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SUPPLEMENTATION WITH VITAMIN D ASSOCIATED TO THE EXERCISE ALTERS DIFFERENTLY TO THE MORPHOLOGY OF THE BROWN ADIPOSE TISSUE IN LEAN AND OBESE RATS

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ABSTRACT

Here was evaluated the effect of chronic VD supplementation associated or not to exercise on fat accumulation in Brown Adipose Tissue (BAT) from lean and obese male rats. Obesity was induced by administration of monosodium glutamate (MSG; 4g/Kg). Control (CON) received saline. At 30th day, MSG and CON rats were weaned and subdivided in VD supplemented (12µg/Kg) or non-supplemented (NS), Exercised (E) or Sedentary (S). At 86 days of life, the rats were euthanized and white adipose tissue (WAT) and BAT depots weighted. The BAT was submitted to histological analyses. Data are mean ± SEM with ANOVA and Tukey post-test ($p<0.05$). The VD associated or not to exercise increased WAT depot in CON rats compared to CON-E_{NS} groups. While VD increased BAT proliferation, the VD combined to exercise resulted in higher lipid accumulation in BAT from CON rats ($p<0.05$); without significant effects in MSG rats. Thus, VD supplementation increases adiposity and induces high proliferation in BAT from CON rats. The association, VD and exercise, promoted higher lipid accumulation in BAT from CON lean rats suggesting an inhibitory effect on BAT function. These responses are absent in BAT from MSG obese rats.

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INTRODUCTION

The adipose tissue (AT) is a critical modulator of energy homeostasis acting asan energy reservoir during the period of caloric excessive ingestion or producing energy expenditure via thermogenesis process (Choe *et al.*, 2016). However, to execute these functions there are three distinct subtypes of adipocytes in rodents and humans, white adipocytes, brown adipocytes and beige adipocytes (Park *et al.*, 2014; Ikeda, *et al.*, 2018). The function of the energy reservoir is exerted primarily by White Adipose Tissue (WAT), which is organized in unilocularadipocytes that store large amounts of triglycerides, besides of releasing several adipokines that influence glucose and lipids homeostasis (Birsoy *et al.*, 2013). The excess of WAT is relatedto obesity and their comorbidities, such as diabetes and hypertension (Tai *et al.*, 2000; Janssen *et al.*, 2002). In contrast, the Brown Adipose Tissue (BAT) is made of multilocular brown adipocytes, it is primarily involved in thermogenesis process, an event that produces heat and protects against hypothermia and obesity (Van Marken Lichtenbelt *et al*, 2009).

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TheBAT is characterized by the presence of numerous mitochondria and high expression of uncoupling protein 1 (UCP1), the protein responsible for non-shivering thermogenesiss (Park *et al.*, 2014). Thus, BAT oxidative metabolism has been shown to be a significant contributor to whole body energy expenditure in rodents and in adult humans (Van Marken Lichtenbelt *et al*, 2009; Virtanen *et al*, 2009). Considering that obesity is a result of an energy imbalance, to increase thethermogenesis, it seems an interesting alternative for the treatment of this disease. It is well established that the Sympathetic Nervous System (SNS) via norepinephrine (NE) and subsequent activation of beta-adrenergic receptor (βAR) promotes stimulation of thermogenesis in BAT (Klingenspor, 2003; Nguyen *et al.*, 2011). Interestingly, many effects of exercise are attributed to activation of SNS, including increases in BAT thermogenesis and energy expenditure (Sanchez-Delgado, 2015). However, an earlier study examined the effects of exercise on BAT, with conflicting results (Stanford; Goodyear, 2016). Thus, regular physical exercise has beneficial effects on metabolic health, including the improvement in glucose tolerance, in the insulin sensitivity, and lowering circulating lipid concentration (Cannel *et al.*, 2009), therefore has been used as a tool against obesity and their comorbidities. Physical exercise, however, has not been

sufficient by its own, with this, several nutrients and vitamins are used as a supplement to improve physical performance and augment energy expenditure favouring weight loss (Stanford; Goodyear, 2016). It is interesting that obesity is often related to hypovitaminosis D, an event that contributes to insulin resistance, dyslipidemia and inflammatory process associated with adiposity (Marcotorchino *et al*., 2014). Thus, the VD supplementation has been used in obese humans and rodents to attenuate or improve metabolic abnormalities, frequently present in obese conditions (Bourlon *et al.*, 2009; Jamka *et al*., 2015). The bioactive form is generated after two hydroxylations; occurring first in the liver (25-hydroxycholecalciferol) and then in the kidney (1,25 dihydroxycholecalciferol) (Marcotorchino *et al*., 2014; Percegoni, Castro,2014). The active form of VD binds to a vitamin D receptor (VDR), which is found in many cellular types including the BAT (Stumpf, 1995; Ding *et al*., 2012), however,the role of VD and VDR activation in BAT physiology and morphology has not been extensively studied. The obesity induction in the adulthood, through the administration of high doses of monosodium glutamate (MSG), which is a neuroexcitatory amino acid, during the neonatal phase, leads to hypothalamic lesions in neuronal regions developing an autonomic imbalance, culminating in endocrine alteration and adiposity (Olney, 1969, Konrad *et al.,* 2012). The MSG-treated rats present massive WAT accumulation, insulin resistance, glucose intolerance and dyslipidemia (Macho *et al*., 2000; Diemen *et al*., 2006). Reduction in SNS activity is characteristic in MSG-treated mice (Konrad, *et al*., 2012), an event that could modify thermo genesis in BAT. However, the data are conflicting and some authors report that treatment with neonatal MSG does not affect the activity of the SNS (Iwase *et al.,* 2000).Moreover, MSG-treated rats are responsive to exercise as well as to VD supplementation (Leite *et al.*, 2013; Marcotorchino *et al.,* 2014). In the present study, we explored the effect of the association between chronic VD supplementation and swimming training on BAT morphology with focus in fat deposition in BAT from lean and MSG-treated rats.

EXPERIMENTAL METHODS

Ethical aspects: During lactation and growth, rats were maintained under adequate conditions, according to the guidelines of the National Council for Control of Animal Experiments (CONCEA), following international norms for animal care and maintenance, as recommended by The Arrive Guidelines (2010). The rats were kept in plastic cages (n=3-4 rats/cage) with a stainless steel cover and housed in a controlled environment; with a 12 h light/cycle, constant temperature (21 ± 2ºC) and free access to food and water.The Ethics Committee on Animals Use (CEUA/November, 15/2015) of the Western Parana State University (UNIOESTE) previously approved all the experimental protocols.

Experimental design: At the first day of birth offspring size was adjusted to 6-8 male pups per dam to avoid influences of caloric ingestion in the lactation phase. From the 2nd to the 6th day of life, male Wistar (n=30) rats received daily subcutaneous injections of monosodium glutamate (MSG) in a dose of 4 g/Kg body weight (BW). In the same period, the Control (CON) rats (n=30) received saline (1.25 g/Kg BW). At 30th day of life, the MSG and CON rats were weaned, and randomly assigned to vitamin D (VD)-supplemented or non-supplemented (NS) groups, the rats were divided into Exercised (E) or Sedentary (S) originating eight experimental groups(n=15 rats/group): CON-S_{NS}: Control sedentary non-supplemented; CON-S_{VD}: Control sedentary VD supplemented; CON-E_{NS}: Control exercised non-supplemented; CON-$E_{VD:}$ Control exercised VD supplemented; MSG-S_{NS}: MSG sedentary non-supplemented; MSG-S_{VD}: MSG sedentary VD supplemented; MSG-E_{NS}: MSG exercised non-supplemented; MSG-E_{VD}: MSG exercised VD supplemented. The VD supplementation and exercise training were applied during the same period (from 30th to 85th day of life) and in the same frequency (3 times per week). Thus, the VD supplemented groups received VD (SupraD®) at a dose of 12µg/Kg (Sadek, Shaheen, 2014) of BW dissolved in corn oil (vehicle). The NS groups also were gavaged with the same volume of the vehicle but without VD in the same frequency that the VD supplemented groups. The exercised rats swan 3 times per week during 30 minutes in a stainless steel tank (57 cm length X 105 cm width X 60 cm depth) and the water temperature was kept at 32±2ºC, according to the previousprotocol (Leite *et al*., 2013). To avoid animal's accommodation, they had an overcharge with 5% of body weight tied on their tails (Leite *et al*., 2013). The swimming was conducted in the afternoon; the rats were dried and then, returned to their cages. Sedentary (S) animals did not swim at any time of the experiment.

Biometric parameters: At the 86th day of life, 48 hours after the last swimming training and VD supplementation, animals were euthanized. Total body weight (BW) and naso-anal length were registered and the Lee Index (LI) calculated according to the formula: *animal BW (g)$^{1/3}$/naso-anal length (cm) X 1000*. The LI is an obesity predictor for rats according to Bernardis e Patterson (1968), which ratified Lee's report (1928). White adipose tissue (WAT) (retroperitoneal, perigonadal, perirenal and inguinal fat pads), were collected to evaluate the efficacy of MSG neonatal treatment on WAT expansion.

Histological analysis of brown adipose tissue (BAT): The interscapular BAT was identified, collected, clean, weighted and immediately transferred to histological fixation solution (ALFAC) for 24h, before storing in 70% alcohol. After a week, the BAT was dehydrated in progressive concentrations of alcohol solution from 70 to 100%, then it was diaphonized in xylol and impregnated with paraffin. The tissues were sectioned at 5µm on a Reichert Jung rotary microtome (Leica RM 2025 Microsystems Inc., Wetzlar, Germany) and Hematoxylin and Eosin (H&E) staining. Three microscopic fields per section and three sections per animal (6 rats per group) were analyzed. Afterward, stained preparations were photographed (10x objective, 50µm scale) in a photomicroscope (OLYMPUS BX60), coupled to the capture chamber (OLYMPUS DP71) and analyzed using Image J software (Bethesda, MD, USA), available on the NIH site (http://rsb.info.nih.gov/ij, accessed on 15 June 2017). The fat accumulation in BAT was quantitatively evaluated by measurement of the adipocyte size. In addition also were counted the number of nucleus by field in BAT. For such, a quadrant (501 µm) was selected and the total nucleus in the area was counted.

Statistical Analysis: Data are presented as mean ± standard error mean (SEM). To analyze the effect of neonatal MSG treatment on inducing obesity in animals, test t Student's was conducted ($p<0.05$). The impact of VD associated or not with swimming training was evaluated inside of CON lean rats and

MSG-treated rats using Two-way ANOVA. When F values were significantly different ($p<0.05$), the Tukey post-test was applied. The statistical analyses were conducted using Prism for Macintosh, version 5.0 (Graph Pad Software, San Diego, CA, USA).

RESULTS

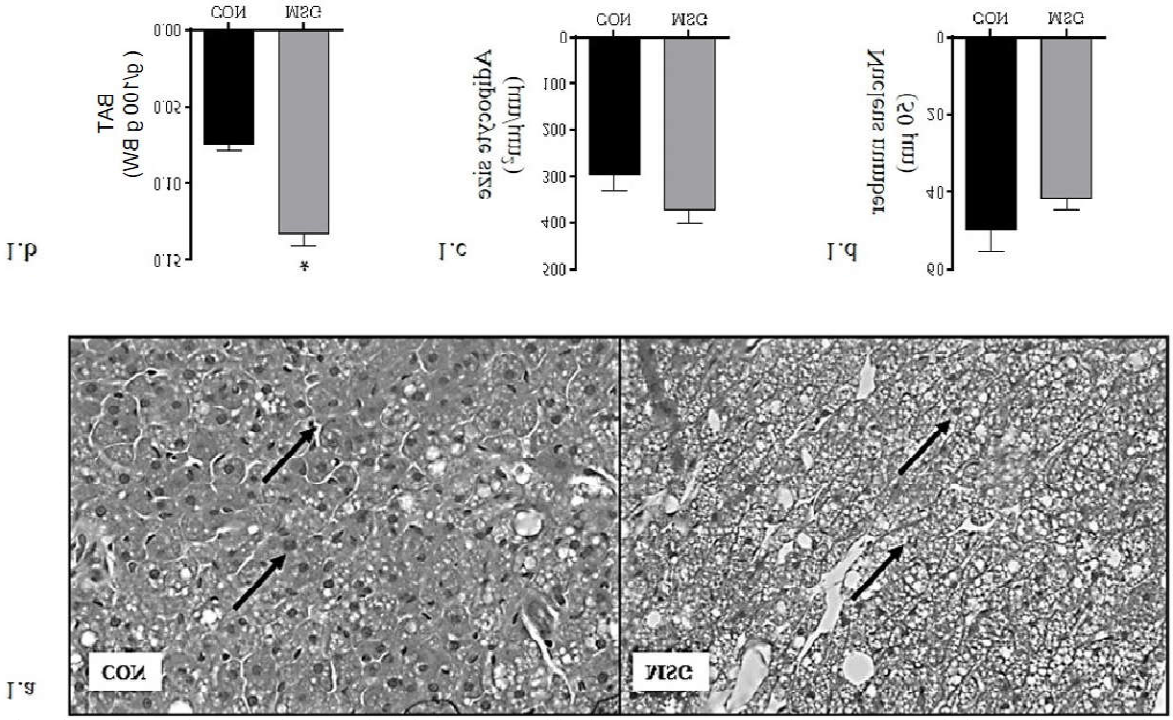
The MSG-treated rats presented a reduction in body weight (26.98%), naso-anal length (9.93%) and higher adiposity (50.04%) in relation to CON animals (Figure 1; $p<0.05$). Despite the measure of the adipocyte size be similar between CON and MSG groups, it is possible to observe (Figures 1 a and b) that rats from MSG group presented more lipid droplets deposition in the cytosol than the ones from CON group (Figure 1.c).

Moreover, CON-E_{VD} also presented higher content of perigonadal fat depot compared to CON-E_{NS} ($p<0.05$). The impact of VD supplementation associated or not to exercise on MSG obese rats are shown in Table 3. The influences of VD supplementation associated or not with exercise in anthropometric parameters from CONlean rats are shown in Table 1. The VD supplementation in sedentary animals exerted effect on body weight ($F_{1,53}$= 8.488; p = 0.0052). Thus, CON-S_{VD} rats presented higher body weight in relation to CON-E_{NS} animals ($p<0.05$).The naso-anal length ($F_{1,53}$ = 0.855; p = 0.359), Lee Index ($F_{1,54}$ = 2.848; p = 0.097), content of retroperitoneal ($F_{1,54}$ = 0.501; p = 0.4817) andinguinal fat depot ($F_{1,53}$ = 0.290; p = 0.592) were not affected significantly neither by VD supplementation nor by exercise ($F_{1,53}$ = 0.039; p = 0.843; $F_{1,54}$ = 3.313; p = 0.074; $F_{1,54}$ = 3.310; p = 0.074; $F_{1,53}$ = 2.871; p = 0.096; $F_{1,54}$ = .268; p =0.265, respectively).

Table 1. Effect of neonatal MSG treatment in biometric parameters and adiposity.

	CON(n=10)	MSG(n=10)
Body weight (g)	335.80 ± 7.00	245.2 ± 8.62*
Naso-anal length (cm)	22.55 ± 0.18	20.30 ± 0.26*
Lee Index	308.15±2.63	312.58 ± 5.10
Retroperitoneal Fat (g/100g BW)	0.38 ± 0.03	0.76 ± 0.04*
Perigonadal Fat (g/100g BW)	0.41 ± 0.02	0.77 ± 0.06*
Perirenal Fat (g/100g BW)	0.22 ± 0.01	0.35 ± 0.04*
Inguinal Fat (g/100g BW)	0.20 ± 0.02	0.49 ± 0.07*

Data are mean±SEM. *p<0.05 test t Student´s. BW: body weight; CON: control and MSG: monosodium glutamate.



Representative photomicrography (1.a) of BAT stained in H&E; magnification (10x); 50.0μm scale. Black arrows indicate adipocyte nucleus. Graphs present the mean±SEM of BAT weight (Fig. 1.b), adipocyte size (Fig. 1.c) and number of adipocyte nucleus (Fig. 1.d) in BAT. The symbol "*" above of bars represent statistical difference in T test Student's ($p < 0.05$); n = 6.CON, control; MSG, monosodium glutamate.

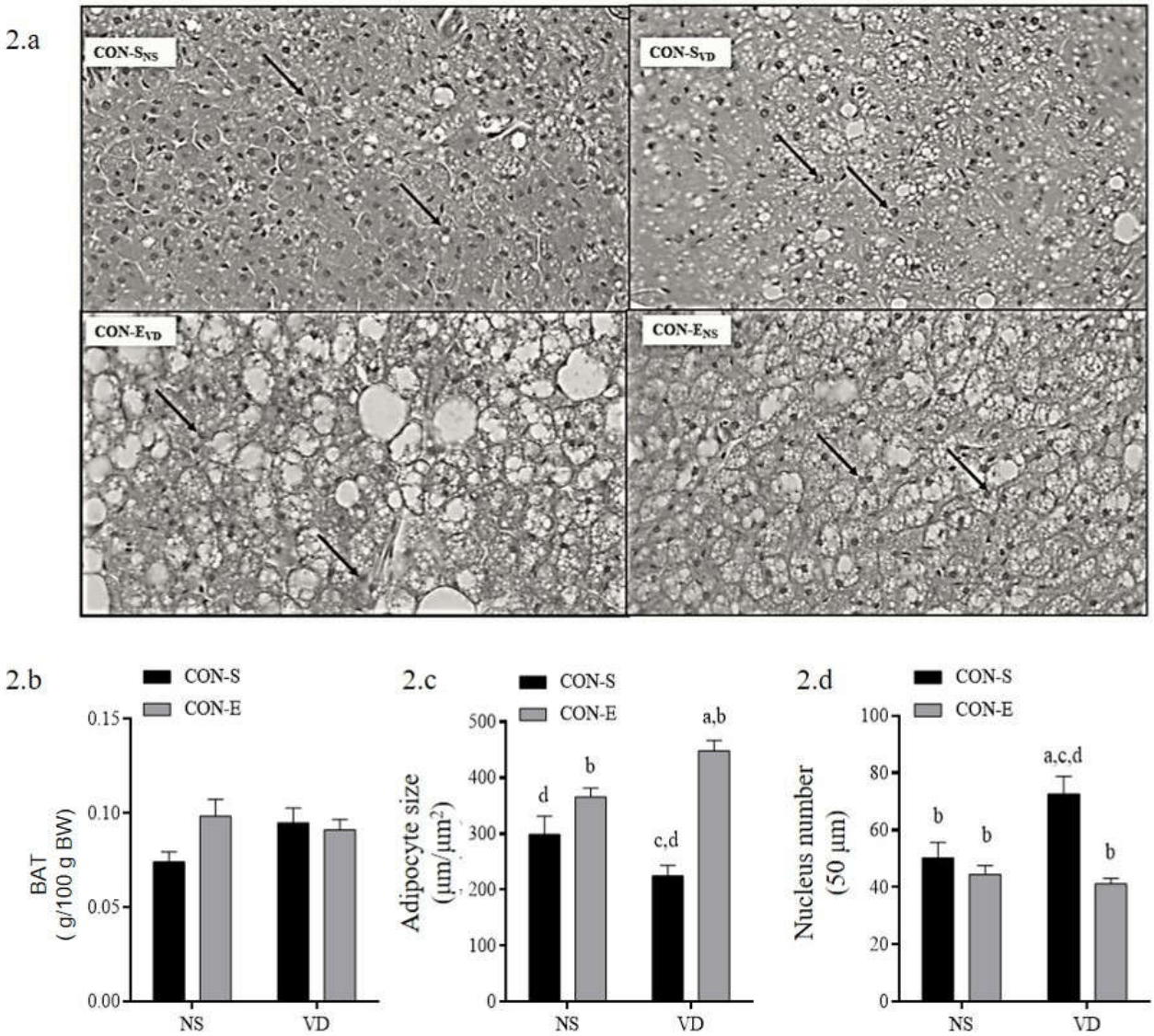
However, the VD supplementation associated or not to exercise, influenced the perirrenal ($F_{1,54}$ = 7.779; p = 0.0773)and perigonadal ($F_{1,54}$ = 11.64; p = 0.0012) fat depots. Thus, the CON-S_{VD} group presented higher content of perirenal and perigonadal fat depots in relation to CON-E_{NS} rats ($p<0.05$).

Neither VD supplementation nor exercise influenced significantly the body weight, naso-anal length, Lee Index or adiposity in MSG-obese rats ($p>0.05$). Despite VD supplementation and exercise had influenced adipocyte size in BAT from MSG-obese rats ($F_{1,20}$ = 5.287; p = 0.032), there

Table 2. Effects of chronic VD supplementation associated or not with exercise on biometrics parameters of CON lean rats at the 86th day of life

	$CONS_{NS}$	$CON\text{-}S_{VD}$	$CON\text{-}E_{NS}$	$CON\text{-}E_{VD}$	p-value VD	p-value Exercise	p-value interaction
Body weight (g)	335.80 ±7.00	351.64±5.60[c]	316.58 ±6.66[b]	345.15 ±10.86	0.005	0.097	0.407
Naso-anal length (cm)	22.55 ±0.18	22.76 ±0.15	22.62 ±0.18	22.76 ± 0.23	0.359	0.843	0.862
Lee Index	308.15±2.63	310.07±2.10	301.17 ±2.05	307.82 ±3.11	0.097	0.074	0.355
Retroperitoneal Fat (g/100g BW)	0.38 ±0.03	0.41 ±0.03	0.32 ±0.03	0.34 ±0.03	0.481	0.074	0.814
Perigonadal Fat (g/100g BW)	0.41 ±0.02	0.48 ±0.03[c]	0.33 ±0.02[b,d]	0.45 ±0.02[c]	0.001	0.059	0.405
Perirenal Fat (g/100g BW)	0.22±0.01	0.28 ±0.02[c]	0.16 ±0.01[b]	0.23 ±0.02	0.007	0.028	0.883
Inguinal Fat (g/100g BW)	0.20 ±0.02	0.18 ±0.02	0.15 ±0.03	0.15 ±0.01	0.592	0.096	0.819

Values represent mean ± SEM; n = 10 – 15 rats by group. ANOVA two-way, followed by Tukey's post-test ($p< 0.05$). Different letters on the same line represent statistical differences between groups. $CON\text{-}S_{NS}$: control sedentary non-supplemented; $CON\text{-}S_{VD}$: control sedentary supplemented with VD; $CON\text{-}E_{NS}$: control exercised non-supplemented; $CON\text{-}E_{VD}$: control exercised supplemented with VD. (a) $CON\text{-}S_{NS}$; (b) $CON\text{-}S_{VD}$; (c) $CON\text{-}E_{NS}$ and (d) $CON\text{-}E_{VD}$.



Representative photomicrography (2.a) of BAT stained in H&E; magnification (10x); 50.0µm scale. Black arrows indicate adipocyte nucleus. Black arrows indicate adipocyte nucleus. Graphs present the mean±SEM of BAT weight (Fig. 2.b), adipocyte size (Fig. 2.c) and number of nucleus (Fig. 2.d) in BAT . The letters above of bars represent statistical difference in ANOVA two-way with *Tukey* post-test ($p < 0.05$); n = 6 rats by group. $CON\text{-}S_{NS}$: control sedentary non-supplemented; $CON\text{-}S_{VD}$: control sedentary supplemented with VD; $CON\text{-}E_{NS}$: control exercised non-supplemented; $CON\text{-}E_{VD}$: control exercised supplemented with VD. (a) $CON\text{-}S_{NS}$; (b) $CON\text{-}S_{VD}$; (c) $CON\text{-}E_{NS}$ and (d) $CON\text{-}E_{VD}$.

Figure 2. Effect of chronic VD supplementation associated or not to exercise in histological aspects of BAT from Control lean rats

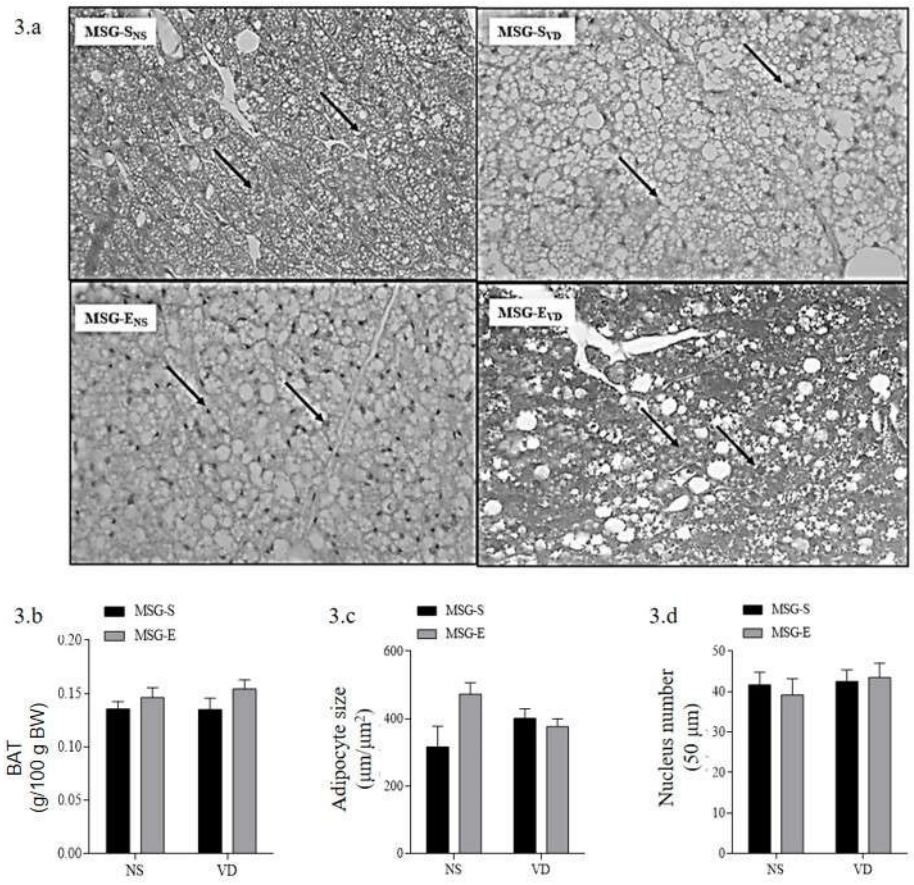
were no statistical differences between the means from obese groups in the post-test. The VD supplementation influenced the proliferation of adipocyte number in BAT ($F_{1,20} = 4.557$; $p = 0.045$) from Control lean rats (Figures2.a-2.d). Thus, rats from CON-SVD group presented lower adipocyte size associated with higher nucleus number in BAT in relation to CON-SNS rats ($p<0.05$).

Moreover, the association of VD and exercise influenced BAT histology ($F_{1,20} = 8.2878$; $p = 0.0093$). Thus, in $CON\text{-}E_{VD}$ ratswe observed the higher size of lipids dropletsin relation to$CON\text{-}S_{NS}$ and $CON\text{-}S_{VD}$ groups ($p<0.05$). Moreover, $CON\text{-}E_{VD}$ also presented reduction of nucleus number in relation to $CON\text{-}S_{VD}$ ($p<0.05$).

Table 3. Effects of chronic VD supplementation associated or not to exercise on biometric parameters from MSG obese rats at the 86th day of life

	MSG-S_{NS}	MSG-S_{VD}	MSG-E_{NS}	MSG-E_{VD}	p-value VD	p-value Exercise	p-value interaction
Body weight (g)	245.21 ± 8.62	249.06 ± 7.83	247.00 ±13.29	222.09 ±7.73	0.259	0.178	0.125
Naso-anal length (cm)	20.30 ± 0.26	20.06 ± 0.25	20.30 ± 0.39	19.81 ± 0.35	0.272	0.691	0.709
Lee Index	312.58 ± 5.10	314.40 ± 3.25	311.37 ± 4.19	313.02 ±2.07	0.620	0.712	0.980
Retroperitoneal Fat (g/100g BW)	0.76 ± 0.04[c]	0.71 ± 0.03	0.55 ± 0.04[a]	0.69 ± 0.04	0.276	0.012	0.029
Perigonadal Fat (g/100g BW)	0.77 ± 0.06	0.79 ± 0.07	0.61 ± 0.06	0.72 ± 0.04	0.053	0.311	0.488
Perirenal Fat (g/100g BW)	0.35 ± 0.04	0.35 ± 0.04	0.29 ± 0.02	0.34 ± 0.03	0.573	0.373	0.448
Inguinal Fat (g/100g BW)	0.49 ± 0.07	0.58 ± 0.10	0.28 ± 0.05	0.44 ± 0.04	0.112	0.022	0.675

Values represent mean ± SEM; n = 10 – 15 rats by group. ANOVA two-way, followed by Tukey's post-test ($p< 0.05$). BAT: brown adipose tissue. MSG-S_{NS}: monosodium glutamate obese sedentary non-supplemented; MSG-S_{VD}: monosodium glutamate obese sedentary supplemented with VD; MSG-E_{NS}: monosodium glutamate obese exercised non-supplemented; MSG-E_{VD}: monosodium glutamate obese exercised supplemented with VD. (a) MSG-S_{NS}; (b) MSG-S_{VD}; (c) MSG-E_{NS} and (d) MSG-E_{VD}.



Representative photomicrography (3.a) of BAT stained in H&E; magnification (10x); 50.0µm scale. Black arrows indicate adipocyte nucleus. Graphs present the mean±SEM of BAT weight (Fig. 3.b), adipocyte size (Fig. 3.c) and number of nucleus(Fig. 3.c) in BAT . The letters above of bars represent statistical difference in ANOVA two-way with Tukey post-test ($p < 0.05$); n = 6. MSG-S_{NS}, monosodium glutamate obese sedentary non-supplemented; MSG-S_{VD}: monosodium glutamate obese sedentary supplemented with VD; MSG-E_{NS}: monosodium glutamate obese sedentary non-supplemented; MSG-E_{VD}: monosodium glutamate obese exercised supplemented with VD. Different letters on the same line represent statistical differences between groups. (a) MSG-S_{NS}; (b) MSG-S_{VD}; (c) MSG-E_{NS} and (d) MSG-E_{VD}.

Figure 3. Effect of chronic VD supplementation associated or not to exercise in histological aspects of BAT from MSG obese rats.

DISCUSSION

The obese population has increased exponentially over the last decades, having obesity reached pandemic status (Diemen; Trindade, 2006). Despite the intensive advances over the comprehension in the role of energy metabolism, it is clear that the actual knowledge and strategies are not enough to attenuate or avoid the overweight and obesity progress and to mitigate the impact of their comorbidities on health. Within this context, different types of nutrients are usually djuvants in reducing excessive body fat or in the combat of metabolic abnormalities resultant from this condition (Bischoff-Ferrari *et al*., 2006). Interestingly, the excess of WAT is associated with hypovitaminosis, including the VD lower bioavailability (Worstman *et al.*, 2000). Insulin sensibility improvement, reduction in the inflammatory process, effects on food intake and better lipid control, have been reported as VD supplementation benefits on obesity (Cannel *et al.*, 2009). Similar effects are also observed in regular physical activity

practice (Carrillo *et al.*, 2013). Increasing energy expenditure to further reduce WAT are key events against obesity (Goodyear; Khan, 1998). In this sense, thermogenesis is an energy expenditure process primary realized on BAT which can influence body weight (Stanford; Goodyear, 2016). The present study evaluated the capacity of chronic VD supplementation, associated with physical exercise on modulating BAT of lean and obese rats. The MSG obesity is resultant from the autonomic activity disruption, particularly a lower SNS activity and hence lower energy expenditure (Diemen; Trindade; Trindade, 2006), events related to a lower thermogenic activity on BAT from these animals (Leitner; Bartness, 2009). Previous data, MSG-treated rats presented diminished body weight and naso-anal length, followed by higher adiposity in relation to control animals (Leitner; Bartness, 2009). In our study, although we had not observed statistical differences in adipocyte size, it is evident that BAT from MSG group presented greater fat amount and adipocyte size compared to CON group. Thermogenesis is the ability to dissipate energy as heat through glucose and lipid oxidation in brown adipocyte, a process regulated by SNS activity. Sympathetic branch activation seems to be evident in situations where an organism needs to keep thermoneutrality, in order to low temperatures (Cannon; Nedergaard, 2004). Recent data suggest that even in humans, BAT reactivation could be possible, favoring weight loss (Dadson *et al*, 2018).

Thus, it is estimated that if 50g of BAT in humans was activated, the subject would expend 5% more energy than in absolute repose (Virtanen *et al*, 2009; Van Marken; Lichtenbelt, Schrauwen, 2011), in other words, BAT activation could influence body fat loss and, consequently, exerting positive metabolic effects. Interestingly, BAT reactivation in humans is primarily promoted by regular physical activity or low temperature exposition (Oh-ishi *et al*., 1996). Additionally, it is important to mention that regular physical activity also stimulates sympathetic branch, elevates catecholamines release, adrenalin, and noradrenaline, favoring lipolysis in WAT and weight loss (Zouhal*et al.*, 2008). However, physical exercise effects on BAT seem contradictory. Several animal model studies showed that during physical activity occurs hypoactivation of BAT (Shibata, Nagasaka, 1987; Yamashita *et al*., 1993; Scarpace *et al*., 1994; Segawa *et al*., 1998). In contrast, other studies reported BAT activation (De Matteis *et al*., 2013) increase in mitochondrial UCP-1 expression (Boström *et al*., 2012; Slocum *et al*., 2013) and in vascularization. The VDR is found in BAT and seems to exert effects about proliferation as well as in the beta-oxidation (Bhat *et al*., 2014). It remains unclear, until this moment, if VD action in BAT is related to SNS activity. Thus, in the present study, we evaluated the VD and physical activity interaction on BAT from lean and obese rats. According to our findings, regular swimming leads to a significant reduction in WAT only in MSG rodents, suggesting more sensibility to a lipolytic effect on adipocyte from these depots. These results confirm previous studies with MSG-obese animals, showing that the activation of sympathoadrenal axis induced by regular swimming is higher in this model (Andreazzi *et al.*, 2012). Nevertheless, in sedentary lean rodents we noticed that chronic VD supplementation increased WAT content, in particular when compared to exercised lean rodents. This probably explains the greater body weight observed in lean rodents supplemented with VD in relation to exercised lean rats.Considering that lean rats have normal VD plasmatic level it is probable that chronic VD supplementation had provoked excessive action in adipocytes from WAT favouring adipogenesis or lipogenesis. In this regard, the WAT presents VDR, which modulates the metabolism and proliferation in this tissue (Bhat *et al*., 2014). VDR knockout (VDRKO) mice exhibit reduced adiposity and resistance to weight gain when fed either with low or high fat diets, supporting a role of VDR in energy metabolism. However, the conditional deletion of VDR in adipocytes does not transmute the lean phenotype observed in global VDRKO mice (Wong *et al.*, 2009). Despite of contradictories results is evident the role of VD on adipocytes from WAT, an event that needs to be clarified in future. In lean and MSG-treated rats regular swimming singly did not provoke significant alterations in BAT weight, fat content or nucleus number in this tissue in relation to sedentary animals. The effects of exercise in BAT occur at nuclear levels. Shibata and Nagasaka (1987) also did not find alterations on BAT weight after exercise training. In contrast, Vosselman (2015) observed reduced BAT activity in exercised subjects in comparison to sedentary ones. In contrast, the swimming training increased BAT mass and its protein content in mice, suggesting hypertrophy and hyperplasia, consequences of UCP1 expression increased, suggesting enhances the thermogenic activity and capacity in BAT of exercised mice (Oh-ishi *et al*., 1996). Here we demonstrated for the first time, that the association of VD and regular exercise in lean rats provoked higher fat accumulation in BAT, suggesting inhibition of thermo genesis process. The physical training profile is important to evaluate BAT responsiveness. Thus, during swimming training occur a higher heat production by muscle activity; this process could induce an unbalance in temperature control (Stanford; Goodyear, 2016). Heat dissipation during exercise practice is an important physiological mechanism that may influence physical performance. In this sense, it is probable that thermo genesis in BAT, from exercised rats, has been inhibited to avoid hyperthermia. Moreover, studies have demonstrated that VDR in BAT could inhibit thermo genesis (Wong *et al.,* 2009). According to data obtained by Sullo *et al*. (2004), swimming can reduce T3-induced thermo genesis in BAT through changes in 5'deiodase activity. Thus, we believe that VD supplementation combined to exercise results in more intense inhibitory effect in beta-oxidation in BAT and, consequently, more lipid accumulation in adipocyte. Corroborating with this hypothesis, experiments using primary BAT culture confirmed that VD directly suppressed the expression of the UCPs (Wong *et al.*, 2009; Ricciardi *et al*., 2015). In the present study, we also demonstrated that solely the VD supplementation in sedentary lean rats provoked an increase in BAT proliferation. Therefore, is evident that combined or not to exercise, VD can modify BAT morphology in lean rats. As mentioned above, MSG-treated rodents present changes in BAT activity, an effect resultant of lower SNS activity (Diemen; Trindade; Trindade, 2006). Here, we did not find a significant effect of VD supplementation in BAT proliferation and lipid accumulation, indicating that MSG-treated rats are unresponsive to VD action in BAT. In contrast, suppressive effects of VD and VDR signalling in BAT adipocytes differentiation and mitochondrial respiration was found by Ricciardi *et al*. (2015) in BAT from obese mice. Taken together, these different results suggest that the role of VD supplementation in regulating BAT development and function in obesity needs further investigation. In addition, in MSG-treated rats, supplementation with VD combined to exercise also did not provoke alteration in BAT morphology. This is a first study that explores the effect of VD combined or not to swimming training in BAT from MSG-treated rats. It is

important to recognize that MSG-treated rats have lesions in hypothalamic regions, important to the central actions of VD. In addition, MSG-treated rats present high adiposity, an event that could reduce VD bioavailability. However, the effects of VD on BAT function in this obese model remain unknown. In conclusion, chronic VD supplementation induces rises in WAT content in lean rats. Moreover, the association of VD supplementation and swimming training resulted in high-fat accumulation in BAT from lean rats, suggesting inhibitory action on thermo genesis. BAT from MSG-treated rats are unresponsive to VD effects, isolate or in combination to exercise.

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