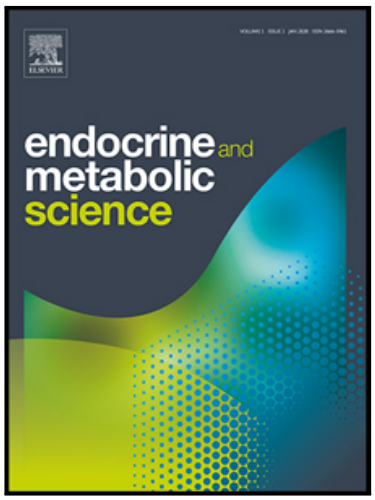

Increased sympathetic tone and hypothalamic–pituitary–adrenal (HPA) axis activation impact in metabolic parameters from hypertensive rats

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Increased sympathetic tone and hypothalamic–pituitary–adrenal (HPA) axis activation impact in metabolic parameters from hypertensive rats

Running title: Energy Metabolism and Stress Hormones in Hypertensive Rats

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HIGHLIGHTS

- Hypertensive rats showed a reduction in adiposity when compared to their controls;
- SHR presented the most evident lipodystrophy;
- SHR exhibited significant alteration in endocrine function of adipose tissue.

ABSTRACT

Increased activity of the sympathetic nervous system (SNS) may play an important role in the development of hypertension and in adiposity disorders. We aimed to investigate the influence of increased sympathetic tone and hypothalamic–pituitary–adrenal (HPA) axis activation in metabolic parameters by evaluating the morphometry of isolated adipocytes from different fat

pads and energy consumption. Serum levels of catecholamines, HPA hormones, T3 and adipokines were measured in 15-weeks-old Wistar (WIS), Wistar-Kyoto (WKY), Spontaneously Hypertensive Rats (SHR) and WIS treated with N^G-nitro-L-arginine methyl ester (L-NAME). L-NAME and SHR showed a reduced adiposity when compared to their controls, which may be related to higher concentrations of T3. However, SHR presented the most evident lipodystrophy, presenting significant changes in the morphometry from isolated adipocytes and the production of adipokines. Thus, our study suggests that endocrine changes in hypertension models may highlight possible therapeutic targets in the treatment of metabolic changes associated with hypertension.

Keywords: Isolated adipocytes; hypertension; SHR; L-NAME-induced hypertension; glucocorticoids.

1. INTRODUCTION

Hypertension is the most common cardiovascular risk factor and remains the leading cause of noncommunicable disease deaths worldwide (Burnier and Egan, 2019). Essential hypertension can be defined as a rise in blood pressure of unknown cause and usually clusters with other cardiovascular risk factors such as ageing, overweight, insulin resistance and hyperlipidaemia (Messerli, et al., 2007). Exposure to chronic stress is a major risk factor for essential hypertension (OMS, 2013). The chronic stress leads the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) to restore allostasis, with consequent activation of the Renin-Angiotensin-Aldosterone System (RAAS) which, associated or not, predisposes to hypertension (Björntorp, et al., 2000, Gold, et al., 2005, Goodwin and Geller, 2012, Hering and Schlaich, 2015, McEwen and Gianaros, 2011, Thomas and Dasgupta, 2015). The chronic activation of SNS and HPA seems to be the central mechanism in essential hypertension, regardless of a well-defined external stressor (Björntorp, Holm, Rosmond and Folkow, 2000, Gold, Dziobek, Rogers, Bayoumy, McHugh and Convit, 2005, Hering and Schlaich, 2015). Furthermore, the activation of RAAS is not only attributable to sympathetic nervous system and renal compression but also to dysfunctional adipose tissue (Borghi, et al., 2021, Schütten, et al., 2017).

Classically, there are two well established models of sympathetic overactivity: the N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension and the spontaneously hypertensive rats (SHR) (Conceição-Vertamatti, et al., 2020). The L-NAME is characterized by intense peripheral vasoconstriction caused by chronic oral administration of N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase (NOS), while SHR develops spontaneous hypertension due to an increase in sympathoadrenal activity between 4 and 6 weeks of age, which is followed by an increase in blood pressure present in adulthood (Bergamaschi, et al., 1999, Conceição-Vertamatti, et al., 2017, Thomas and Dasgupta, 2015). Nevertheless, Wistar-Kyoto rat (WKY), inbred from the SHR strain as a control, is normotensive but it is also associated with HPA activation due to the high catecholamines pattern (Borghi, et al., 2020, Conceição-Vertamatti, Borghi, Canova and Grassi-Kassisse, 2017).

The relationship with the stress hormones remains to be elucidated. At the same time, the stress response can trigger metabolic changes that directly influence adiposity, appetite and energy expenditure. In general, sympathetic activation is considered to mediate short-term and more immediate responses, whereas HPA axis activation is considered to mediate long-term and sustained responses (Shariq and McKenzie, 2020, Tank and Lee Wong, 2015).

The acute stress response produces a synergistic action between glucocorticoids and catecholamines to induce a lipolytic response to ensure energy supply for the required demands, which may reflect a decrease in adiposity (Sacta, et al., 2015). This signaling is followed by suppression of appetite and increased energy expenditure triggered by corticotropin-releasing hormone (CRH), initiating the activation of the HPA axis and the production of catecholamines, which will act as positive feedback (Messina, et al., 2013, NICOLAIDES, et al., 2015). When the stimulus becomes chronic, glucocorticoids promote adipogenesis and adipose tissue expansion, mainly central regions, predisposing to obesity (Fardet and Fève, 2014, Lee, et al., 2014). The high concentrations of glucocorticoids also triggers an increase in appetite, mainly for fatty and high-calorie foods, decreasing energy expenditure (Fardet and Fève, 2014). A key component of the decrease in energy expenditure is reduced adaptive thermogenesis, due to the uncoupling proteins (UCPs) activity (Fuller-Jackson and Henry, 2018). These proteins participate in energy expenditure, thermogenesis and regulation of free fatty acids (Oliveira, et al., 2016). UCP3 is primarily expressed in skeletal muscle and is involved in energy metabolism regulation and weight control (Oliveira, Pinhel, Nicoletti, Oliveira, Quinhoneiro, Noronha, Marchini, Marchry, Junior and Nonino, 2016).

The exact mechanisms behind the development of metabolism disorders in response to high levels of glucocorticoids are not completely elucidated. Therefore, the aim of this study was to investigate the influence of increased sympathetic tone and HPA axis activation in metabolic parameters in normotensive and hypertensive rats.

2. METHODS

2.1 Animals

Studies were conducted using 15-week-old Wistar (HanUnib:WH; n=6 - WIS), Wistar-Kyoto (NTacUnib:WKY; n=6 - WKY) and Spontaneously Hypertensive Rats (SHR/NTacUnib; n=6 - SHR) male rats (*Rattus norvegicus*) weighed since 7th week and before the experimental procedures. For the L-NAME cohort (n=6), we inhibit nitric oxide synthesis by L-NAME (Enzo Life Sciences International, Inc.5120 Butler Pike, Plymouth Meeting, PA 19462) 40 mg/kg/day, for 5 weeks in the drinking water, started at the 10th week of life of WIS (Paulis and Unger, 2010). Water was exchanged three times a week, with correction in dose per weight. All animals were provided by the Multidisciplinary Center for Biological Research (CEMIB - UNICAMP). Animals were housed in collective cages (3 rats per cage) at 22°C on a 12 h light-dark cycle (lights on at 06:30 a.m.) with *ad libitum* access to standard chow (Labina Purina®) and filtered water. Food and water intake were measured each three days by weighing what remained in the food cup and quantifying what remained in the water bottle and the values consumed were expressed by g/g or mL/g, respectively, of each animal in the cage. The epididymal and retroperitoneal adipose tissue were weighed to calculate the weight percentage of the fat pad in relation to body weight. All animal housing, care, and experimental procedures were approved by the Committee for Ethics in Animal Experimentation (CEUA) of the Institute of Biology from Unicamp in Campinas, Brazil (no. 2616-1), in accordance with NIH guidelines. The rats under fasting were anesthetized with tiletamine 29 mg/kg and zolazepam 29 mg/kg, i.p. (Zoletil 50® - Virbac Laboratories, Carros, France); and xylazine 12.88 mg/kg, i.p. (Anasedan® - Sespo Ind. e Com. Ltda, Paulínia, Brazil).

2.2 Blood pressure

Blood pressure was measured under anesthesia after 15 weeks, when hypertension has been established (Conceição-Vertamatti, Borghi, Canova and Grassi-Kassisse, 2017). Blood catheterization was performed with introduction of a cannula (PE 50) into the right carotid artery and connected to a pressure transducer strain-gauge type connected to a MLS370 amplifier/7 blood pressure Module (AD Instruments, Sydney, Australia). Data acquisition was performed by Power Lab 8/30 and results analysis were performed using LabChart Pro 7 (ADInstruments,

Sydney, Australia). The mean blood pressure was defined as: [systolic blood pressure + (2 X diastolic blood pressure)]/3.

2.3 Resting metabolic rate

Resting metabolic rate (RMR) of fed animals was analyzed by the measurement of oxygen consumption and carbon dioxide exhaled over a period of 4 minutes inside of a hermetically sealed respirometer. Data acquisition was performed by PAC CHECK™ 650 (Ametek-Mocon, Minneapolis, USA) at 7th and 15th week. RMR was defined as: CO_2 production (%) /O_2 consumption (%) x 20292.4 (J) x /body weight (g) / 4 (min).

2.4 Serum analysis

Five mL of blood samples were collected from anesthetized rats by cardiac puncture. We waited 20 min to perform cardiac punction to avoid stress effects of manipulation of the anesthesia procedure (Lee and Goosens, 2015). Serum was obtained by centrifugation of blood samples at 10,000 rpm, 15 min, 4ºC after 2 h at room temperature. Serum aliquots were frozen until quantification. Levels of corticosterone, adrenocorticotropic hormone (ACTH), triiodothyronine (T3), leptin and adiponectin were measured using Milliplex Map Kit (Cat. #RSHMAG-69K; #RTHYMAG-30K-01; #RADPKMAG-80K-03; #RADPNMAG-81K-01; Millipore, Billerica, MA), following the manufacturer's recommendations. Serum catecholamines were quantified fluorometrically (excitation 420 nm, emission 510 nm) according to Kelner et al. (Kelner, et al., 1985).

2.5 Adipocyte isolation and morphometry

Adipocyte isolation was performed as described by Borghi et al. (Borghi, et al., 2019). 1-1.5 g of epididymal, retroperitoneal and mesenteric adipose tissue was fragmented and digested with 1 mg/mL collagenase (type II, from *Clostridium histoliticum*), in polyethylene tubes with 6 mL of Krebs-Ringer bicarbonate buffer (KRBA) containing Hepes (25 mM), glucose (6 mM), and bovine albumin (3%, BSA fraction V fatty-acid free), pH 7.4 (KRBA), at 37°C with shaking (60 cycles/min) during 45 min. The isolated adipocytes were filtered through a nylon mesh and washed 3 times with 6 mL KRBA buffer (3% BSA). The final volume of cellular suspension was adjusted to 50 mL with KRBA buffer (3% BSA). A 100 µL aliquot of cellular suspension were

adjusted with KRBA to a 10% suspension:10 μL of this suspension were transferred to a Mallassez chamber for adipocytes counting and morphometry through light microscopy Carl Zeiss Axiolab re 10x objective magnification (Zeiss, Oberkochen, Germany). Then, we used the software IMAGE J (National Institute of Health, USA) to perform the morphometry.

2.6 Western Blotting

Fragments of gastrocnemius muscle (100 mg) were separately pulverized in liquid N_2 and homogenized in cold RIPA lysis buffer (Merck Millipore, Billerica, MA, USA) containing protease inhibitor cocktail (PIC, Sigma-Aldrich) to obtain total protein extracts. Protein was obtained by centrifugation of homogenized tissue at 11,000 rpm, 40 min, 4ºC to remove insoluble material. Equal amounts of protein (0.050 mg) were used as total extracts, followed by SDS-PAGE and Western Blot analysis with anti-UCP3 (SC-7756 1:1,000; Santa Cruz Biotechnology) antibody. Anti-α tubulin (T5168 1:1,000; Sigma-Aldrich) antibody was used as loading control to normalize band intensity of UCP3. Immunocomplexes were detected using a luminol peroxidase chemiluminescence kit (Clarity Max™, Bio-Rad) and acquired using the Syngene GBox imaging system (Synoptics Group, Cambridge, England). Protein band intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, USA).

2.7 Statistical analysis

Data are presented as means ± SEM. The normality was confirmed by Kolmogorov-Smirnov. Unpaired Student's t-test for data between controls (WIS vs. L-NAME; WKY vs. SHR) and for same condition (WIS vs. WKY; L-NAME vs. SHR) and paired Student's t-test for comparisons in the same group (7^{th} week vs. 15^{th} week). All statistical analyses were performed using GraphPad Prism version 8.00 (GraphPad Software, San Diego, California, USA). The acceptance level of significance was set at $p<0.05$.

3. RESULTS

3.1 Body weight, resting metabolic rate, food and water intake

SHR exhibited lower body weight when compared to WKY and L-NAME for all checked timepoints. During the same period, WKY showed higher body weight when compared to WIS

and the treatment with L-NAME did not influence the body weight in relation to its WIS control. As the strains exhibited different weights, we considered the allometry of the animals to normalize metabolic and consumption parameters. At 7th week, SHR presented higher water intake when compared to WKY and L-NAME. WKY presented higher water intake when compared to WIS, but L-NAME did not present difference to WIS. At the 15th week, SHR presented the same water intake as WKY and L-NAME. WKY and L-NAME presented higher water intake when compared to WIS. Over time, all strains showed decreased water intake, except L-NAME, which maintained the same water intake. At 7th week, L-NAME presented same food intake as WIS, but WKY showed a decreased food intake when compared do WIS. SHR presented higher food intake when compared to WKY and L-NAME. At 15th week, all strains decreased the food intake when compared to 7th week. L-NAME presented a decreased in food intake when compared to WIS, but at this age presented the same food intake as WKY. SHR maintained the increased food intake when compared to WKY and L-NAME. SHR presented higher resting metabolic rate (RMR) when compared to WKY and L-NAME for all checked timepoints. L-NAME did not show difference in RMR to WIS in any timepoint. At 7th week, WKY did not show difference in RMR when compared to WIS, but at 15th week it showed lower RMR values when compared to WIS. All strains showed a decrease in RMR values when compared to the younger form (Table 1).

3.2 Changes in anthropometry and energy expenditure

At week 15, SHR and L-NAME exhibited higher mean arterial pressure when compared to their controls, WKY and WIS respectively. There is no difference between normotensives (WIS vs. WKY) or hypertensives rats (L-NAME vs. SHR). The longitudinal analysis showed that WIS and L-NAME displayed the same values in weight gain, RMR and food intake reduction. However, SHR displayed a higher value in weight gain, food intake and RMR reduction when compared to WKY. Among the normotensive rats, WKY displayed lower value in weight gain and food intake reduction, but no reduction in RMR when compared to WIS. Finally, regarding the hypertensive rats, SHR displayed the same value in weight gain, but lower food intake reduction and greater reduction of RMR when compared to L-NAME (Table 2).

3.3 Hormones measurement

Serum T3 were significantly elevated in SHR and L-NAME when compared to its controls, but without differences between hypertensives (L-NAME vs. SHR) and normotensive (WIS vs. WKY) rats. WKY exhibited higher ACTH levels when compared to WIS and SHR, but L-NAME did not show any difference when compared to WIS and SHR. Serum corticosterone and catecholamines were significantly elevated in L-NAME when compared to its control, WIS, but there was not significantly difference between WKY and SHR. In the normotensive group, WKY showed higher levels of serum corticosterone and catecholamines when compared to WIS, but any differences were observed in between the hypertensive group (Table 3). L-NAME showed same serum leptin levels than WIS, but SHR showed lower concentrations when compared to WKY. There is no difference in serum leptin between normotensives (WIS vs. WKY) or hypertensives (L-NAME vs. SHR) rats. Serum adiponectin concentration was not significantly different between WIS and L-NAME, but SHR exhibited higher adiponectin levels when compared to WKY. In normotensives, WKY exhibited lower adiponectin levels than WIS, while among hypertensives, SHR showed higher levels when compared to L-NAME.

3.4 Fat pads and morphometry

The relative epididymal fat pad (EFP) is lower in SHR when compared to WKY and L-NAME, however, L-NAME and WKY showed no significant difference when compared to WIS. The relative retroperitoneal fat pad (RFP) is lower in hypertensive rats when compared to their normotensive controls. Considering each group, WKY and SHR exhibited lower RFP when compared to WIS and L-NAME, respectively. This work evaluated the adipocyte morphometry of isolated adipocytes from three different adipose pads. L-NAME exhibited smaller retroperitoneal and mesenteric adipocytes when compared to WIS. SHR exhibited smaller epididymal, retroperitoneal and mesenteric adipocytes when compared to WKY. When compared to WIS, WKY showed smaller mesenteric adipocytes, with no difference in the retroperitoneal and epididymal adipocytes sizes. When comparing hypertensive rats, the only difference observed was the smaller mesenteric adipocytes in SHR when compared to L-NAME (Table 3, Figure 1).

3.5 Western blotting analysis

The L-NAME and WKY showed lower uncoupling protein 3 (UCP3) expression when compared to the normotensive WIS (Figure 2A/C). However, there were no differences in UCP3 expression between SHR and its normotensive control WKY (Fig. 2B).

4. DISCUSSION

The allometry is intrinsic related to properties whose proportions change as a function of size but some metabolic parameters, as food consumption and metabolic rate, do not vary linearly with body weight. This is attributed to the fact that the surface/weight ratio is directly linked to the dissipation of heat into the environment and the expenditure of energy to maintain body temperature (Huang and Riviere, 2014, Tschöp, et al., 2012). Over time, WIS and L-NAME did not presented any differences in body weight gain, and reduction in food intake considering aging and RMR. This occurs probably because L-NAME group is a WIS rat treated with a NO inhibitor, indicating that the treatment does not interfere with these parameters. On the other hand, SHR showed greater weight gain when compared to WKY over time, despite to be smaller. Despite the greater reduction in RMR and food intake, these rats continue to show higher RMR, reflecting a greater food intake when compared to WKY. In the normotensive group, WKY showed lower reduction in body weight and food intake that not affect reduction in RMR when compared to WIS, due to lower RMR that WKY exhibited at 15th week. The hypertensive rats did not show differences in weight gain, but SHR showed a greater reduction in RMR when compared to L-NAME, probably due to its body weight, since resting metabolic rates are highly correlated with body weight in general (Byrne, et al., 2003).

The HPA axis plays a role in the regulation of energy balance (Nieuwenhuizen and Rutters, 2008). The cascade starts when hypothalamus produces and releases corticotropin-releasing hormone (CRH), which subsequently stimulates the synthesis and release of adrenocortiocotropin (ACTH) from the anterior pituitary (Nieuwenhuizen and Rutters, 2008). Insular response leads to a decrease in appetite, that can also be modulated by the HPA axis leading to a further decline in food intake (Bou Khalil, et al., 2017). In addition to HPA, this hypophagic effect can also be triggered by SNS activation (Tentolouris, et al., 2006). At 15th

week, L-NAME rats exhibited higher serum concentrations for corticosterone and catecholamines when compared to WIS. This hormonal environment stimulates continuous activation of the HPA axis, justifying the decrease in food intake in these animals. An increase in catecholamine and corticosterone concentrations also rises basal lipolysis rates (Thorp and Schlaich, 2015, Wang, et al., 2012), resulting in decreased RFP weight and retroperitoneal and mesenteric adipocytes size. T3 also may have contributed to fat reduction because it potentiates catecholamine-induced lipolysis (Pucci, et al., 2000). Corticosterone exerts a less potent lipolytic effect that of catecholamines at the same time as being delayed, but induce lipogenesis in visceral adipose tissue (Fruhbeck, et al., 2014). The effects of glucocorticoids on energy metabolism may depend on the duration of exposure (Nieuwenhuizen and Rutters, 2008). Despite the chronic treatment of L-NAME, this group exhibited common metabolic changes related to acute stress. This can be attributed to the short period of treatment compared to the literature or due to the use of strains more susceptible to metabolic changes, such as Sprague Dawley rats (Cardoso, et al., 2013, Higaki, et al., 2001, Roy, et al., 1998, Shankar, et al., 1998).

The L-NAME treatment clearly increased the water intake at 15th week. The inhibition of endogenous NOS enhances drinking behavior and cardiovascular responses induced by the central administration of angiotensin II (AII) (Reis, et al., 2010). The increased blood pressure during L-NAME treatment leads to higher concentrations of aldosterone, increasing sodium reabsorption and consequently thirst, in addition to increase adrenal sensitivity to activation of the renin-angiotensin-aldosterone system (RAAS) (Brem, 2009, Muldowney, et al., 2004).

Interestingly, WKY showed higher serum concentrations for catecholamines and corticosterone as L-NAME when compared to WIS. Despite the higher concentrations of hormones linked to the activation of the HPA axis, these animals did not show increased blood pressure values. WKY rats showed increased serum ACTH levels when compared to WIS and SHR, a metabolic disorder related to a hormonal abnormality of the HPA and hypothalamic-pituitary-thyroid (HPT) axes (Solberg, et al., 2001). The increased ACTH and corticosterone levels in the WKY are more likely due to defective glucocorticoid negative feedback than to increased CRH (Solberg, Olson, Turek and Redei, 2001).

WKY showed differences in food intake when compared to WIS, even with higher body weight. At 7th week, WKY presents lower food intake, which may be a hypophagic response to stress caused by CRH and catecholamines, as observed in L-NAME. However, at 15th week, the food intake was equivalent to WIS, a consistent response to the corticosterone effect. Higher concentrations of corticosterone inhibits CRH production and stimulates neuropeptide Y (NPY) synthesis in hypothalamus, which increases appetite and diminishes energy expenditure, also resulting in a RMR reduction (Crespo, et al., 2014). This hyperphagic response is common to chronic stress and usually follows the hypophagic response caused by acute stress (Yau and Potenza, 2013), which was observed in L-NAME. One of the reasons for this occurred in WKY may be the longer exposure to high concentrations of glucocorticoids than those experienced in L-NAME.

Furthermore, studies with rodents suggest that prolonged exposure to catecholamines and the chronic central action of glucocorticoids may produce a marked decrease in UCP3 expression (Depieri, et al., 2004, Zakrzewska, et al., 1999). This information corroborates with the decreased expression of UCP3 showed by WKY and L-NAME when compared to WIS, which may directly affected the reduction in RMR of WKY due to its greater effect on the thermogenesis of skeletal muscle, a tissue that represents 40% of the metabolic active mass and which contributes to energy allostasis (Depieri, Pinto, Catarin, de Carli and Garcia Júnior, 2004, Oliveira, Pinhel, Nicoletti, Oliveira, Quinhoneiro, Noronha, Marchini, Marchry, Junior and Nonino, 2016). Even presenting higher values for weight than WIS, WKY presented lower RFP weight and smaller mesenteric adipocytes. This result may be triggered by fasting, potentiating the lipolytic effects of high concentrations of catecholamines, corticosterone and ACTH (Chaves, et al., 2011, Thorp and Schlaich, 2015, Vendrame, et al., 2016, Wang, Gray, Kuo and Harris, 2012). Additionally, the overstimulated HPA axis leads to RAAS activation, increasing AII and consequently aldosterone, which triggers a greater increase in water consumption (Kishi and Hirooka, 2013, Rossier, et al., 2015).

The SHR also exhibits elevated thyrotropin releasing hormone (TRH) activity associated with lower levels of leptin (Borghi, Silva, da Silva, Ferrucci, Morais, Conceição-Vertamatti,

Carvalho, Fonseca, Vieira and Grassi-Kassisse, 2020, Duntas and Brenta, 2012). These rats have shown a clear correlation between the HPT axis and the development of hypertension, besides metabolic alterations and hypolipodystrophy (Berta, et al., 2019, Borghi, Silva, da Silva, Ferrucci, Morais, Conceição-Vertamatti, Carvalho, Fonseca, Vieira and Grassi-Kassisse, 2020). The higher serum T3 concentration shown by these rats may have triggered their higher food and water intake and higher metabolic rates (Duntas and Brenta, 2012), and their enhanced catecholamine-induced lipolysis (Pucci, Chiovato and Pinchera, 2000). In addition to T3 effect, resting metabolic rate may have increased due to SNS activation (Tentolouris, Liatis and Katsilambros, 2006). The higher lipolysis and metabolic rate may have caused their more pronounced hypolipodistrophy showed by the decrease in all adipose parameters compared to WKY (relative EFP and RFP weights and adipocyte area of epididymal, retroperitoneal and mesenteric fat pads). T3 also up regulates UCP3 expression and stimulates uncoupling activity of UCP3 (Lombardi, et al., 2015). Thus, it was expected that SHR would present a greater expression of UCP3 than WKY, but it was not observed. However, high concentrations of glucocorticoids decrease the expression of UCP3 in the muscle, highlighting T3 as a compensating factor in this strain (Zakrzewska, Cusin, Stricker-Krongrad, Boss, Ricquier, Jeanrenaud and Rohner-Jeanrenaud, 1999). As expected, SHR rats showed alterations in fat mass related adipocytokines: high adiponectin and low leptin levels, corroborating respectively with their negative and positive correlation with adiposity, the latter possibly amplifying their higher food intake, elicited by T3 (Friedman and Halaas, 1998, Gavrila, et al., 2003). Figure 3 summarizes our proposed mechanisms by which altered hormones in hypertensive groups may have led to lipodystrophy and disturbed secretion of adipose tissue.

5. CONCLUSIONS

In summary, we correlate the metabolic alterations in hypertensive rats and WKY normotensive rats to the overstimulated HPA axis and the linked hormones. Hypertensive rats showed a reduction in adiposity when compared to their controls, which may be related to their altered hormonal environment, characterized by high concentrations of corticosterone, catecholamines and T3 and it is not directly correlated with body weight gain or metabolic rate.

Adiposity can be reduced by these hormones directly, due to the stimulation of lipolysis, and indirectly, due to its action on food and water intake and metabolic rate. However, SHR presented the most evident lipodystrophy, which could be observed not only by the reduction in all adiposity parameters, but also by the alteration of the endocrine function of adipose tissue, with significant changes in the production of leptin and adiponectin. Thus, our study suggests that endocrine changes in hypertension models may highlight possible therapeutic targets in the treatment of metabolic changes associated with hypertension.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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8. TABLES

Table 1 - Body weight, food and water intake and RMR in WIS, L-NAME, WKY and SHR rats at 7 and 15 weeks of age.

	7th Week				15th Week			
	WIS	L-NAME	WKY	SHR	WIS	L-NAME	WKY	SHR
Body weight (g)	251.4±9.3	268.7±5.6	313.1±6.7$^{\#}$	183.7±6.0$^{\$\#}$	436.8±7.2*	446.6±7.2*	488.6±6.3$^{\#*}$	314.2±11.9$^{\$\#*}$
Water intake (mL/g of rat)	0.12±0.005	0.14±0.011	0.16±0.003$^{\#}$	0.17±0.004$^{\$\#}$	0.09±0.001*	0.11±0.002$^{\$}$	0.12±0.004$^{\#*}$	0.12±0.008*
Food intake (g/g)	0.11±0.012	0.10±0.009	0.09±0.002$^{\#}$	0.11±0.003$^{\$\#}$	0.06±0.001*	0.05±0.002$^{*\$}$	0.06±0.001*	0.07±0.003$^{*\$\#}$
RMR (J/g/min)	18.4±0.8	17.3±0.3	17.5±0.6	32.9±1.8$^{\$\#}$	10.3±0.3*	10.3±0.4*	9.2±0.3$^{\#*}$	14.6±0.6$^{\$\#*}$

Body weight, food and water intake and RMR for WIS, L-NAME, WKY and SHR rats. Data are presented as mean ± SEM. *$p<0.05$ compared in the same strain over the time; $^{\$}p<0.05$ compared to control at the same week (WIS vs. L-NAME; WKY vs. SHR); $^{\#}p<0.05$ compared to the same condition (WIS vs. WKY; L-NAME vs. SHR); WIS = Wistar; L-NAME = N^{G}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

Table 2 – Changes in anthropometry and energy expenditure in WIS, L-NAME, WKY and SHR rats at 15 weeks of age.

	WIS	L-NAME	WKY	SHR
Mean blood pressure (mmHg)	106.9±9.2	145.2±12.4*	107.1±5.9	127.1±4.9*
Weight gain (%)	74.6±5	66.3±1.2	56.3±2.7$^{\#}$	71±2.5*
Reduction in food intake (%)	44.7±5.7	46.7±2.6	31.3±1.0$^{\#}$	39.2±2$^{*\#}$
Reduction in RMR (%)	43.9±1.9	40.4±1.8	47.2±1.6	55.3±1.4$^{*\#}$

Evaluation of blood pressure, weight gain and reduction in food intake and RMR. Data are presented as mean ± SEM. *p<0.05 compared to control (WIS vs. L-NAME; WKY vs. SHR); $^{\#}$p<0.05 compared to the same condition (WIS vs. WKY; L-NAME vs. SHR); WIS = Wistar; L-NAME = N^{G}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

Table 3 – Serum values for T3, ACTH, corticosterone, catecholamines and adipokines in WIS, L-NAME, WKY and SHR rats at 15 weeks of age.

	WIS	L-NAME	WKY	SHR
T3 (pmol/L)	5368±1107	8261±676*	7579±246	9623±732*
ACTH (pg/mL)	0.8±0.1	1.0±0.2	5.1±1.2#	1.1±0.1*
Corticosterone (pg/mL)	31572±7175	73491±19322*	78202±10373#	81890±4017
Catecholamines (µmol/L)	45.1±2.4	65.2±2.4*	64.8±4.6#	59.5±1.6
Leptin (pg/mL)	731±157	620±142	942±79	544±96*
Adiponectin (ng/mL)	28441±2435	22676±2242	21728±1552#	36050±2824*#

Serum values for T3, ACTH, corticosterone, catecholamines and adipokines. Data are presented as mean ± SEM. *$p<0.05$ compared to control (WIS vs. L-NAME; WKY vs. SHR); #$p<0.05$ compared to the same condition (WIS vs. WKY; L-NAME vs. SHR); WIS = Wistar; L-NAME = N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

Table 4 – Fat pads and morphometry parameters in WIS, L-NAME, WKY and SHR rats at 15 weeks of age.

	WIS	L-NAME	WKY	SHR
Relative EFP (mg/g)	17.2±0.9	16.4±1.5	19.1±1.0	9.1±0.6$^{*\#}$
Relative RFP (mg/g)	24.1±2.3	17.1±2.4*	16.9±1.2$^{\#}$	12.1±1.0$^{*\#}$
Epididymal adipocyte area (μm^2)	4848±531	3783±357	4621±360	3141±232*
Retroperitoneal adipocyte area (μm^2)	4798±371	3412±295*	5105±681	3623±326*
Mesenteric adipocyte area (μm^2)	3206±252	2373±77*	2691±140$^{\#}$	1997±47$^{*\#}$

Fat pads and morphometry parameters. Data are presented as mean ± SEM. *p<0.05 compared to control (WIS vs. L-NAME; WKY vs. SHR); $^{\#}$p<0.05 compared to the same condition (WIS vs. WKY; L-NAME vs. SHR); WIS = Wistar; L-NAME = N^{G}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats. EFP = Relative Epididymal Fat Pad; RFP = Relative Retroperitoneal Fat Pad.

9. FIGURE LEGENDS

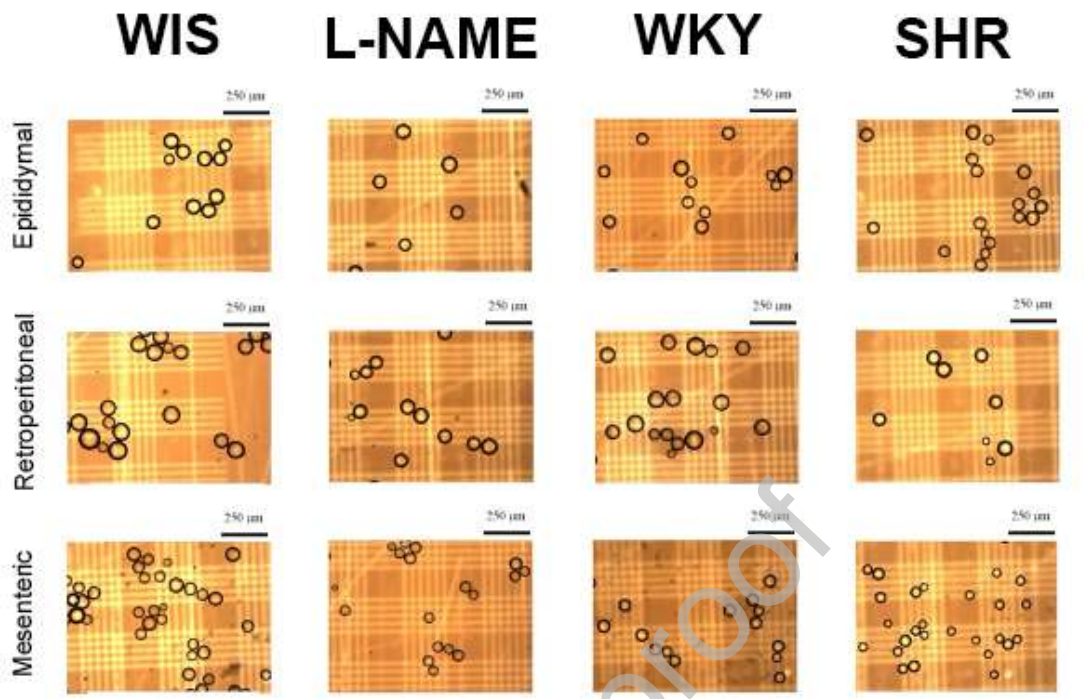

Figure 1 – Isolate adipocytes from epididymal, retroperitoneal and mesenteric fat pads of WIS, L-NAME, WKY and SHR rats at 15th week. WIS = Wistar; L-NAME = N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

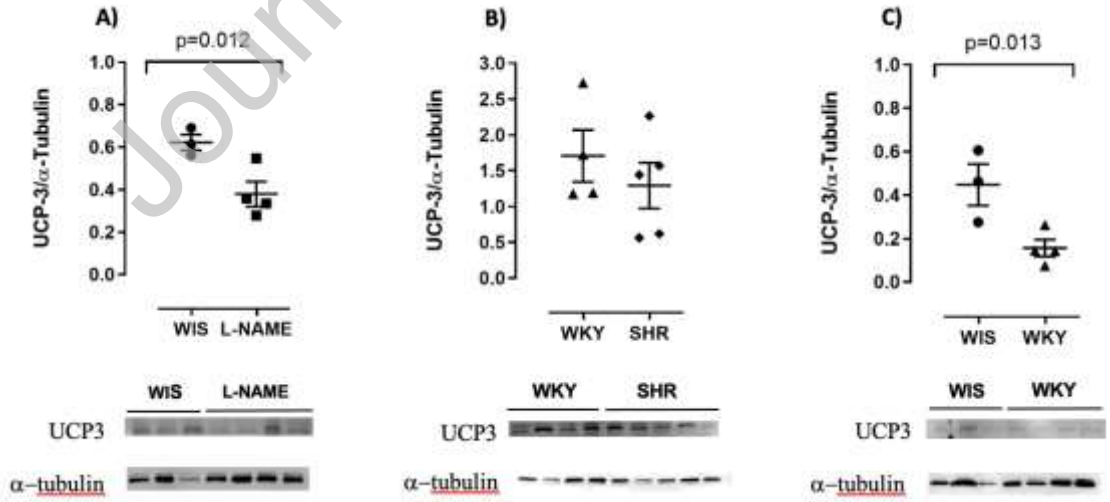

Figure 2 – UCP-3 expression in gastrocnemius muscle from WIS, L-NAME, WKY and SHR rats at 15th week. WIS = Wistar; L-NAME = N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension; WKY = Wistar-Kyoto; SHR = Spontaneously Hypertensive Rats.

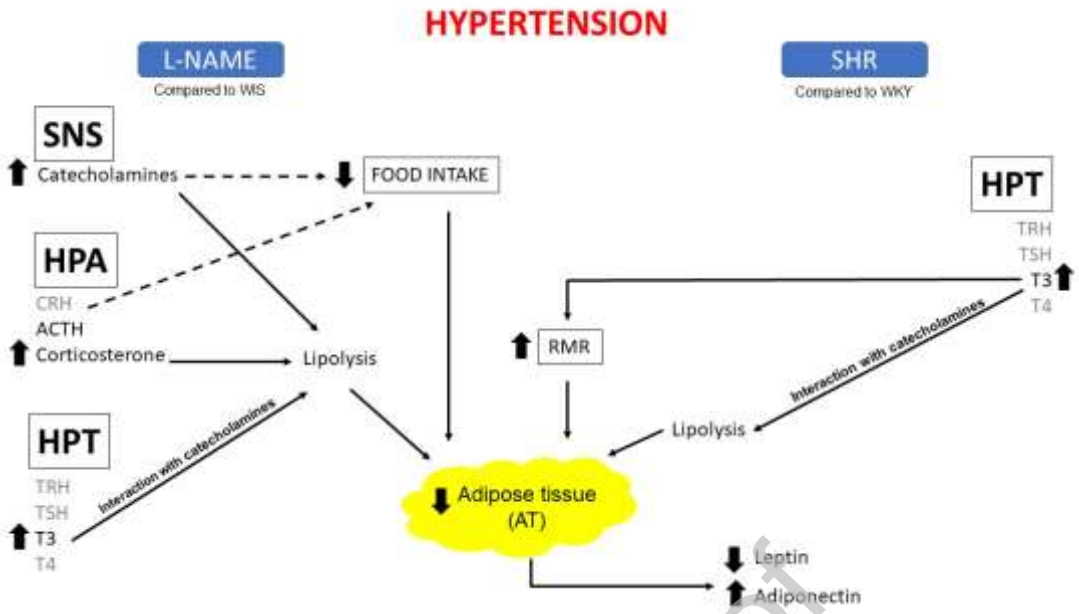

Figure 3 - Proposed mechanisms by which altered hormones may lead to lipodystrophy of adipose tissue in hypertensive L-NAME and SHR rats compared to their normotensive controls. L-NAME rats showed decreased adiposity when compared to WIS rats, which may have been caused by higher levels of lipolytic hormones and lower food intake related to SNS, HPA and HPT activation. SHR rats presented reduced adiposity when compared to WKY rats, which may have been triggered by higher levels of T3, probably due to higher catecholamine-induced lipolysis and higher RMR. Hypolipodystrophy was more evident in SHR, which may have altered the secretion of fat mass related adipocytokines: leptin and adiponectin. SNS: sympathetic nervous system; HPA: hypothalamic–pituitary–adrenal axis; CRH: corticotropin releasing hormone; ACTH: adrenocorticotropic hormone; HPT: hypothalamic-pituitary-thyroid axis; TRH: thyrotropin-releasing hormone; TSH: thyroid-stimulating hormone; T3: triiodothyronine; T4: thyroxine; RMR: resting metabolic rate; WIS: Wistar; L-NAME = N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats; WKY: Wistar Kyoto; SHR: Spontaneously Hypertensive Rats; black text: measured parameters; gray text: non-measured parameters; (↑) increase; (↓) decrease; solid arrow: stimulation; dashed arrow: inhibition.