt on gene expression and synthesis of molecules involved in the control of BP, such as leptin, was also evaluated.

Research Methods and Procedures

Animals

Male Wistar rats from the Animal Resource of the Institute of Biomedical Sciences of the University of Sao Paulo were fed from weaning to adulthood with either normal-sodium (NS; 0,5% Na^+; $n = 10$), low-sodium (LS; 0,06% Na^+; $n = 10$), or high-sodium (HS; 3,12% Na^+; $n = 10$) diet (RHOSTER Industry and Limited Commerce, Vargem Grande Paulista, Brazil). Rats (two per cage) were housed in a controlled-temperature environment (25 $\pm$ 2 °C), under a 12/12-hour light/dark cycle (lights on at 6:00 AM) and with food and water ad libitum. Body weights, as well as food and water consumption, were measured weekly. All experimental procedures reported here were in accordance with the Guidelines for Ethical Care of Experimental Animals and were approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences of the University of Sao Paulo (No. 218/02). Tail-cuff systolic BP and heart rate (HR) were determined during the 6th week of the study period. Animals were killed (8:00 AM) at the end of the 3rd, 6th, and 9th weeks, by decapitation under pentobarbital sodium anesthesia (4 mg/100 g body weight, intraperitoneally) after a 12-hour fast. Trunk blood was collected in heparinized tubes and used for plasma leptin measurement. The subcutaneous (SC) WAT from abdominal and inguinal depots and both periepididymal (PE) and retroperitoneal (RP) fat pads were totally excised, weighed, and processed for adipocyte isolation and measurement of in vitro rates of lipolysis and incorporation of D-[U-^{14}C]-glucose into lipids. Samples of SC and PE WAT were also removed for evaluation of glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme maximal activities, as well as G6PDH and leptin mRNA expression.

Tail-Cuff BP and HR Measurements

BP and HR were measured using a computerized tail-cuff system (Kent Scientific, Torrington, CT). For BP measurement, rats were placed in a warm rat-restraining apparatus. A cuff was placed around the rat's tail and insufflated until blood flow was occluded and then released until the first pulses of arterial flow could be detected and recorded (systolic BP) on a microcomputer (AT/CODAS, 100-Hz sampling rate; DataQ Instruments, Inc., Akron, OH). Three BP measurements were performed, and BP was determined for each animal by averaging the obtained values. HR was obtained from BP pulse records.

Adipocyte Isolation

SC and PE fat pads were minced with fine scissors, digested at 37 °C in Earle's salts, 25 mM HEPES (N-2-hydroxyethylpiperazine′-N-2-ethanesulfonic acid), and 4% bovine serum albumin, pH 7.4 (EHB buffer) containing collagenase type II (1.25 mg/mL), and the adipocytes were isolated according to Rodbell (15). The isolated adipocytes ($\sim$7 to 8 $\times$ 10^5 cells/mL) were suspended in EHB buffer (Earle's salts, 20 mM HEPES, 1% bovine serum albumin, 2 mM sodium pyruvate, and 4.8 mM sodium bicarbonate), pH 7.4, at 37 °C. Cell size and number were determined as previously described by DiGirolamo et al. (16).

D-[U-^{14}C]-Glucose Incorporation Into Lipids

From a 10% adipocyte suspension in Krebs/Ringer/phosphate buffer pH 7.4, with 1% bovine serum albumin and 2 mM glucose, at 37 °C and saturated with a gas mixture of CO_2 (5%)/O_2 (95%), 450-μL aliquots were transferred to polypropylene test tubes containing 5 μL (0.05 μCi/tube) of

D-[U-^{14}C]-glucose, in the presence or absence of insulin (10 nM). These samples were then incubated (final volume = 500 μL) for 1 hour at 37 °C in a water bath. After incubation, the mixture was acidified with 0.2 mL sulfuric acid (8 N) and incubated for an additional 30 minutes. At the end of incubation, the reaction mixture was treated with 2.5 mL of Dole's reagent (isopropanol:*n*-heptane:sulfuric acid, 4:1:0.25 vol/vol/v) for lipid extraction (15). The results were expressed as nanomoles of glucose incorporated into lipids/10^6 cells/h.

Measurement of G6PDH and Malic Activities

Maximal G6PDH and malic activities were evaluated in samples of SC (~0.3 g) and PE (~1.0 g) WAT according to protocols described elsewhere (17,18). Enzymatic activity was determined as the amount of enzyme that reduced 1 nmol of nicotinamide adenine dinucleotide phosphate /min/mg protein.

Measurement of Lipolysis

From a 20% adipocyte suspension in Krebs/Ringer/Phosphate buffer pH 7.4, with 1% bovine serum albumin, 170-μL aliquots were transferred to microfuge tubes (1.5 mL) containing 20 μL of adenosine deaminase (0.2 U/mL) and were incubated at 37 °C for 5 minutes to allow the degradation of endogenous released adenosine, which is a potent inhibitor of lipolysis (19). After this period, these samples were incubated for 1 hour at 37 °C in the presence or absence of 10 μL of isoproterenol (10^{-5} mol/L), in a final volume of 200 μL. At the end of incubation, the reaction was blocked by moving the tubes to a cold water bath, followed by centrifugation at 3500 g for 5 minutes at 4 °C. The infranatant was carefully transferred to microtubes containing 150 μL of silicone oil and recentrifuged at 3500 g for 2 minutes. The glycerol content of the incubation medium was measured using an enzymatic-colorimetric assay (Glycerol Reagent from TAG enzymatic kit; CELM-Cia, São Paulo, Brazil). It was used as an index of lipolysis and was corrected to be expressed as nanomoles/10^6 cells/h.

Plasma Leptin Measurement

Plasma leptin levels were quantified using a specific rat leptin radioimmunoassay kit (Linco Research, St. Charles, MO). The estimated intra-assay coefficient of variation was <5%.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Assay for G6PDH and Leptin Gene Expression

Total RNA was extracted from WAT by use of guanidine isothiocyanate-based TRIzol solution, according to the manufacturer's specifications (20), and quantified spectrophotometrically at 260 nm. Superscript II RT was used to reversely transcribe 5 μg of total RNA isolated using an oligo(dT)$_{10n}$ primer in a total reaction volume of 20 μL. After the RT reaction, 4-μL aliquots were used as cDNA template for PCR amplifications with 2.5 U (0.5 μL) *Taq* DNA polymerase, 2.5 mM $MgCl_2$, 0.25 μM each of the sense and antisense primers, and 10 mM deoxy-NTP mixture (21). PCR reactions (50 μL) were performed in a Gradient Mastercycler (Eppendorf, Hamburg, Germany) with the following profile: 95 °C for 45 seconds, 60 °C for 45 seconds, and 72 °C for 30 seconds, 35 and 32 cycles for leptin in SC and PE WAT, respectively, and 95 °C for 45 seconds, 62 °C for 45 seconds, and 72 °C for 30 seconds, 32 cycles for G6PDH in SC and PE WAT. The primers used (rat leptin sense primer, 5′-CTC AGC ATT CAG GGC TAA GG-3′; rat leptin antisense primer, 5′-AAG CCT CGC TCT ACT CCA CA-3′; rat G6PDH sense primer, 5′-CCA TAG ACA TAC GGG ATG GG-3′; rat G6PDH antisense primer, 5′-CAA CCC TGA GGA GTC TGA GC-3′) amplified 102-bp fragments for leptin and 216 bp for G6PDH. Rat RPL-37a was chosen as the internal control for the integrity of the mRNA, and the primers used (rat RPL-37a sense primer, 5′-CAA GAA GGT CGG GAT CGT CG-3′; rat RPL-37a antisense primer, 5′-ACC AGG CAA GTC TCA GGA GGT G-3′) resulted in a product of 290 bp. The reaction products were separated by agarose gel (2%) electrophoresis, stained with ethidium bromide, and analyzed by scanning densitometry (Eagle Eye-Stratagene, model 401,304, software Eagle Sight 3.2). Samples were normalized to RPL-37a mRNA levels and presented as arbitrary units of leptin and G6PDH mRNA relative to control.

Statistical Analyses

Statistical procedures were performed using one-way ANOVA, followed by Bonferroni post-tests for multiple comparisons among groups. The Pearson correlation coefficient (r) was used for correlation of plasma leptin levels with PE and SC adipocyte volume and SC, PE, and RP fat pad weights. Data are expressed as mean ± standard error (SE). p Values <0.05 were considered statistically significant. The analysis was performed by the statistical software package GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA).

Results

Body Weight, Food and Water Intake

No significant difference ($p > 0.05$) was observed in the body weights of the NS, LS, and HS groups at weaning (3 weeks) and during the 9-week follow-up period of study (Figure 1A). The measurements of food and water consumption were performed two to three times a week, and the results were determined for each group of rats by averaging the values obtained during that week. From the second to the fifth week of diet, the food ingestion was lower in the LS group ($p < 0.05$) than in the HS group. LS rats showed lower food consumption than NS rats at the third and fourth

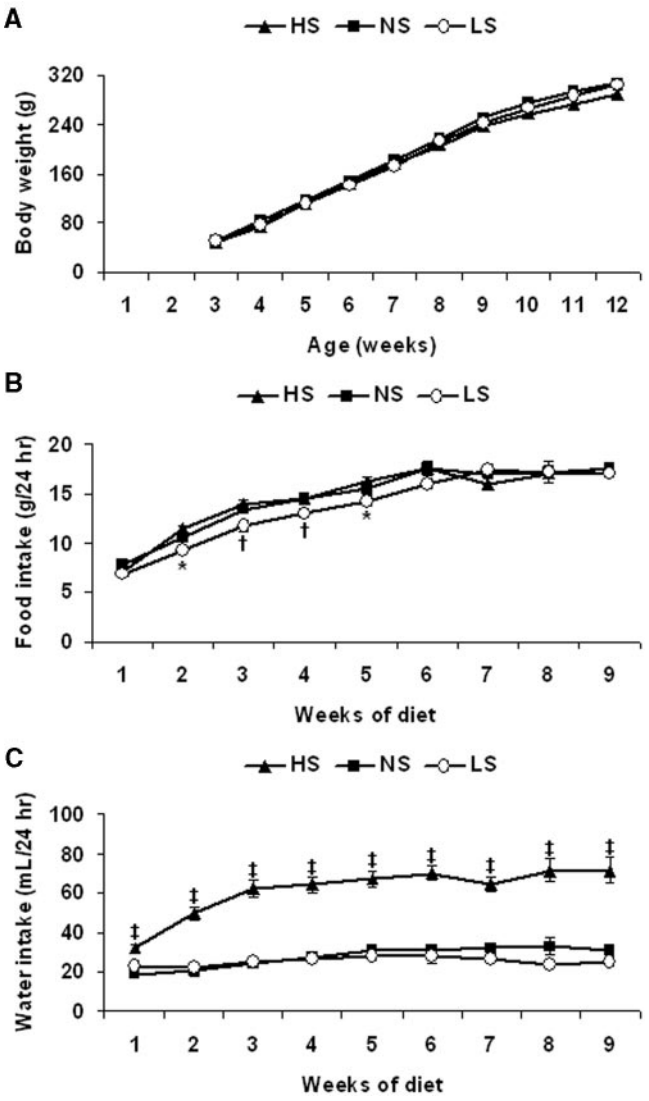

Figure 1: Effect of chronic administration of LS, NS, and HS diets on (A) body weight, (B) food intake, and (C) water intake of rats. Values are mean ± SE, $n = 8$ or 9 rats/group. * $p < 0.05$ vs. HS values of the same week; † $p < 0.05$ vs. NS and HS values of the same week; ‡ $p < 0.001$ vs. NS and LS values of the same week.

weeks of observation (Figure 1B). The area under the curve of food intake was significantly ($p < 0.05$) reduced for the LS group (110.85 ± 1.40 g/9 weeks) compared with the HS group (119.18 ± 2.58 g/9 weeks). Throughout the 9 weeks of the experimental protocol, the water intake was higher in HS rats compared with NS and LS rats ($p < 0.001$) (Figure 1C).

BP and HR

In the sixth week of diet, systolic BP was higher ($p < 0.001$) in rats on the HS diet than in those on the NS or LS diet (Figure 2). No significant difference was observed in HR among the groups in this period (data not shown).

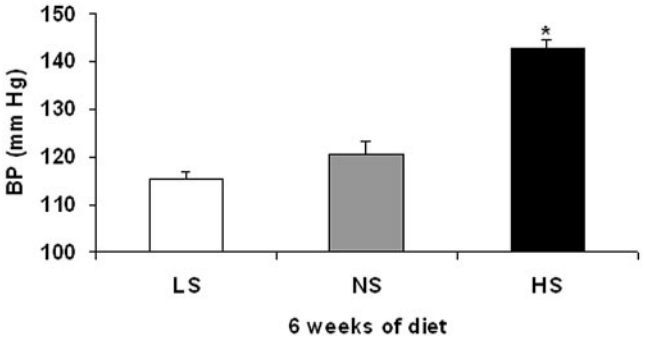

Figure 2: Effect of chronic (6 weeks) administration of LS, NS, and HS diets on systolic BP of rats. Values are mean ± SE, $n = 7$ or 8 rats/group. * $p < 0.001$ vs. NS and LS.

Fat Pad Weights and Adipocyte Size

As indicated in Table 1, the SC, PE, and RP WAT weights were higher in HS rats than in NS and LS rats ($p < 0.01$) after 6 weeks of treatment. The volume of PE adipocytes was increased in HS rats ($p < 0.01$) compared with NS and LS rats, at the same week of study, whereas SC adipocyte size did not differ significantly among the three groups throughout the nine-week period.

Leptin Levels and mRNA Expression

HS animals exhibited higher plasma leptin levels ($p < 0.001$) than NS and HS rats after 6 weeks of diet (Figure 3A). The leptin values obtained for rats on the HS diet were positively correlated with SC ($r = 0.7091$, $p < 0.0001$), PE ($r = 0.5920$, $p < 0.01$), and RP ($r = 0.6963$, $p < 0.0001$) fat pad weights and with PE ($r = 0.5832$, $p < 0.01$) adipocyte volume, in the sixth week of study. Although administration of the HS diet resulted in a significant increase in plasma leptin concentration in the sixth week after the beginning of the diet, leptin mRNA levels were similar among the three experimental groups ($p > 0.05$) in both SC and PE fat depots (Figure 3B).

Insulin-stimulated Rates of D-[U-^{14}C]-Glucose Incorporation Into Lipids

SC adipocytes isolated from HS animals showed the highest level of basal and maximal insulin-induced glucose incorporation into lipids in relation to the cells of LS animals after 6 weeks of diet (Figure 4). HS animals showed a significant increase in the basal (121.66 ± 17.70 nmol/10^6 cells/h, $n = 7$), as well as in the maximal (325.39 ± 31.93 nmol/10^6 cells/h, $n = 8$), rates of glucose incorporation into lipids in PE adipocytes in comparison with NS rats (42.30 ± 4.04 and 135.51 ± 15.74 nmol/10^6 cells/h, $n = 7$ or 8, respectively), and in the maximal rate in comparison with LS rats (197.25 ± 21.32 nmol/10^6 cells/h, $n = 7$). The difference between maximal and basal rates was signifi-

Table 1. Effect of chronic (3, 6, and 9 weeks) administration of LS, NS, and HS diets on fat pad weights and adipocyte volume

Group	SC fat pad weight (g)	PE fat pad weight (g)	RP fat pad weight (g)	SC adipocyte volume (pL)	PE adipocyte volume (pL)
LS					
3 weeks	1.86 ± 0.19	0.76 ± 0.06	0.49 ± 0.07	92.22 ± 11.16	86.09 ± 8.49
6 weeks	4.05 ± 0.36	2.17 ± 0.17	1.89 ± 0.25	155.56 ± 14.09	166.70 ± 7.01
9 weeks	5.95 ± 0.38	4.35 ± 0.29	3.16 ± 0.34‡	192.51 ± 14.32	243.15 ± 14.80‡
NS					
3 weeks	1.59 ± 0.17	0.62 ± 0.06	0.29 ± 0.03	86.76 ± 8.90	77.02 ± 7.73
6 weeks	4.07 ± 0.32	2.64 ± 0.18	2.13 ± 0.24	176.28 ± 13.25	177.14 ± 11.82
9 weeks	6.99 ± 0.49	5.14 ± 0.38	4.62 ± 0.43	222.62 ± 11.42	329.02 ± 28.42
HS					
3 weeks	2.25 ± 0.14*	0.87 ± 0.08	0.54 ± 0.05*	101.60 ± 7.58	88.53 ± 5.34
6 weeks	5.94 ± 0.39†	4.14 ± 0.22†	3.33 ± 0.25†	197.61 ± 13.37	236.97 ± 16.82†
9 weeks	6.73 ± 0.50	5.24 ± 0.48	4.02 ± 0.45	229.95 ± 19.38	318.19 ± 22.29

* $p < 0.05$ vs. NS values of the same week.
† $p < 0.01$ vs. NS and LS values of the same week.
‡ $p < 0.05$ vs. NS values of the same week.
Values are mean ± SE, $n = 7$ to 10 rats/group.

cantly increased in PE cells from HS animals in comparison with the other groups ($p < 0.05$) (Figure 4).

Malic and G6PDH Activities and G6PDH mRNA Expression

In the sixth week of study, the SC WAT from rats fed the HS diet showed a higher maximal activity of malic (Figure 5A) and G6PDH (Figure 5B) enzymes compared with fat pads of both LS and NS rats. Malic enzyme activity in the PE fat pad did not differ significantly among the groups. G6PDH activity in the PE adipose depot (Figure 5B) of the HS group (21.87 ± 2.36 nmol/min/mg protein, $n = 7$) was increased ($p < 0.05$) in comparison to the NS group (12.90 ± 1.44 nmol/min/mg protein, $n = 7$). As depicted in Figure 5C, G6PDH mRNA expression was similar among the three experimental groups ($p > 0.05$) in both fat depots.

Lipolysis

After 6 weeks of the diets, PE adipocytes isolated from HS animals showed a significant rise in basal and isoproterenol-stimulated lipolytic responses (Figure 6). The rates of lipolysis in SC cells did not differ significantly among the groups.

Discussion

This study demonstrated that a high dietary sodium intake increased BP in rodent animals, thus confirming previous data reported by others (22–24). However, the results presented here showed for the first time, to our knowledge, that, in addition to the higher BP, increased WAT mass and plasma leptin concentration were induced by the HS diet.

Aside from the repeatedly demonstrated HS-induced changes in water intake (13,25), one of the findings of the present study was the lower food intake observed during the first weeks on the LS diet. It was shown before that the LS diet raises plasma angiotensin II levels (26) and that this peptide reduces food intake in rats after both systemic and intra-cerebroventricular administrations (27,28). Thus, considering that the LS diet affected our animals in the same way, the effect of high angiotensin II levels on the central nervous system (29) could explain, in part, the lower food intake. Reduced food intake associated with high plasma angiotensin II concentration in rats on an LS diet was also observed by Coelho et al. (23) in a similar experimental protocol. Although the area under the curve of food consumption was significantly reduced for LS compared with HS group, there was not a statistically significant correlation between body weight gain and food intake ($r = 0.079$; $p > 0.05$) throughout the 9 weeks of observation.

Although the LS and HS diets did not alter the ponderal evolution of animals when compared with the NS diet, the HS rats showed higher SC, PE, and RP WAT masses after 6 weeks, which can indicate a great increase in body adiposity induced by the salt overload. The morphometric

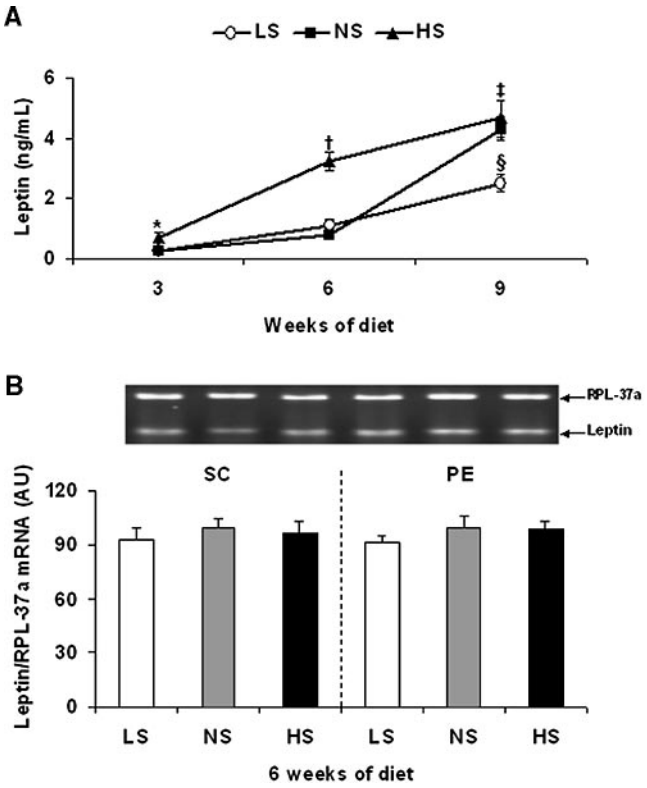

Figure 3: Effect of chronic administration of LS, NS, and HS diets on (A) leptin plasma levels and (B) leptin mRNA expression in SC and PE fat pads from rats. Values are mean ± SE, $n = 6$ to 9 rats/group. * $p < 0.05$ vs. NS and LS values of the same week; † $p < 0.001$ vs. NS and LS values of the same week; ‡ $p < 0.01$ vs. LS values of the same week; § $p < 0.05$ vs. NS values of the same week.

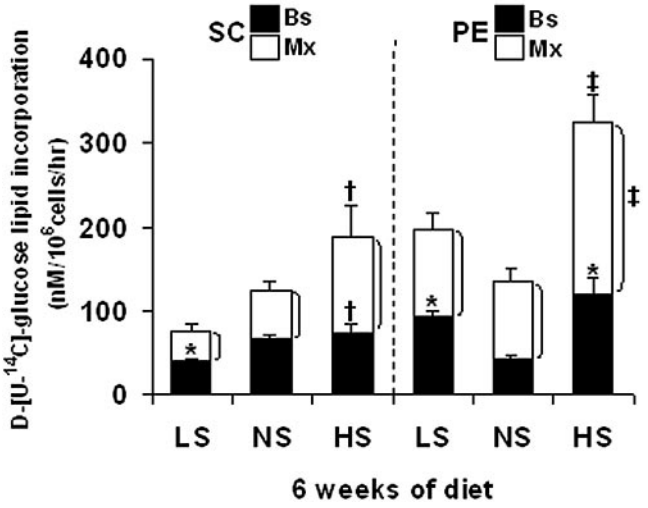

Figure 4: Effect of chronic (6 weeks) administration of LS, NS, and HS diets on incorporation of D-[U-^{14}C]-glucose into lipids in SC- and PE-isolated adipocytes from rats. Bs, basal; Mx, maximal. Values are mean ± SE, $n = 6$ to 8 rats/group. * $p < 0.05$ vs. NS; † $p < 0.01$ vs. LS; ‡ $p < 0.05$ vs. NS and LS.

analysis of WAT also revealed that the HS diet enhanced the volume of visceral adipocytes. As both muscle tissues and visceral and SC WAT depots contribute to total body mass, we consider it imprecise to determine body weight as the only parameter by which to evaluate the influence of salt intake on body composition. However, since fat masses were more developed in these rats, we suggest that there was a reduction in lean body mass, which could explain the similar body weights of NS, LS, and HS groups even considering the higher WAT mass in HS rats.

WAT development depends on a balance between food consumption and energy expenditure, and it involves both cellular hypertrophy (increase in cell size) and hyperplasia (increase in cell number) (30). The higher adiposity in HS rats may not be explained by an increased energy intake because these animals consumed the same amount of food as the NS animals throughout the experimental period.

Glucose is an important lipogenic substrate since it allows the synthesis of pyruvate and glycerol-3-phosphate, which will be transformed by the action of several enzymes, including acetyl-CoA carboxylase and fatty-acid synthase, into FA that will be stored as TAG into lipid droplets within the adipocytes. The required reduced form of nicotinamide adenine dinucleotide phosphate for fatty acid synthesis is produced by both G6PDH (the first enzyme of the pentose-phosphate pathway) and malic enzyme (31). These anabolic pathways are mainly under the control of insulin, a lipogenic hormone that among other actions elevates the mRNA expression and activity of lipogenic enzymes such as acetyl-CoA carboxylase, fatty-acid synthase, G6PDH, and malic enzyme (3,32,33).

HS rats showed higher insulin-stimulated rates of glucose incorporation into lipids in both SC and PE adipocytes in comparison with the other groups after 6 weeks of diet. In addition, glucose incorporation into lipids in the absence of insulin was also enhanced by salt overload.

Similar results were obtained by Lima et al. (22) in visceral adipocytes, but they did not include an NS group in their study. Okamoto et al. (13) verified a higher glucose transporter-4 gene expression, as well as basal and insulin-stimulated glucose transporter-4 translocation to the plasma membrane in WAT of HS rats in comparison with LS and NS rats. These previous works and our study with isolated SC and visceral adipocytes showed an increase in glucose metabolization and in the insulin responsiveness in WAT of rats on a high salt intake. Thus, this HS-induced augmented capacity of adipocytes to metabolize glucose might have contributed to the increase in adipose mass.

Rats on the HS diet displayed higher G6PDH and malic enzyme activities in WAT, which helps to explain the increase in lipogenesis justifying the higher accumulation of adipose depots in this group. Although G6PDH gene ex-

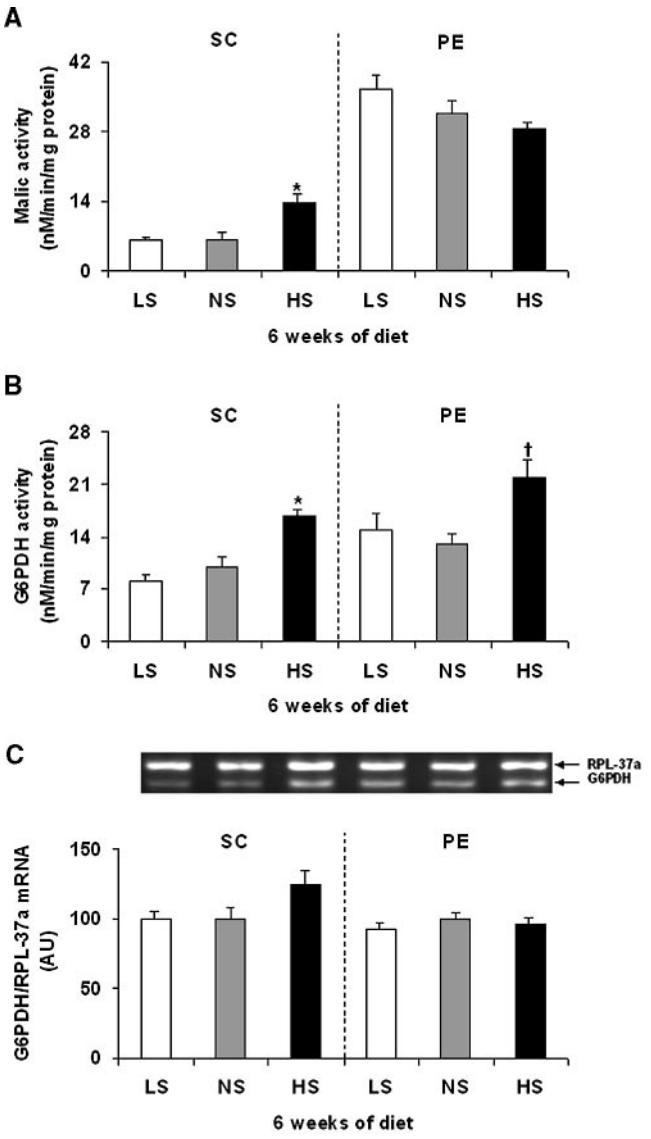

Figure 5: Effect of chronic administration (6 weeks) of LS, NS, and HS diets on (A) malic activity, (B) G6PDH activity, and (C) G6PDH gene expression in the SC and PE fat pads from rats. Values are mean ± SE, $n = 6$ or 7 rats/group. * $p < 0.01$ vs. NS and LS; † $p < 0.05$ vs. NS.

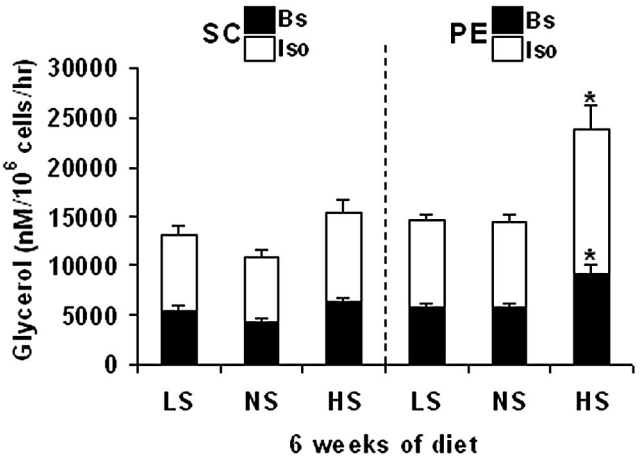

Figure 6: Effect of chronic (6 weeks) administration of LS, NS, and HS diets on lipolysis in SC- and PE-isolated adipocytes from rats. Bs, basal; Iso, isoproterenol. Values are mean ± SE, $n = 6$ to 8 rats/group. * $p < 0.05$ vs. NS and LS.

pression was not altered by sodium overload, the enhanced activity of these two lipogenic enzymes in the WAT of HS rats may have resulted from a more accentuated insulin action in this process. Therefore, these data provide other evidence that reinforces the hypothesis that higher insulin responsiveness is induced by high sodium intake.

It is well established that leptin gene expression and plasma levels are proportional to total body fat stores in humans (6) and rodents (7). In agreement with the study of Dobrian et al. (34), HS diet induced a significant increase in PE adipocyte size (volume) that may have accounted for the higher PE fat pad weight in the sixth week of diet. Dobrian et al. also reported higher plasma leptin concentration in rats fed a 4% sodium chloride diet for 10 weeks compared with rats on 0.8% sodium chloride, without detecting a significant difference in the PE and RP fat depot weights between the groups (34). Based on their results, Dobrian et al. (34) suggested that high sodium chloride content could modulate leptin levels, independently of obesity. In our investigation, the HS group showed higher circulating leptin concentration concomitant with an increase in body adiposity. Although sodium overload did not change leptin gene expression in the WAT, leptin levels positively and significantly correlated with SC, PE, and RP tissue weight and with PE adipocyte volume in the sixth week of study, which allows us to infer that hyperleptinemia may be the result of excessive fat accumulation in rats on the HS diet. Moreover, since insulin stimulates leptin secretion from WAT of rats, the HS-induced higher insulin responsiveness of adipocytes may contribute to enhancing leptinemia in these animals (35).

Intra-cerebroventricular and systemic administration of leptin have been shown to augment sympathetic outflow and to increase BP and HR in rats (36–38). Thus, leptin-induced activation of the SNS has been proposed as one of the elements to explain the mechanisms underlying the association between increased fat mass and hypertension (8). Although a recent report has suggested that leptin may not contribute to arterial pressure sensitivity to salt in hyperleptinemic obese rats (39), we propose that increased leptin production may have contributed to HS-induced hypertension probably due to leptin stimulation of SNS activity in the central nervous system (8,39). Further studies are needed to determine a possible relationship between high-salt intake and leptinemia in the development of arterial hypertension in both humans and animals.

The present study showed that high plasma leptin concentration in HS rats did not reduce food intake, body weight, or SC, PE, and RP fat depot masses, suggesting the presence of a selective leptin resistance in these animals. In accordance with this recently conceived concept regarding leptin physiology (40), animals on an HS diet are resistant to metabolic (satiety, body weight, and adipose mass-reducing) effects of leptin in rodents (41,42), but leptin's sympatho-excitatory actions still remain intact.

Our results also demonstrated that the salt overload increased visceral adipocyte basal and isoproterenol-stimulated lipolytic response, however, without promoting any significant loss in body weight and adiposity. The enhancement in the lipolytic activity may be explained by the higher PE adipocyte volume (43) or by the low serum levels of angiotensin II, a lipolysis peptide inhibitor (44,45), in animals fed a sodium-rich diet. In addition, the HS-induced hyperleptinemia may stimulate the lipolytic process by a direct action (46) or by SNS activation (47).

In summary, in the present study, we demonstrated that prolonged administration of an HS diet increased BP, body adiposity, and leptinemia in rats. The augmented capacity to incorporate glucose into lipids and the higher lipogenic enzymatic activity may have promoted adipocyte hypertrophy and excessive fat accumulation. Although the increased leptin production did not reduce food intake or visceral and SC fat depot masses, it may have contributed to the elevation of BP and the adipose lipolytic response in HS rats, probably due to an increase in central sympathetic activity. This work provides evidence that high sodium intake may contribute to the development of obesity, which, in turn, possibly raises the propensity for a hypertensive clinical picture in rats.

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References

1. **Kopelman PG.** Obesity as a medical problem. *Nature.* 2000; 404:635–43.
2. **Brown CD, Higgins M, Donato KA, et al.** Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res.* 2000;8:605–19.
3. **Girard J, Ferre P, Foufelle F.** Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu Rev Nutr.* 1997;17:325–52.
4. **Langin D.** Control of fatty acid and glycerol release in adipose tissue lipolysis. *C R Biologies.* 2006;329:598–607.
5. **Kershaw EE, Flier JS.** Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab.* 2004;89:2548–56.
6. **Considine RV, Sinha MK, Heiman ML, et al.** Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996;334:292–5.
7. **Hiraoka J, Hosoda K, Ogawa Y, et al.** Augmentation of obese (*ob*) gene expression and leptin secretion in obese spontaneously hypertensive rats (obese SHR or Koletsky rats). *Biochem Biophys Res Commun.* 1997;231:582–5.
8. **Montanaro MS, Allen AM, Oldfield BJ.** Structural and functional evidence supporting a role for leptin in central neural pathways influencing blood pressure in rats. *Exp Physiol.* 2005;90:689–96.
9. **No author listed.** Effects of weight loss and sodium reduction intervention on blood pressure and hypertension incidence in overweight people with high-normal blood pressure: The Trials of Hypertension Prevention, phase II. The Trials of Hypertension Prevention Collaborative Research Group. *Arch Intern Med.* 1997;157:657–67.
10. **Whelton PK, Appel LJ, Espeland MA, et al.** Sodium reduction and weight loss in the treatment of hypertension in older persons: a randomized controlled trial of nonpharmacologic interventions in the elderly (TONE Collaborative Research Group). *JAMA.* 1998;279:839–46.
11. **Sacks FM, Svetkey LP, Vollmer WM, et al.** Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med.* 2001;344:3–10.
12. **Prada PO, Okamoto MM, Furukawa LNS, Machado UF, Heimann JC, Dolnikoff MS.** High- or low-salt diet from weaning to adulthood: effect on insulin sensitivity in Wistar rats. *Hipertension.* 2000;35:424–9.
13. **Okamoto MM, Sumida DH, Carvalho CRO, et al.** Changes in dietary sodium consumption modulate GLUT4 gene expression and early steps of insulin signaling. *Am J Physiol Regul Integr Comp Physiol.* 2004;286:R779–85.
14. **Prada PO, Coelho MS, Zecchin HG, et al.** Low salt intake modulates insulin signaling, JNK activity and IRS-1ser307 phosphorylation in rat tissues. *J Endocrinol.* 2005;185:429–37.
15. **Rodbell M.** Metabolism of isolated fat cells: effects of hormones on glucose metabolism and lipids. *J Biol Chem.* 1964; 239:357–80.
16. **DiGirolamo M, Medlinger S, Fertig JW.** A simple method to determine cell size and number in four mammalian species. *Am J Physiol.* 1971;221:850–8.
17. **Bergmeyer HU, Bernt E.** D-glucose determination with hexoquinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. London, U.K.: Academic Press; 1974, pp. 1196–201.
18. **Newsholme EA, Williams T.** The role of phosphoenolpyruvate carboxykinase in amino acid metabolism in muscle. *Biochem J.* 1978;176:623–6.
19. **Honnor RC, Dhillon GS, Londos C.** cAMP-dependent protein kinase and lipolysis in rat adipocytes: cell preparation, manipulation, and predictability in behavior. *J Biol Chem.* 1985;200:15122–9.
20. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156–9.

21. **Meadus WJ.** A semi-quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on porcine muscle PPAR gene expression. *Biol Proced Online.* 2003;5:20–8.
22. **Lima NKC, Lima FB, Santos EA, et al.** Chronic salt overload increases blood pressure and improves glucose metabolism without changing insulin sensitivity. *Am J Hypertens.* 1997;10:720–7.
23. **Coelho MS, Passadore MD, Gasparetti AL, et al.** High- or low-salt diet from weaning to adulthood: effect on body weight, food intake and energy balance in rats. *Nutr Metab Cardiovasc Dis.* 2006;16:148–55.
24. **Lima NKC, Lima FB, Santos EA, et al.** Effect of lifelong high- or low-salt intake on blood pressure, left ventricular mass and plasma insulin in Wistar rats. *Am J Med Sci.* 2006; 331:309–14.
25. **Stricker EM, Hoffmann ML, Riccardi CJ, Smith JC.** Increased water intake by rats maintained on high NaCl diet: analysis of ingestive behavior. *Physiol Behav.* 2003;79:621–31.
26. **Ingert C, Grima M, Coquard C, Barthelmebs M, Imbs J.** Effects of dietary salt changes on renal-angiotensin system in rats. *Am J Physiol Renal Physiol.* 2002;283:F995–1002.
27. **English V, Cassis L.** Facilitation of sympathetic neurotransmission contributes to angiotensin regulation of body weight. *J Neural Transm.* 1999;106:631–44.
28. **Porter JP, Potratz KR.** Effect of intracerebroventricular angiotensin II on body weight and food intake in adult rats. *Am J Physiol Regul Integr Comp Physiol.* 2004;287:R422–8.
29. **McKinley MJ, Allen AM, Burns P, Colvill LM, Oldfield BJ.** Interaction of circulating hormones with the brain: the roles of the subfornical organ and the organun vasculosum of the lamina terminalis. *Clin Exp Pharmacol Physiol.* 1998; 25(suppl):61–7.
30. **Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ.** The biology of white adipocyte proliferation. *Obes Rev.* 2001;2:239–54.
31. **Hillgartner FB, Salati LM, Goodridge AG.** Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiol Rev.* 1995;75:47–76.
32. **McHugh KM, Drake RL.** Insulin-mediated regulation of epididymal fat pad malic enzyme. *Mol Cell Endocrinol.* 1989; 62:227–33.
33. **Wagle A, Jivraj S, Garlock GL, Stapleton SR.** Insulin regulation of glucose-6-phosphate dehydrogenase gene expression is rapamycin-sensitive and requires phosphatidylinositol 3-kinase. *J Biol Chem.* 1998;273:14968–74.
34. **Dobrian AD, Schriver SD, Lynch T, Prewitt RL.** Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity. *Am J Physiol Renal Physiol.* 2003;285: F619–28.
35. **Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW.** Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology.* 1997;138:4463–72.
36. **Dunbar JC, Hu Y, Lu H.** Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes.* 1997;46:2040–3.
37. **Casto RM, VanNess JM, Overton JM.** Effects of central leptin administration on blood pressure in normotensive rats. *Neurosci Lett.* 1998;246:29–32.
38. **Shek EW, Brands MW, Hall JE.** Chronic leptin infusion increases arterial pressure. *Hypertension.* 1998;31:409–14.
39. **Correia MLG, Morgan DA, Sivitz WI, Mark AL, Haynes WG.** Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension.* 2001;37:936–42.
40. **Mark AL, Correia MLG, Rahmouni K, Haynes WG.** Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. *J Hypertens.* 2002;20:1245–50.
41. **Levin N, Nelson C, Gurney A, Vandlen R, de Sauvage F.** Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci USA.* 1996;93:1726–30.
42. **Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rosseti L.** Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest.* 1997;100:3105–10.
43. **Engfeldt P, Arner P.** Lipolysis in human adipocytes: effects of cell size, age and of regional differences. *Horm Metab Res Suppl.* 1988;19:26–9.
44. **Boschmann M, Ringel J, Klaus S, Sharma AM.** Metabolic and hemodynamic response of adipose tissue to angiotensin II. *Obes Res.* 2001;9:486–91.
45. **Goossens GH, Blaak EE, Saris WH, van Baak MA.** Angiotensin II-induced effects on adipose tissue and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects. *J Clin Endocrinol Metab.* 2004;89:2690–6.
46. **Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, et al.** Direct effects of leptin on brown and white adipose tissue. *J Clin Invest.* 1997;100:2858–64.
47. **Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL.** Sympathetic and cardiorenal actions of leptin. *Hypertension.* 1997;30:619–23.