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Browning of white adipose tissue by melatonin

Eleen Zarebidaki

BROWNING OF WHITE ADIPOSE TISSUE BY MELATONIN

by

ELEEN ZAREBIDAKI

Under the Direction of Timothy Bartness, PhD

ABSTRACT

There are two distinct types of adipose tissue which have different functions within the body, white (WAT) and brown (BAT). Browning of WAT occurs with increases in the WAT sympathetic nervous system (SNS) drive. In this regard we previously reported that melatonin (MEL) stimulation of MEL receptor 1A (MEL_{1a}) within the SNS outflow to the WAT might be implicated in a naturally-occurring reversal of obesity (by ~30% of total body fat). Therefore, in this study we tested the hypothesis that MEL causes browning of WAT through the stimulation of SNS drive to WAT. This was done by comparing specific browning and lipolytic markers in WAT following 10 weeks of MEL treatment, short day housing (SD), and long day housing with saline injections (LD+VEH). Browning effects of a 5 day treatment of a β3-adrenergeric (β3-AR), CL 316, 243, were also measured. We found that CL 316, 243, MEL treatment, and SD housing had increased expressions of browning markers within WAT and lipolytic activity in MEL treated animals was increased in specific WAT.

INDEX WORDS: Brown adipose tissue, Lipolysis, obesity, UCP1, PGC-1α

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1 INTRODUCTION

1.1 Seasonal changes within Siberian hamsters and MEL

Obesity has shown to be one of the leading causes of death in the United States, being a primary risk factor for diseases such as diabetes, cardiovascular disease, and hypertension[1,2]. These health complications account for at least 85% of the obesity-related economic burden[3], therefore, a lot of research has been going into finding a methods for its reversal. An ideal animal model for obesity reversal research is the Siberian hamster (*Phodopus sungorus*) due to its innate ability to express an obese phenotype with only a change in photoperiod. No genetic or dietary changes are needed for this phenomenon to occur. When exposed to short-day (SD) "winter-like" photoperiods (8hr light:16hr dark) Siberian hamsters express lean body mass (~20 % body fat) however, if the light cycle is altered to a long day (LD) photoperiod (16hr light:8hr dark) resembling "summer-like" conditions, their physiology is transformed to express an obese phenotype (~50 % body fat)[4-7].

The mechanism behind this seasonal change is controlled by melatonin (MEL); a neuropeptide hormone released from the pineal gland whose principal function is to maintain a natural circadian rhythm within the body. Studies have shown that MEL causes a decrease in obesity, without affecting food intake and is hypothesized to be a thermogenic regulator[8-10]. When retinal ganglionic cells of the eyes detect darkness, a signal is sent to the suprachiasmatic nucleus (SCN) of the brain, which in turn, through a series of pathways, triggers the pinealocytes to release MEL into the bloodstream. The amount of MEL released over time is directly proportional to the duration of darkness[11,12]. Obstruction of the signal from the retinal ganglion or pineal gland disrupts this seasonal change[13]. When the pineal gland is removed, it has been seen that photoperiodic changes are blocked in Syrian hamster, including body and lipid mass

changes within Siberian hamsters[4,11,12]. This intrinsic system is vital for the determination of seasonal changes, which triggers physiological transformations necessary for survival and reproduction.

It was previously thought that seasonal changes in circulating hormones such as gonadal steroids, prolactin, thyroid hormones, glucocorticoids, and epinephrine cause the seasonal lipolytic changes however, that was proven to not be the case and the changes are possibly neuronal rather than hormonal[14]. The lipolytic activity of MEL can easily be measured by analyzing the ratio of hormone-sensitive lipase (HSL) and phosphorylated hormone-sensitive lipase (pHSL). HSL is the rate limiting step in the breakdown of triglycerides to free fatty acids (FFA) within adipose tissue which in turn allow for the thermogenic effects to occur. When HSL hydrolyzes triglycerides, it becomes phosphorylated, therefore, by taking the ratio of unphosphorylated to phosphorylated HSL we can determine the degree to which lipolysis is occurring within the given fat pad[15,16].

1.2 Fat depots and their innervations

Fat cells are not distributed equally throughout the body; they are accumulated in clumps known as fat depots. Siberian hamsters have many fat pads situated subcutaneously [inguinal white adipose tissue (IWAT) and interscapular brown adipose tissue (IBAT)], as well as more internally located [retroperitoneal white adipose tissue (RWAT), epididymal white adipose tissue (EWAT), and mesenteric white adipose tissue (MWAT)]. There are two different types of adipose tissue, brown and white, which play significantly different roles within the body.

Brown adipose tissue (BAT) is unique in that it is responsible for quick access to energy in the form of heat through non-shivering thermogenesis, signaled by uncoupling protein-1 (UCP1), a protein specific to BAT. In contrast, WAT is responsible for the long-term storage of

energy in the form of triglycerides which when activated by the SNS, undergoes lipolysis. BAT, which contains a higher concentration of mitochondria compared with WAT, is activated in times of physiological thermogenic challenge such as exposure to cold, food deprivation, and exercise.

It has previously been shown that the SNS release of norepinephrine (NE) triggers lipolysis in vitro[17]. Neuroanatomical evidence for the link between MEL the SNS increase in lipolysis was shown through the injection of a retrograde transneuronal tract tracer, Pseudorabies virus (PRV), into WAT which labels the SNS pathway from the brain to fat pad. Regions of the brain that were connected to the fat pad showed infection of the PRV virus in areas hypothesized to contain MEL receptors, such as the SCN, optic chiasm, dorsomedial hypothalamic nucleus, periventricular hypothalamic nucleus (PVH), as well as several other nuclei. These areas infected with PRV were later tested for co-localization of MEL1a. Major areas such as the SCN, pars tuberalis, PVH, and nucleus reuniens, showed double labeling of SNS innervation and MEL1a receptor. These findings imply the importance of MEL in SNS innervation of fat pads and in the effects of seasonal changes within them, such as browning of white adipose tissue[7].

1.3 Browning of white adipose tissue

Approximately three decades ago the idea of browning of white adipose tissue (WAT) was introduced, but it was not until recently that it has become an area of increasing interest. It has been shown that WAT can contain traces of brown adipocytes under conditions of thermogenic hardships, among many others, not only in rodents, but also in humans[18]. Brown adipocytes within WAT, referred to as brite cells[19], exhibit the same thermogenic characteristics found in BAT adipocytes.

In order to differentiate at a molecular level WAT from BAT, markers specific to BAT must be identified. UCP1 is a protein found in the mitochondria of brown adipocytes that allows for the heat generating property of BAT by altering the permeability of the inner mitochondrial membrane. In the unstimulated state UCP1, also found in WAT, does not generate heat, as UCP1 containing mitochondria do not exhibit a natural leaky membrane to protons necessary for heat production[20]. However, we believe that when sympathetically challenged, conversion of white adipocytes into 'brite,' as assessed by increases in UCP1 mRNA and protein synthesis, occurs. Thus, UCP1 serves as an excellent browning marker.

In addition to UCP1, Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1-α (PGC-1α) regulates the production of mitochondria within a fat pad. PGC-1α is a major transcriptional cofactor in charge of regulating many of the PPAR proteins responsible for metabolic changes; most importantly, PPARγ, which plays a key role in adipocyte formation, differentiation, and mitochondrial biogenesis[21,22]. Due to its regulation of UCP1, BAT thermogenesis, and involvement in the production of mitochondria, PGC-1α is found more abundantly in BAT as compared with WAT and can be used as an accurate marker for brown adipocyte presence[23]. These above receptor proteins are factors whose change in expression can shed light onto the mystery that is the browning of white adipose tissue and therefore are being measured for this experiment.

Increases in the SNS drive to WAT leads to release of NE from WAT-associated SNS postganglionic nerve terminals thereby stimulating brown adipocyte conversion via β3-AR[17] that ultimately activate UCP1. This NE effect is mimicked by CL316, 243, a highly selective β3-AR agonist both in vivo[24,25] and in vitro[26]. CL 316,243 has proven to induce browning in mice and rats[24,27]. We hypothesized that MEL-induced browning of WAT depots underlying seasonal

changes in adiposity may be mediated via increases in the SNS drive to WAT and with the measurement of these markers, we can test the extent to which browning is occurring.

The SCN, however, is not the only area mainly responsible for these seasonal changes. The Dorsal medial Hypothalamus (DMH) has been shown to also be highly involved in seasonal physiological changes that occur within Syrian and Siberian hamsters[28,29]. The DMH was found to cause browning in an experiment where the NPY neurons within the DMH were knocked down. Animals with this knockdown had an increase in browning of their WAT which also increased the rate of thermogenesis[30].

MEL has been directly linked to the browning of WAT in laboratory rats and Siberian hamsters, perhaps by increasing the SNS drive through MEL in the brain and, specifically, the SCN and DMH[31,32]. This suggests that MEL could be playing a major role in the regulation of brown adipocytes. Unlocking the process in which browning occurs can be a key to obesity reversal research; however, the question still stands; how are these traditionally white adipocytes redirected into becoming brown adipocytes with very different functions?

1.4 Experimental Aim

Experiment 1: In order to mimic SNS activation, CL316, 243 will be exploited for its potential browning effect in Siberian hamsters. We hypothesize that CL316, 243 administration will increase the SNS drive to WAT, causing an increases in brown adipocyte markers. Based on our previous study browning will most likely occur within the retroperitoneal WAT (RWAT), inguinal WAT (IWAT), and, perhaps, epididymal WAT (EWAT) as well as causing an increase in mitochondriogenesis within IBAT[14].

Experiment 2: In this experiment we aim to test the effects of prolonged (10-week) MEL administration to mimic the effect of photoperiodic SD on WAT browning in Siberian hamsters.

The presence of 'brite" adipocytes in WAT will be revealed by identifying BAT-specific markers such as UCP1 and PGC-1α. We hypothesize that daily MEL injections to animals stored in LD photoperiod will trigger the occurrence of physiological changes normally seen in animals stored in SD photoperiods, specifically the production of multilocular brite adipocytes within WAT in relation with increases in the SNS efferent drive to WAT.

2 MATERIALS & METHODS

2.1 Animal Model

Adult male Siberian hamsters used for this study were obtained from the lab breeding colony and were single housed at room temperature (22 ± 2 °C) with exposure to a LD photoperiod with free access to water and regular chow. They were allowed to acclimate for one week and then transferred to their appropriate light cycle room depending on the experimental grouping. Animal care was in accordance with humane standards and all animal procedures were conducted under the regulation of Georgia State University.

2.2 Experiment 1 -CL316, 243 injections

Three month old Siberians hamsters were divided into two separate groups (n = 5/group) and housed in LD light cycle. One group received daily intraperitoneal (i.p.) injections of CL316, 243 at a dose of 1 mg/kg for five days while the control group received saline (SAL) injections. Animals were then terminated with sodium pentobarbital (Sleep Away: 300 mg/kg), fat pads (BAT, IWAT, RWAT) excised, weighed, and snapped frozen in liquid nitrogen.

2.3 Experiment 2 -MEL injections

After a one week acclimation period in a LD photoperiod light cycle, two month old Siberian hamsters were housed in two separate rooms with different light cycles, SD photoperiod

with 16 hours dark and 8 hours light (n = 15) and LD photoperiod with 16 hours light and 8 hours dark (n = 22) with free access to water and regular chow. In the LD room, half of the animals (n = 11) received subcutaneous (s.c.) MEL injections (0.4 mg/kg) daily 3 hours prior to lights off for a total period of 10 weeks while the other half received s.c. (SAL) and served as a control group. Therefore, overall, there were a total of 3 experimental groups: LD + SAL, LD + MEL and SD (Fig.1). Weekly food intake and body mass were monitored. After 10 weeks, the animals were sacrificed as mentioned above and fat pads (BAT, RWAT, IWAT, EWAT) excised, weighed, and snap frozen in liquid nitrogen and later stored in -80 °C freezer for later Western Blot analysis. Testes were also excised and weighed for confirmation of regression. All SD animals that did not respond to the SD photoperiod, as seen by the absence of testicular regression, were considered no-responders and therefore excluded from the study.

2.4 Western blot analysis

Fat tissue were homogenized using ~120 g of sterile Zirconium beads and a 1:2 ratio of homogenization buffer, and 415 µl of protease and phosphatase inhibitor cocktail (Halt). Tissues were then added to their individual tubes and were mechanically homogenized using a bullet blender 2 times for one minutes. Tubes were then centrifuged for 10 minutes at 13,000 x g. Protein extracts (supernatant) were then aliquoted into 3 tubes and stored in -80 °C. Protein concentration for each sample was determined using known concentrations of bovine serum albumin standards. Desired concentrations for each sample was calculated and made with 4x Loading buffer and Millipore water. The appropriate concentration for each sample was loaded onto a 10% acrylamide gel along with 10 µL of broad range protein ladder (Thermo) in the first and last wells. Sample concentrations for experiment 1- CL were 10 µg/µl for IWAT and IBAT and 5 µg/µl for RWAT; all fat pads in experiment 2-MEL were at a concentration of 5 µg/µl.

Following the SDS-page gel run, gels were transferred to a PVDF membrane, washed 2x for 10 mins in TBS, and blocked for 2 hours in blocking solution (4 % nonfat dry milk in TBS). Membranes were then incubated for ~48 hours in a [1:1000] of the appropriate primary antibody, UCP1, PGC-1α, HSL, and pHSL. Following the completion of the primary antibody incubation, the membranes were washed 3x for 5 minutes with TBS, then incubated in goat anti rabbit secondary antibody [1:1000] for 2 hours. Finally, they were washed 3x for 10mins with TTBS and incubated in chemiluminescent kit for 5 min followed by gel imaging.

Data were analyzed by the Student's t-test and one-way analysis of variance (ANOVA) followed by the post-hoc Bonferroni's and Holm-Sidak's tests using NCSS (version 2007, Kaysville, UT). Significance was set at $P<0.05$. For simplicity and clarity, values with $P<0.05$, $P<0.01$ and $P<0.001$ were all indicated with a single asterisk. All values are presented as mean ± standard error of the mean.

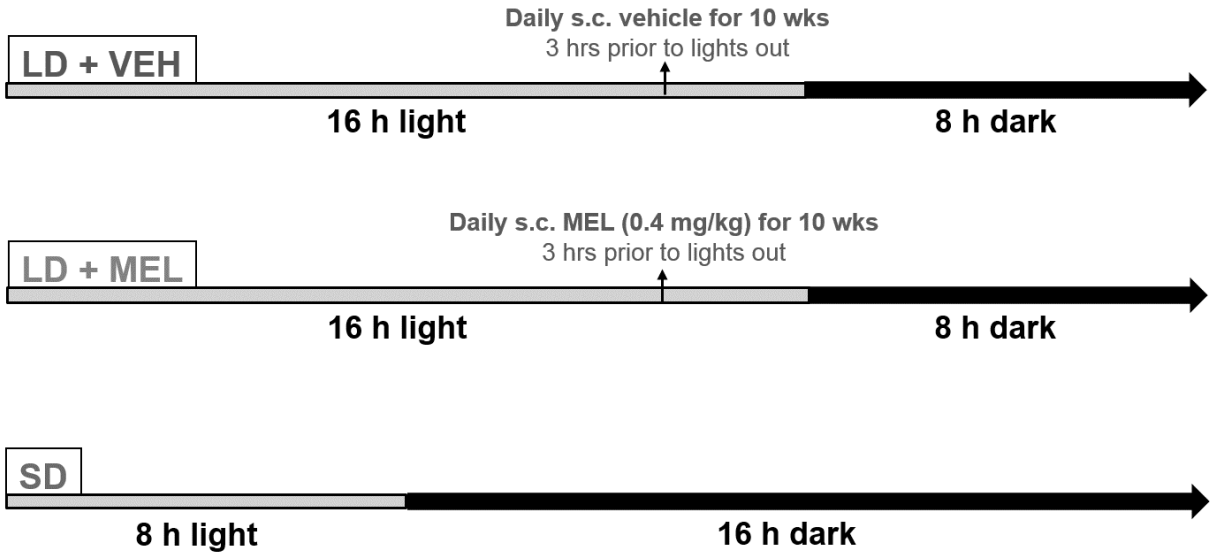


Figure 1: *Schematic representation of MEL experimental design.*

3 RESULTS

3.1 CL 316,243 injections

CL 316,243, tended to decrease BM over course of the 5 day injections, however without statistical significance (Fig. 2A). The fat pad mass individually as well as testes showed no significant difference between the groups (Fig. 2B). CL316, 243 treatment significantly increased protein levels for browning markers UCP1 and PGC-1α (Ps<0.05) both in RWAT and IWAT as compared with the saline control (Fig. 3,4,5). IBAT however, only had an increase in UCP1 expression for CL 316, 243 animals with IBAT PGC-1α protein unchanged between the groups.

3.2 MEL injections

Although it was previously seen that MEL does not affect food intake for up to 5 weeks[28,33], in this study, SD and s.c injections of MEL daily for 10 weeks markedly decreased food intake starting from week 5 until the end of the experimental period compared with LD+VEH animals (Ps <0.05; Fig. 6A). Body mass was also lower for LD+MEL animals once again resembling the SD animals (Ps<0.05; Fig. 6B). One of the most obvious physiological changes that occurs during the photoperiod triggered seasonal changes is testes regression[4,6]. Indeed SD and MEL treatment resulted in a profound testicular regression. Occasionally, SD and chronic MEL caused a complete testicular regression. As hypothesized, the testes mass for LD+MEL animals was regressed to a size similar to the SD group confirming MEL's effect on the reproduction. Focusing more closely, individual fat depot masses were measured which showed significant decreases in IWAT, RWAT, and EWAT masses (p<0.05), but not IBAT, for the LD+MEL group as compared with LD+VEH (Fig. 6C).

As for the occurrence of browning UCP1 protein levels in RWAT, IWAT, and IBAT were lowest in the LD+VEH group while UCP1 protein expression in SD hamsters seemed to be higher than that of the LD+MEL hamsters, however, without statistical significance (Fig. 7A,8A,9A). When looking at EWAT UCP1 expression, the trend was reversed, with LD+MEL animals having the highest expression and SD animals having an expression similar to LD+VEH animals (Fig. 10).

PGC1-α was also expressed at higher levels in IWAT and RWAT of LD+MEL and SD animals (Fig.8B, 9B). RWAT and IWAT showed a stepwise increase for PGC1-α ($P < 0.05$) while EWAT PGC1-α was the opposite of this with LD+VEH showing the highest amount of expression (Fig, 8B, 9B, 10B). Lastly, IBAT had no significant difference in PGC1-α within all groups (Fig 7B).

Lipolytic activity, measured by the ratio of pHSL/HSL, was increased stepwise within IWAT ($p < 0.05$), while showing no significant amounts in RWAT and BAT. EWAT, contrary to our hypothesis, showed a decrease in lipolysis in the SD group as compared with the LD+ MEL. The LD+MEL and SD group however did show higer lipolytic activity then the control LD+VEH animals ($p < 0.05$; Fig.11).

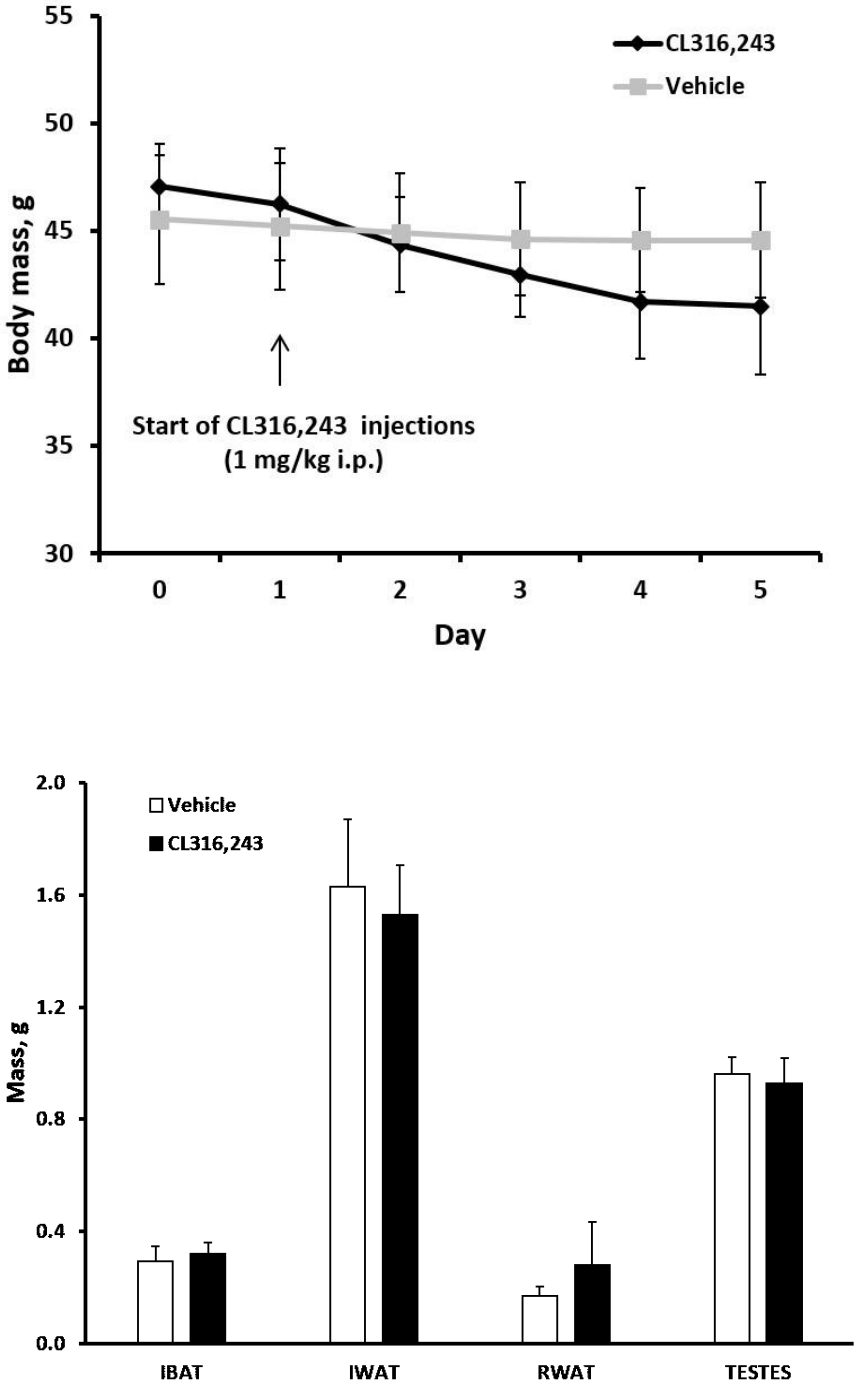


Figure 2: ***A)*** *Body mass of animals during 5 day CL316, 243 and saline injections.* ***B)*** *Paired fat pad masses and testes mass.*

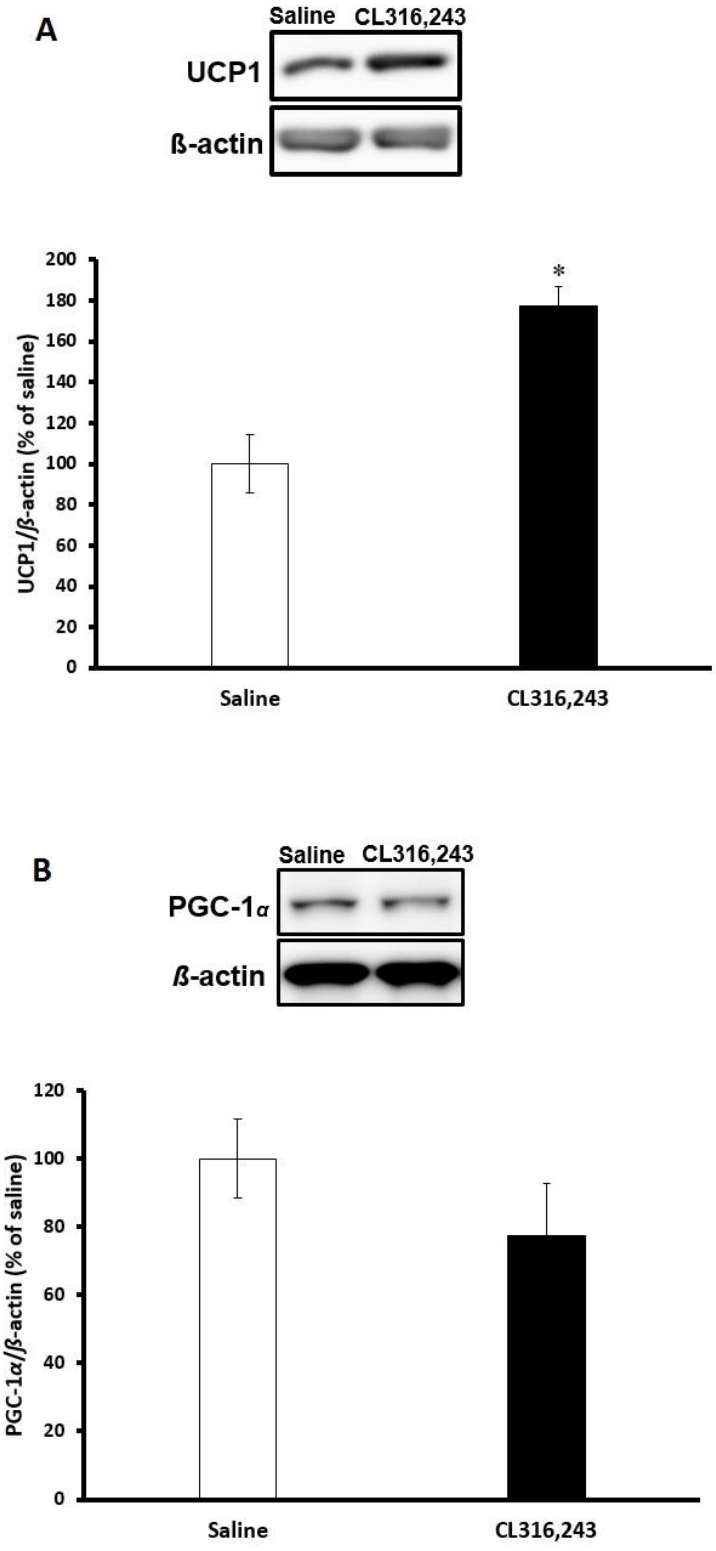


Figure 3: *Western blot analysis of IBAT UCP1* ***(A)*** *and PGC-1α* ***(B)*** *in CL and saline Injected for duration of 5 days. *p<0.05 vs. saline.*

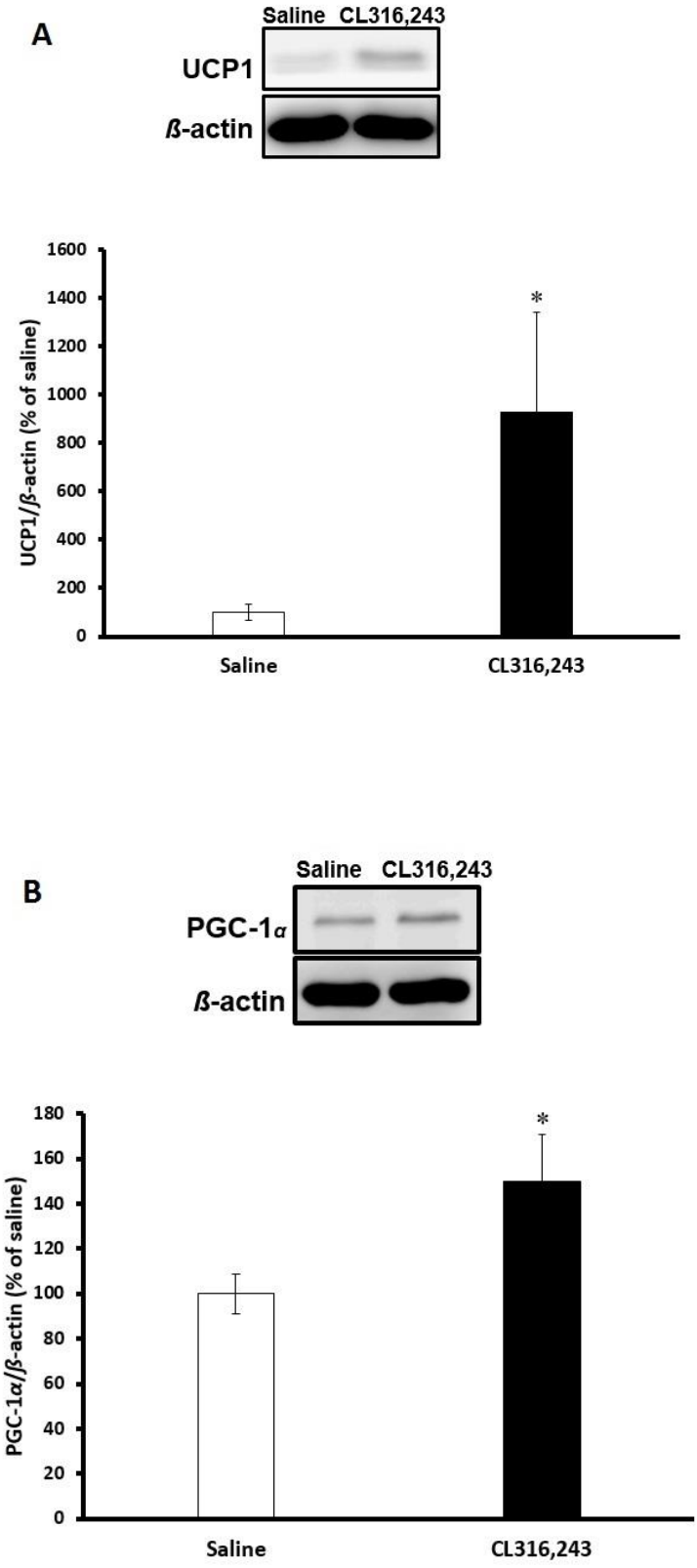


Figure 4: *Western blot analysis of IWAT UCP1* ***(A)*** *and PGC-1α* ***(B)*** *in CL and saline Injected for duration of 5 days. *p<0.05 vs. saline.*

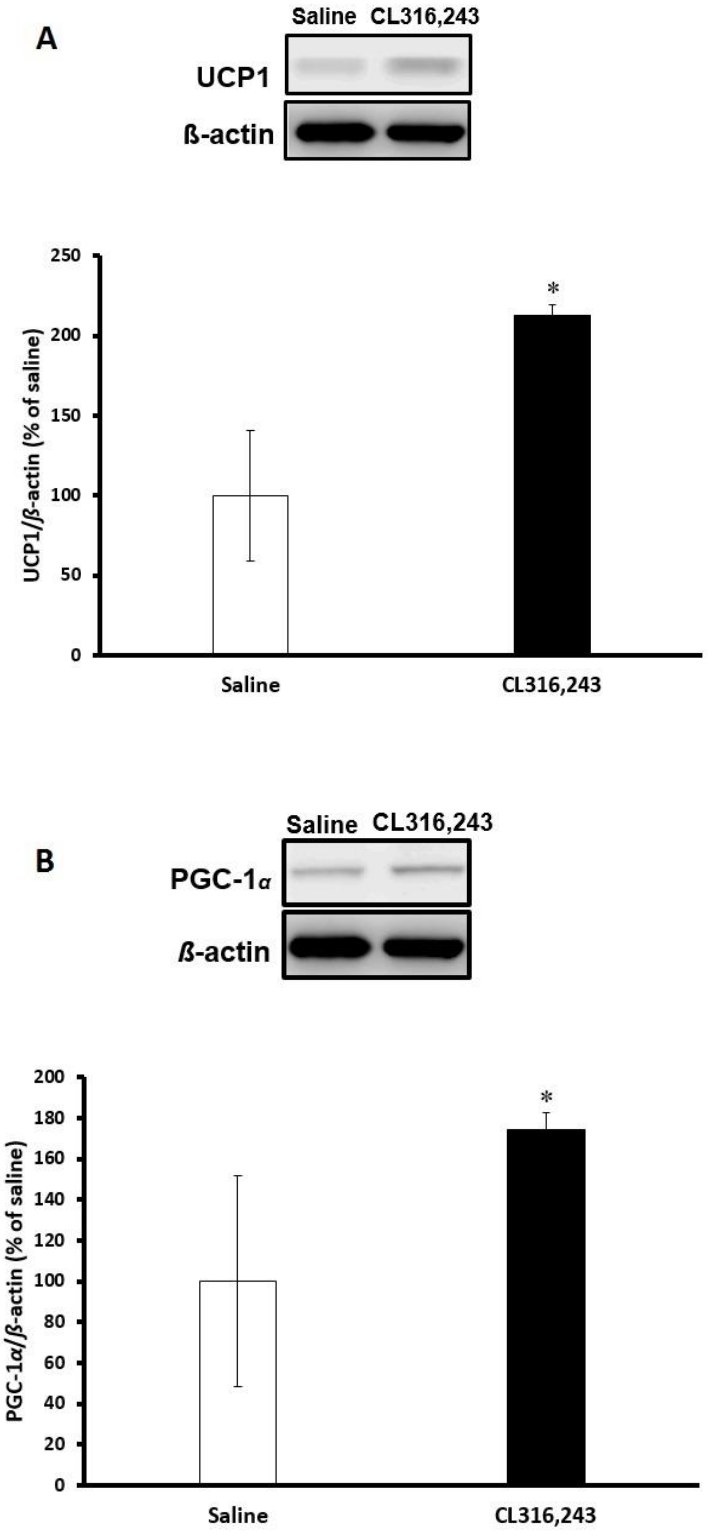
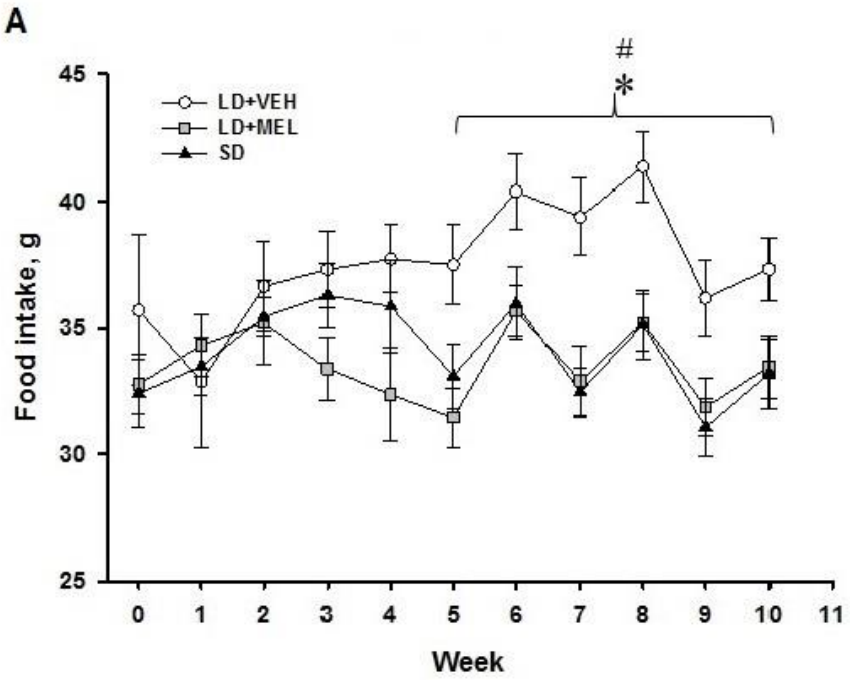


Figure 5: *Western blot analysis of RWAT UCP1* ***(A)*** *and PGC-1α* ***(B)*** *in CL and saline Injected for duration of 5 days. *p<0.05 vs. saline.*


A
#
*
LD+VEH
LD+MEL
SD
Food intake, g
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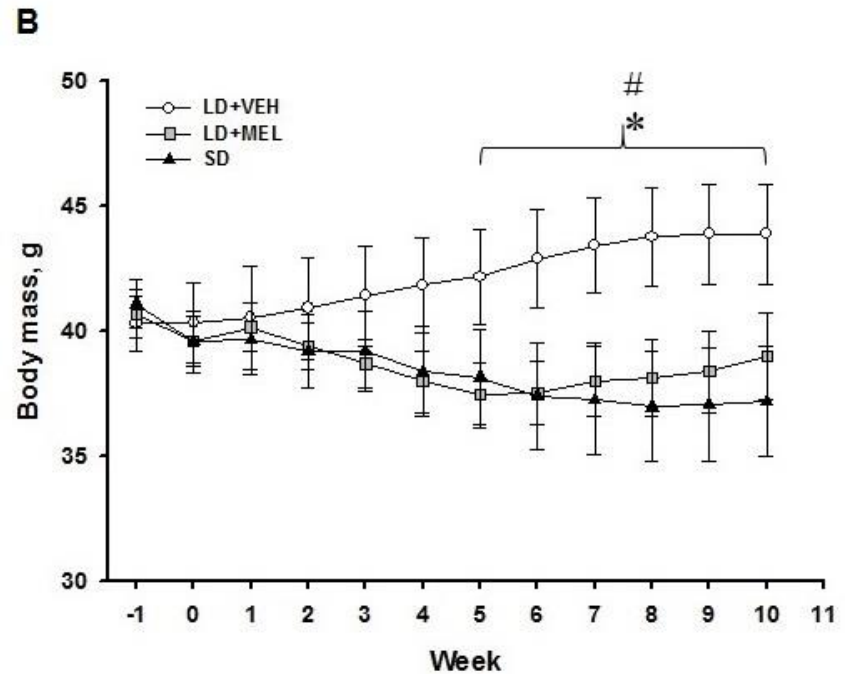
11
Week


B
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*
LD+VEH
LD+MEL
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Body mass, g
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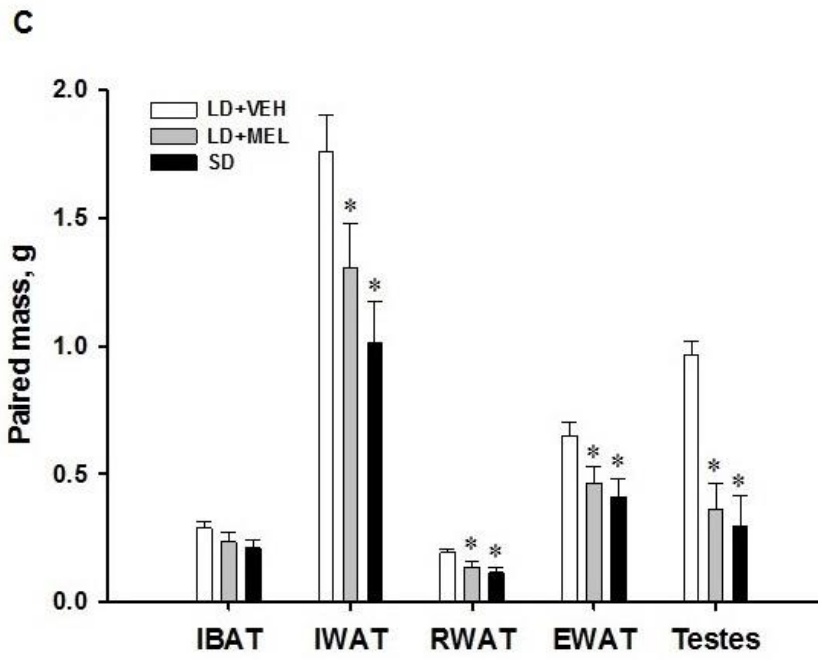


Figure 6: A*) Food Intake over 10 week period in animals given MEL or VEH injections.* ***B****) Body mass change.* ***C****) Paired fat pad masses and testes mass.* $^{*}p<0.05$ *vs. SD;* $^{\#}p<0.05$ *vs. LD+MEL.*

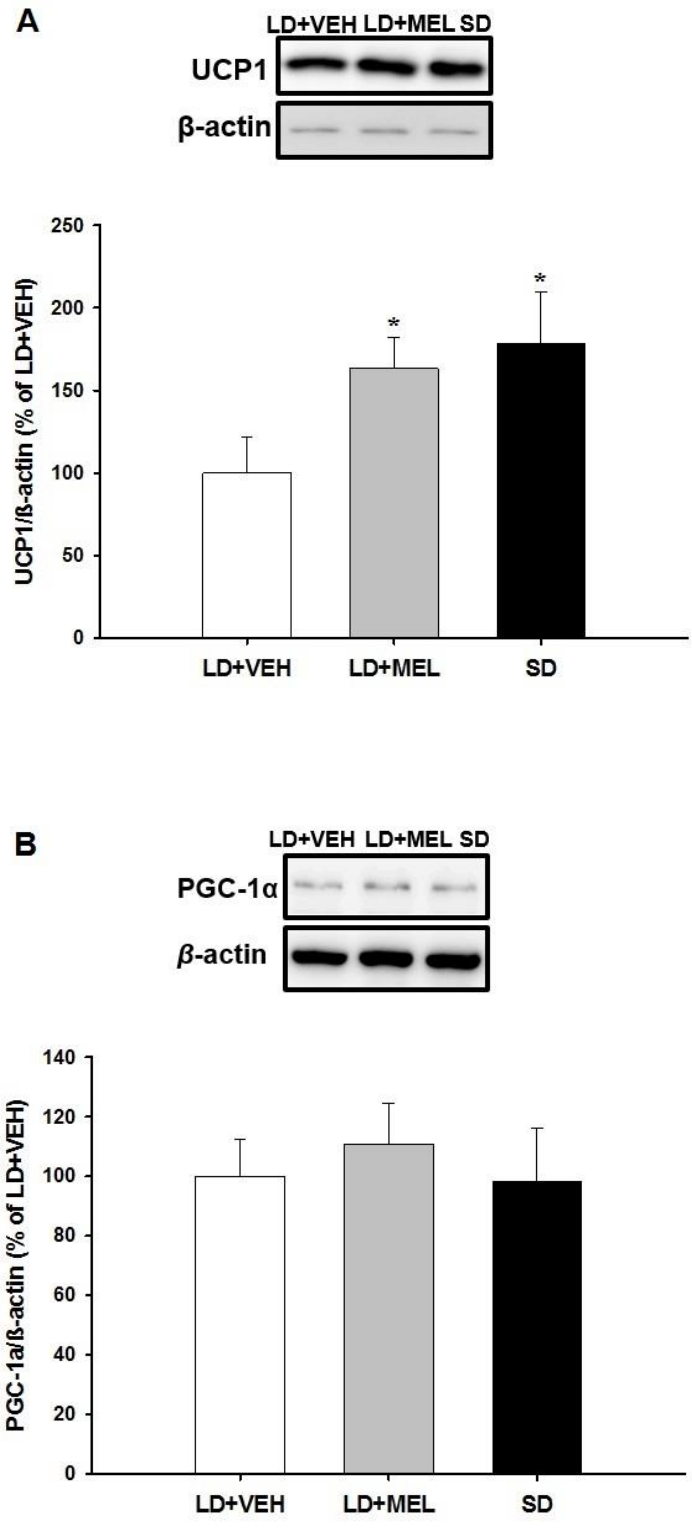


***Figure 7**: Western Blot Analysis of UCP1 and PGC-1α in IBAT**. A)** Expression on UCP1 **B)** Expression of PGC-1α. *p<0.05 vs. LD+VEH*

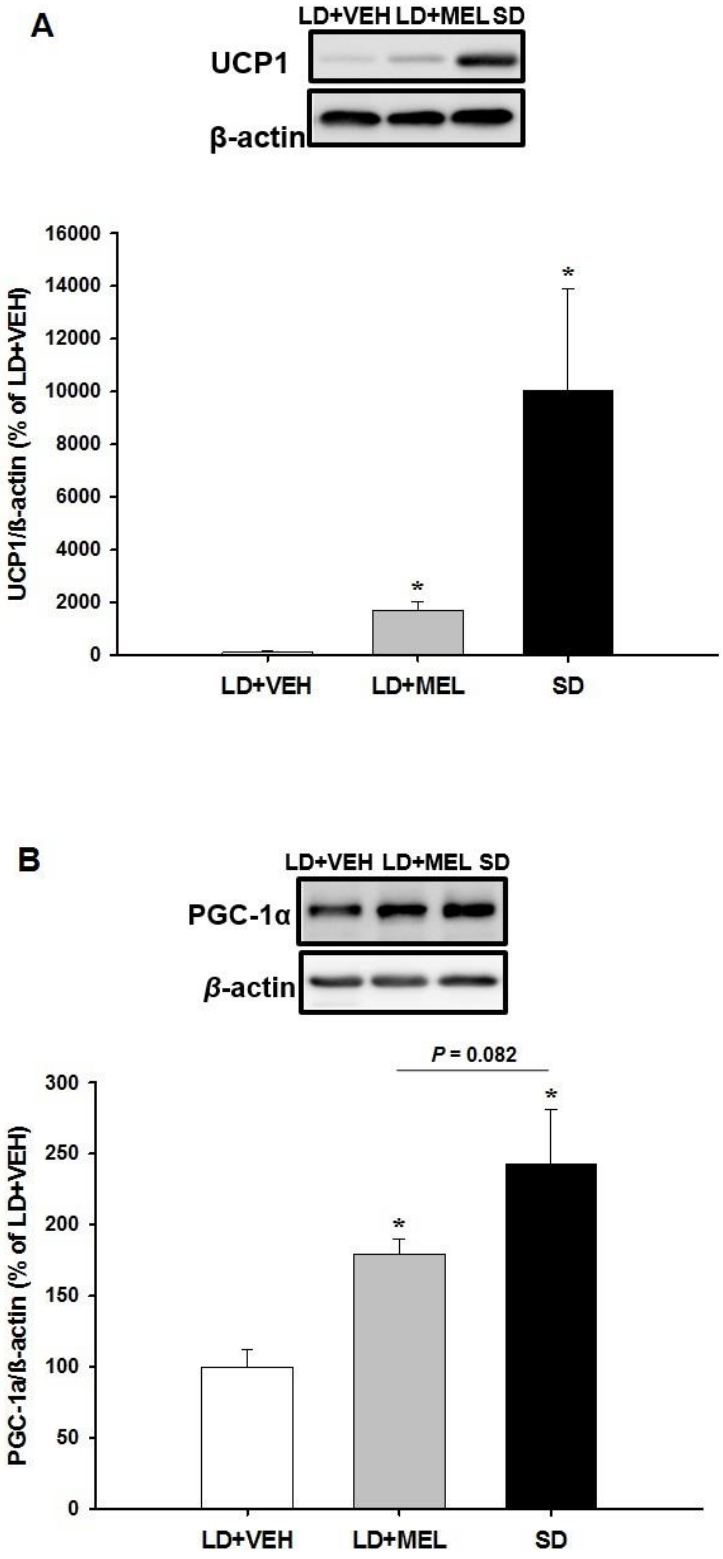


Figure 8 *: Western Blot analysis of UCP1 and PGC-1α in IWAT* ***A)*** *Percent UCP1 expression.* ***B)*** *Percent PGC-1α expression. Percent calculated as ratio of protein tested to* β*-actin control.* $^{*}p<0.05$ *vs. LD+VEH.*

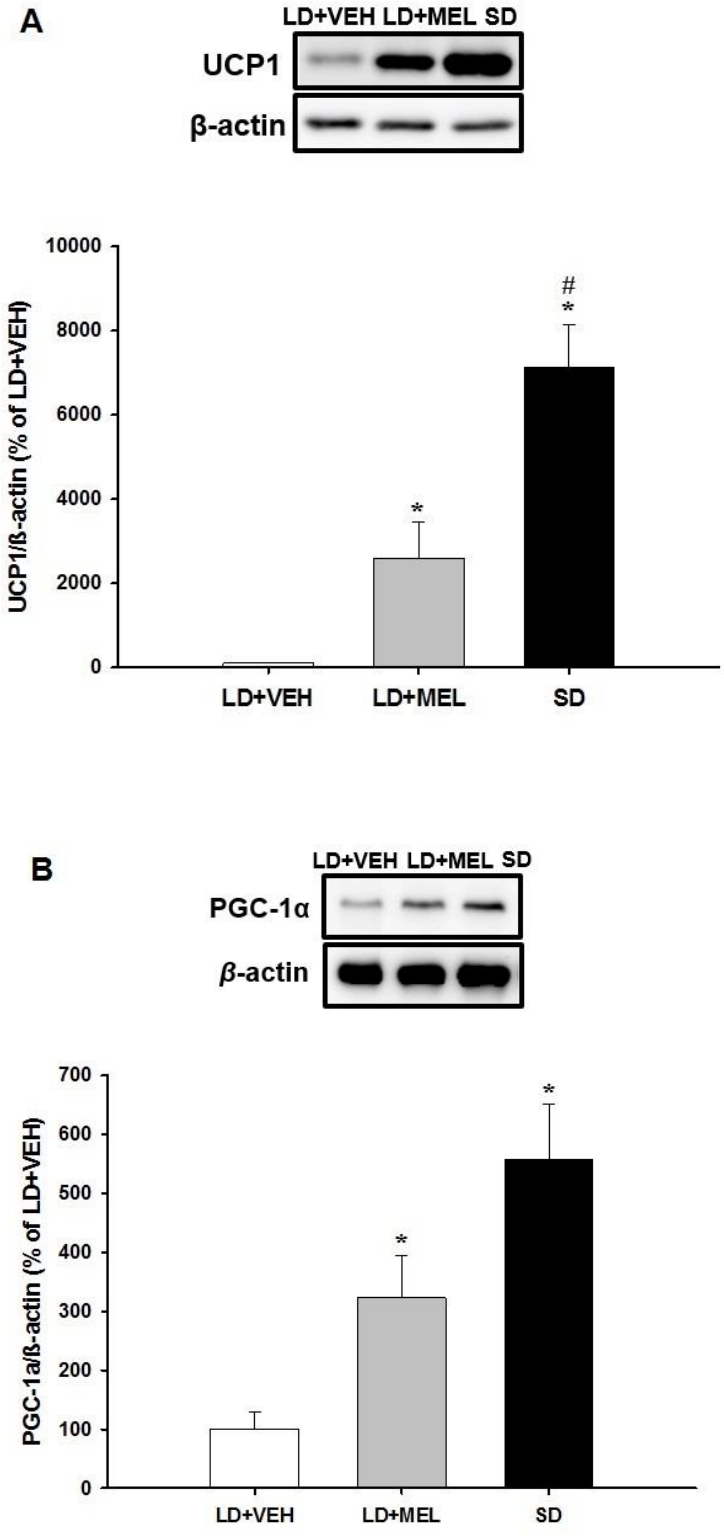


Figure 9: *Western Blot analysis of UCP1 and PGC-1α in RWAT* ***A)*** *Percent UCP1 expression.* ***B)*** *Percent PGC-1α expression. Percent calculated as ratio of protein tested to* β*-actin control.* $^{*}p<0.05$ *vs. LD+VEH* $^{\#}p<0.05$ *vs. LD+MEL* $^{*}p<0.05$ *vs. LD+VEH*

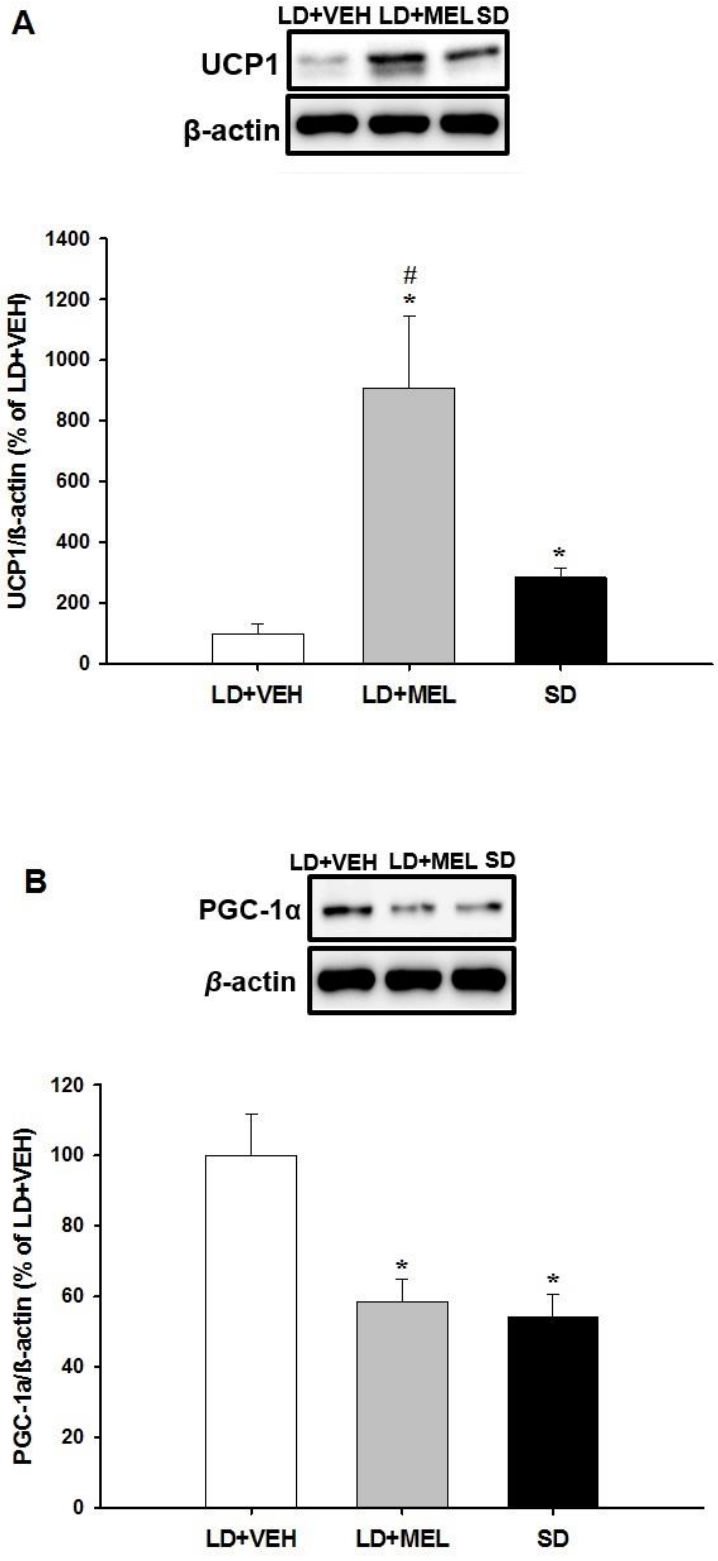
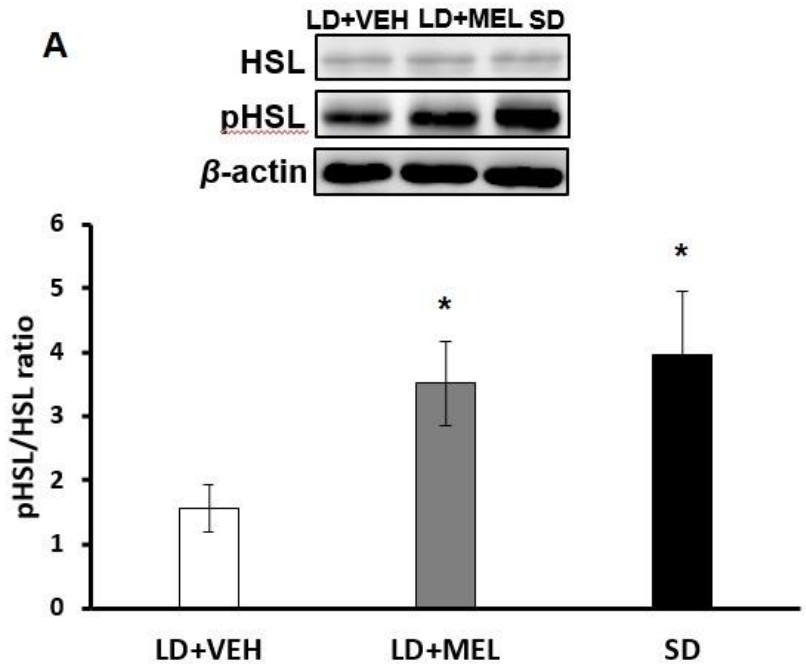
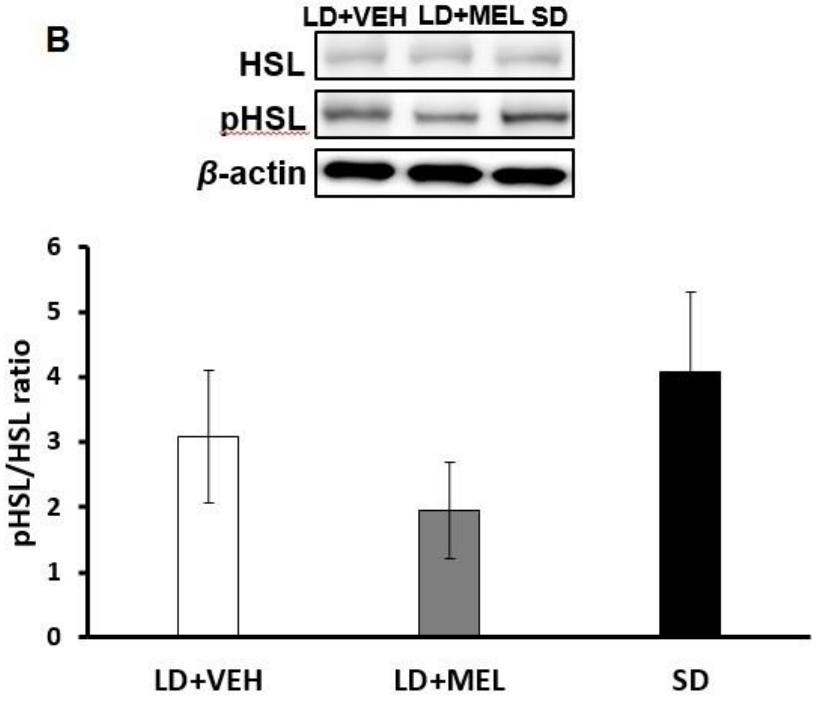


Figure 10: *Western Blot analysis of UCP1 and PGC-1α in EWAT.* ***A)*** *Expression on UCP1* ***B)*** *Expression of PGC-1α.* $^{*}p<0.05$ *vs. LD+VEH* $^{\#}p<0.05$ *vs. SD*


A
LD+VEH LD+MEL SD
HSL
pHSL
β-actin
pHSL/HSL ratio
0
1
2
3
4
5
6
*
*
LD+VEH
LD+MEL
SD


B
LD+VEH LD+MEL SD
HSL
pHSL
β-actin
pHSL/HSL ratio
0
1
2
3
4
5
6
LD+VEH
LD+MEL
SD

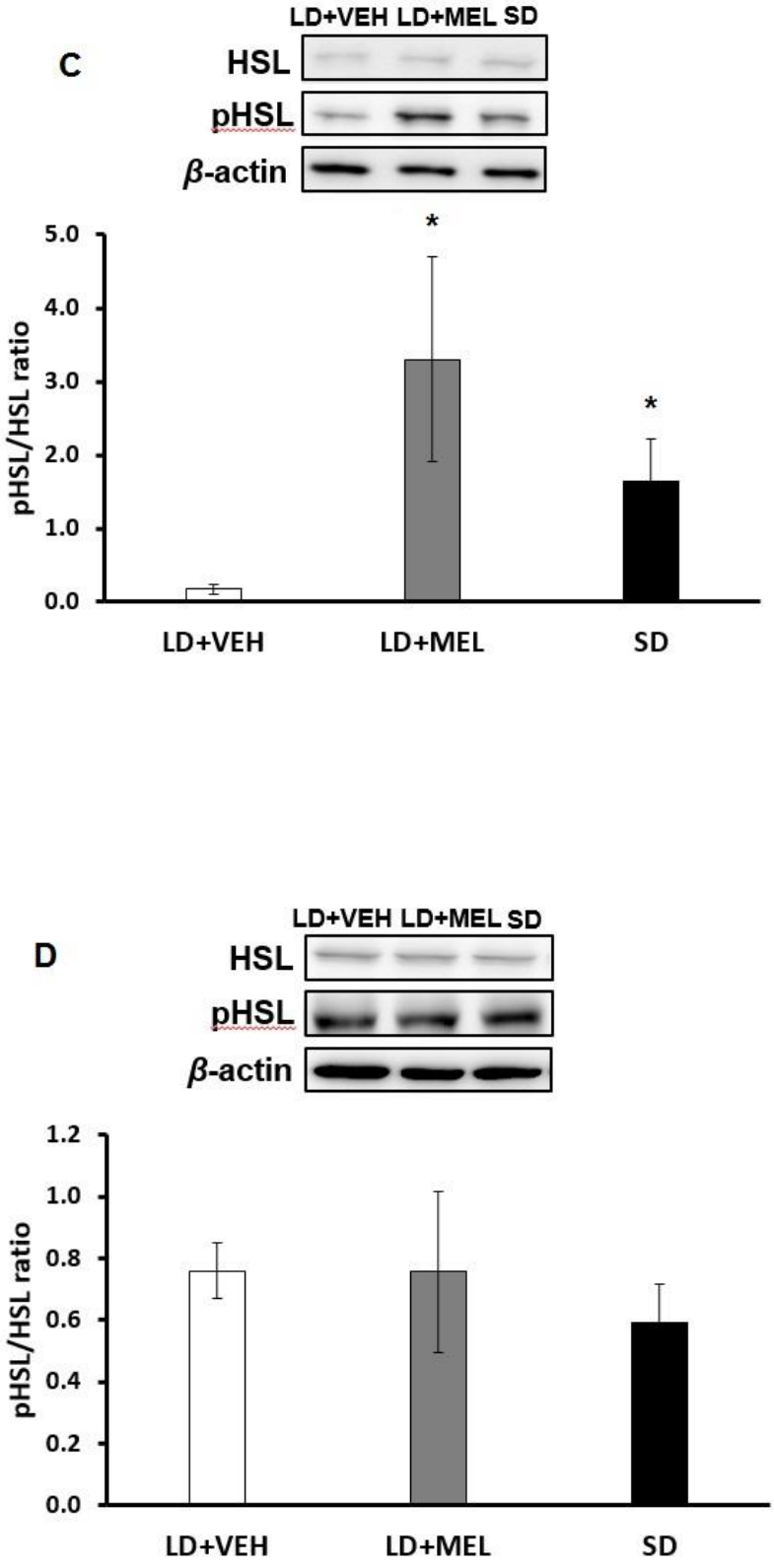


Figure 11: *Western blot analysis of HSL and pHSL. IWAT **(A)**, RWAT **(B)**, EWAT **(C)**, and BAT **(D)** western blot with ratio of HSL/pHSL taken as percent of LD+VEH. *P < 0.05 vs. LD+VEH.*

4 DISCUSSION

In this study we effectively presented the browning and lipolytic effects of CL316, 243 and MEL within WAT of Siberian hamsters. In accordance with our hypothesis, overall expression of browning markers were elevated in CL316, 243 and MEL treated animals as compared with controls. MEL animals showed browning expression similar to SD animals, confirming that chronic MEL injections in animals housed in LD light cycles is capable of replicating SD physiology within Siberian hamsters. CL316, 243 confirmed its role in SNS stimulation leading to browning of WAT.

CL316, 243 and MEL treatment caused a decrease in overall BM as predicted. Although MEL injected rodents were reported as reducing BM independent of food intake[34], our data in Siberian hamsters show that food intake of MEL injected animals was lower compared to the control. Although it is plausible that decreases in BM of our hamsters were the result of a decreased food consumption, we do not exclude the possibility that this change in food intake could be secondary to the decrease in BM after 5 weeks. Paired fat pad masses as well as testes mass show significant differences between the MEL animals and saline treated ones ($p < 0.05$), however not for the CL316, 243 injected group. This could have been due to the relatively short duration of CL 316,243 injections. If the number of days were to be increased, it is possible that a more dramatic change will be observed, specifically within individual fat pads. Testicular regression of the LD+MEL animals decreased to a mass extremely close to SD animals. The results of this study are in concordance with our previous findings where chronic MEL induced similar testicular regression despite the different dose of MEL used in the study[35]. All fat pads with the exception of BAT in LD+MEL animals show significant decreases in mass. This set of data confirms MEL's ability to induce SD seasonal changes within Siberian hamsters.

Since browning has been confirmed of occur in mice and rats following CL 316, 243 injections[24], we measured expression of UCP1 and PGC-1α in Siberian hamsters receiving the same treatment. As expected, all pads tested showed an increase in UCP1 expression, and all except for IBAT showed elevated PGC-1α expression. Since PGC-1α is a marker of mitochondria biogenesis, it could be that IBAT has an abundance of mitochondria, and with an increase in SNS signaling to IBAT, only UCP1 is further stimulated within the already present mitochondria. MEL browning marker expression had a similar trend as CL316, 243 treated animals for UCP1 and PGC-1α in IWAT, IBAT, and RWAT (Fig. 7-10), thus, further supporting the idea that β3-AR stimulation of SNS to WAT plays a role in the browning phenomenon.

Although LD+MEL animals show a significant increase in browning, similar to that of SD animals ($p<0.05$), it is possible that with longer exposure to s.c. MEL, the LD+MEL could obtain a higher degree of browning that would match that of SD housed animals more closely. UCP1 expression in BAT for CL316, 243, LD+MEL, and SD animals shows a significant elevation in expression ($p<0.05$), however differences in PGC-1α expression between the groups is not significant. An explanation for this could be that UCP1 and PGC-1α, although both markers of browning, represent different parts of the pathways leading to browning. UCP1 is responsible for altering the permeability of mitochondria while PGC-1α expression represents mitochondrial biogenesis.

EWAT had the least predictable data out of all the pads, with UCP1 expression being highest in LD+MEL and SD having an expression more closely resembling LD+VEH (Fig. 10). This difference between browning marker trends in IBAT PGC-1α and EWAT UCP1 and PGC-1α could be due to the presence of more SNS innervation within RWAT and IWAT pads, specifically RWAT due to its small size. When comparing this data to lipolysis markers, as

measured by the ratio of pHSL to HSL, we observed differential correlation between browning marker expression and lipolysis for RWAT, IWAT, BAT, and EWAT. The reason for this could be that there is an lipolysis does not occur at the same time period of all fat pads and at the 10 week time point, when fat depots were extracted for this study, IWAT and EWAT were at their peak lipolytic activity, as compared with BAT and RWAT, therefore more epinephrine was being released resulting in the elevation of lipolytic markers. In support of this notion, Demas and colleagues previously demonstrated that mRNA for β3-adrenoceptor was significantly increased after 5 weeks of daily MEL treatment indicating MEL-induced SNS outflow to adipose tissues to trigger lipolytic responses.[36]. It could be possible that RWAT and BAT will show peak lipolytic activity at a time point earlier than 10 weeks. Given this, our test animals most likely had already depleted their stores and the markers could not be detected at 10 weeks.

In conclusion, we have validated the browning effects of chronic exogenous MEL on WAT and from CL316, 243 data determined that this browning effect could possibly be through increased SNS signaling to WAT. Markers of 'brite' adipocytes, UCP1 and PGC-1α, were significantly increased in IBAT, RWAT, IWAT, and partially in EWAT following CL316, 243, MEL, and SD treatments inferring MEL ability to trigger browning. Overall, we conclude that MEL has shown to be a major contributor to the pathway causing the seasonal changes within Siberian hamsters by altering SNS drive and through this, induce browning of WAT. With further research, MEL role could potentially shed light on a method for reversing obesity.

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