Roles for the sympathetic nervous system, renal nerves, and CNS melanocortin-4 receptor in the elevated blood pressure in hyperandrogenemic female rats.

By

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Women with polycystic ovary syndrome (PCOS) have hyperandrogenemia and increased prevalence of risk factors for cardiovascular disease, including elevated blood pressure. We recently characterized a hyperandrogenemic female rat (HAF) model of PCOS (chronic dihydrotestosterone (DHT) beginning at 4 weeks of age) that exhibits similar characteristics as women with PCOS. In the present studies we tested the hypotheses that the elevated blood pressure in HAF rats is mediated in part by sympathetic activation, renal nerves, and melanocortin-4 receptor (MC4R) activation. Adrenergic blockade with terazosin and propranolol or renal denervation reduced mean arterial pressure (MAP by telemetry) in HAF rats, but not controls. Hypothalamic MC4R expression was higher in HAF rats than controls and central nervous system MC4R antagonism with SHU-9119 (1nmol/h icv) reduced MAP in HAF rats. Taking a genetic approach, MC4R null and wild type (WT) female rats were treated with DHT or placebo from 5 to 16 weeks of age. MC4R null rats were obese and had higher MAP than WT control rats, and while DHT increased MAP in WT controls, DHT failed to further increase MAP in MC4R null rats. These data suggest that increases in MAP with chronic hyperandrogenemia in female rats are due, in part, to activation of the sympathetic nervous system, renal nerves, and MC4R, and may provide novel insights into the mechanisms responsible for hypertension in women with hyperandrogenemia, such as PCOS.

Key words: hypertension, female, dihydrotestosterone, melanocortins, sympathetic nervous system, renal denervation.
Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive dysfunction, affecting approximately 10% of reproductive age women, and is characterized by hyperandrogenemia with or without cystic ovaries and oligomenorrhea (2). Women with PCOS often present with cardiovascular risk factors, such as, insulin resistance, type II diabetes, hyperlipidemia, and endothelial dysfunction (13). They also have an increased prevalence for elevated blood pressure (16,18), but are not always treated with antihypertensive drugs since their blood pressure may not reach the levels required by treatment guidelines. However, an increase in mean arterial blood pressure of even 5-10 mm Hg is significant since normotensive young women typically have arterial blood pressures in the 70-80 mm Hg (mean) range, and even small elevations in their blood pressure may predispose them to later cardiovascular disease and target organ damage.

The mechanisms responsible for elevated blood pressure in young women with PCOS are not clear; however, there is evidence that they have sympathetic nervous system activation (21,24), and renal sympathetic nerve activation. In support of a role for the latter in the elevated blood pressure, radiofrequency renal nerve ablation attenuated hypertension in two women with PCOS, although the procedure was more successful in lowering blood pressure in one than in the other (21).

The mechanisms responsible for sympathetic activation in women with PCOS have not been determined. Some but not all women with PCOS are overweight or even obese (1,18). Hall and colleagues determined that obesity-related hypertension and the concomitant sympathetic activation are mediated, in part, by the melanocortin-4-receptor (MC4R) in the brain (9,10,12). However, the MC4R can contribute to sympathetic activation and hypertension even if the subject is not obese, as shown by da Silva and colleagues in male SHR (8). Thus MC4R
activation may play a key role in contributing to sympathetic nervous system activation and hypertension, although its importance in PCOS is still unclear.

Over the past few years, we have been studying a model of hyperandrogenemia in female rats (HAF) first described by Manneras and colleagues (19) that mimics many of the characteristics of women with PCOS (26). In this model, female normotensive rats are treated with dihydrotestosterone (DHT, 7.5 mg pellet/90 d) from the age of 3-4 weeks. We found that this dose of DHT in female Sprague Dawley rats caused an approximately 3-4 fold increase in plasma DHT, with normal to slightly decreased estradiol levels compared to placebo treated controls. The model also exhibits many of the metabolic and cardiovascular characteristics found in women with PCOS (19,26). For example, hyperandrogenemic female (HAF) rats are hyperphagic compared to placebo-treated controls (approximately 3 grams of food/day) and gain weight and at 14-16 weeks of age HAF rats are approximately 20-30% heavier than placebo controls (26). In addition, HAF rats exhibit increases in plasma cholesterol and insulin, and have abnormal oral glucose tolerance tests (19,26). They also have approximately 10 mm Hg higher blood pressure than placebo controls, and reductions in glomerular filtration rate (26).

In the present study we tested the hypotheses that both the sympathetic nervous system and the renal sympathetic nervous system contributes to the hypertension in our HAF rats model, and that sympathetic nervous system activation could be due to activation of MC4R.

**Methods**

**Animal models:** Female Sprague Dawley (SD) rats were obtained from the vendor (Harlan Sprague Dawley, Indianapolis, IN) at 3 weeks of age. MC4R−/− rats on a Wistar background and Wistar wild type rats were obtained at 4-6 weeks of age from the colony maintained by Drs.
Frank Spradley and Joey Granger at the University of Mississippi Medical Center (22). The MC4R\(^{-/-}\) was originally bred by Transposagen, and exhibits hyperphagia, obesity, hyperinsulinemia and elevated leptin levels. Wild type (WT) rats are Wistar Hannover strain.

Rats were maintained throughout the studies on standard rat chow (Harlan 8640, Harlan SD, Indianapolis, IN), and housed in temperature-controlled rooms with a constant light/dark cycle (12 hr/12 hr) and free access to water. At 4-6 weeks of age, rats were implanted subcutaneously on the back of the neck with non-aromatizable, continuous-release dihydrotestosterone pellets (DHT 7.5 mg/90 days (daily dose = 83 \(\mu\)g)), Innovative Research, Sarasota, FL) or placebo pellets (Innovative Research) (26). Rats were allowed to age to 12-16 weeks before study. These rats, termed hyperandrogenemic females (HAF), were compared with their placebo control Sprague Dawley counterparts, termed “controls”. Female MC4R\(^{-/-}\) and WT rats were also treated with DHT, and termed “DHT-treated” or “control” rats. The protocols complied with the Guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health, and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Measurement of adipose tissue distribution by computed tomography. At 14-16 weeks of age, rats (n=4/group) were individually placed in the prone position inside cardboard boxes, and imaged in the prone position using a Siemens Sensation 64 CT scanner. The field of view (FOV) was made as small as possible in the X-Y direction (approximately 10 cm) and included the whole body (nose through the hindfeet) in the Z direction. A modification of a clinical abdominal protocol was selected, and included the following parameters: helical mode, pitch 1.0, 120 kVp, 400 mAs, 1 sec gantry rotation, 0.6 mm collimator, 0.6 mm axial slice thickness,
0.6 mm axial slice interval, 512 X 512 pixel matrix, and B50s (medium sharp) reconstruction algorithm. The estimated voxel size was 0.2 mm by 0.2 mm in the X-Y plane and 0.6 mm in the Z direction. For measurement of abdominal fat, each rat was scanned twice. Five sequential 0.6 mm slices (3 mm slab) located immediately cranial to the tip of the iliac crests were loaded into the Phillips Brilliance 3-dimensional software viewer. Total abdominal fat (TAF) was selected using Hounsfield Unit (HU) threshold technique assuming that fat attenuation is between -30 and -190 HU. The subcutaneous abdominal fat (SAF) and visceral abdominal fat (VAF) compartments were separated by a region of interest (ROI) drawn along the abdominal wall musculature as an anatomic landmark separating the two compartments. TAF, VAF, and subcutaneous abdominal fat (SAF) volumes were measured using the two separate image datasets for each rat, and the mean of each compartment was used for comparison between rats. Data are presented as mean +/- SEM with units: mm$^3$ factored for body weight (g) or femur length (cm).

**Measurement of mean arterial blood pressure (MAP):** Under gas anesthesia with isofluorane (Mallinckrodt Veterinary, Hazelwood, CA) and with aseptic technique, HAF rats and controls were implanted at 14 weeks of age with radiotelemetry transmitters (TA11PA-C40; Data Sciences International, St. Paul, MN) into the abdominal aorta below the renal arteries, as we previously described (20,26). Rats were placed into individual cages above a receiver (RLA-3000) and allowed two weeks of recovery. Mean arterial pressure (MAP) measurements were obtained during a 10-second sampling period (500 Hz), recorded and averaged every 5 min for 24 hrs per day. Data are presented as mean +/- SEM, and averaged for each group of rats.

**MAP in HAF rats treated with α1, β1,2-adrenergic receptor antagonists.** HAF rats and controls, aged 14 weeks (n=5/group) were implanted with telemetry transmitters as described above. After two weeks recovery period, baseline MAP was measured 24 hrs per day for 5
days. Then rats were given terazosin (10mg/kg/d sc) and propranolol (10 mg/kg/d sc) for 6
days, and MAP was measured. Data were averaged for the group of rats and are presented as
mean +/- SEM.

MAP in HAF rats subjected to renal denervation. Renal denervation or sham surgery was
performed in HAF and control rats (n=5-6/group), as we have previously described (15). Telemetry
transmitters were also implanted at the same time. Following two weeks recovery, MAP was
measured for 5 days, and averaged for each group. Data are presented mean +/- SEM.

Treatment of HAF rats with MC4R antagonist, SHU-9119. HAF (n=6) and control rats (n=6)
were implanted with radiotelemetry transmitters using isoflurane anesthesia, as described
below. After transmitter implantation, intracerebroventricular (icv) cannulae were implanted into
the right lateral cerebral ventricle, as previously described (20). Cannula placement accuracy
was tested by measuring the dipsogenic response (immediate drinking of at least 5 ml of water
in 10 min) to an icv injection of 100 ng of angiotensin II.

After two weeks recovery, MAP was measured during a 5-day baseline period, and then an
osmotic minipump (Alzet) was implanted subcutaneously in the scapular region and connected
to the icv cannula using polyethylene tubing for infusion of the MC3/4R antagonist, SHU-9119 (1
nmol/h in saline, 0.5 μl/h icv) for 5 days. The dose of SHU-9119 was based on our previous
studies showing that this dose effectively blocks MC3/4R and markedly increases food intake
(9,10,12,20). During SHU-9119 infusion, HAF and control rats were food restricted to the level
of diet eaten during baseline blood pressure measurements. Body weights were measured
throughout baseline and treatment periods, and were similar during SHU-9119 infusion and
baseline.
**MAP in DHT-treated female MC4R−/− and wild type rats.** Female MC4R−/− and wild type rats were implanted at 5-6 weeks with DHT or placebo pellets (“controls”) (n=5/group), as described above. At 14 weeks of age, rats were implanted with radiotelemeters, as described below, and two weeks later, MAP was measured for 7 days. Data are presented as mean +/- SEM over the 7 days. At the end of the study, blood samples were drawn for DHT levels.

**Measurement of plasma DHT:** Plasma DHT was measured in all HAF and control rats, using a commercially available radioimmunoassay kit (Diagnostic Systems Laboratories, Inc., Webster, TX), as we previously described (26). Data are only presented for MC4R null and WT rats since we have previously shown that DHT levels are increased by 3-4 fold in Sprague Dawley rats.

**Western blot for MC4R:** Protein levels of MC4R were evaluated in hypothalamic homogenates from HAF and control rats (n = 3/group) by Western blot analysis, using goat anti-MC4R (R-19) antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) and donkey anti-goat IgG-HRP-sc2020 secondary antibodies (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA). Rabbit anti-GAPDH antibody served as a loading control (1:30,000; Sigma-Aldrich, Germany) with donkey anti-rabbit IgG as secondary antibody (1:60,000; Sigma-Aldrich, Germany).

**Statistical analyses:** All data are expressed as mean ± S.E.M. Data were analyzed by Student’s T test (for two groups) or two way Analysis of Variance (ANOVA) with Dunnett’s post hoc test. Differences were considered statistically significant at p<0.05. Statistical analyses were performed with SigmaStat software package version 3.1 (Systat Software Inc., San Jose, CA).

**Results**
Measurement of adipose tissue distribution by computed tomography. As shown in Table 1, body weight was higher in HAF than control rats. Regardless of whether factored for body weight or femur length, subcutaneous abdominal fat (SAF) was higher in HAF than control rats. Total abdominal fat (TAF) was only higher in HAF rats when factored for femur length. Visceral abdominal fat (VAF) was not different between the groups.

Effect of adrenergic blockade on MAP in HAF rats. As shown in Figure 1, treatment of HAF rats with $\alpha_1, \beta_1,2$-adrenergic antagonists, terazosin and propranolol, reduced the MAP by 14-18 mm Hg, compared with baseline levels. There was no effect of adrenergic blockers in controls (data not shown).

Effect of renal denervation on HAF and control rats. As shown in Figure 2, MAP was higher in sham HAF rats than control sham rats (101±4 vs. 120±6 mm Hg, $p<0.05$), and renal denervation reduced MAP in HAF rats to the same level as treated or untreated controls, but had no effect in control rats.

Measurement of MC4R: As shown in Figure 3, MC4R expression in the hypothalamus was two fold higher in HAF rats than in controls.

Treatment of HAF and placebo controls with MC4R antagonist, SHU-9119. In the baseline period, HAF rats had higher MAP than controls (Figure 4) (112±1 vs 105±2, $p<0.05$), and SHU-9119 decreased MAP in HAF rats to 94±4 mm Hg, but had no effect in SHU-9119 treated control rats. Heart rate was higher in control rats than HAF rats during baseline (375±6 vs
351±6 bpm, *p*<0.05). SHU-9119 reduced heart rate in both groups to similar levels (339±12 vs 335±9 bpm, *p*=NS; *p*<0.05 compared to baseline for both groups).

**Effect of DHT on MAP in MC4R<sup>-/-</sup> and WT controls.** DHT treatment increased plasma DHT levels by approximately 5 fold in both WT rats and MC4R<sup>-/-</sup> rats (DHT-treated WT: 20.7±3.2, control WT: 4.4 ±1.1 ng/dL; DHT-treated MC4R<sup>-/-</sup>: 26.7±2.2, control MC4R<sup>-/-</sup>, 5.4±1.6 ng/dl; *p*<0.01 for DHT treated rats vs control rats). Body weights were significantly higher in untreated MC4R<sup>-/-</sup> rats compared to WT controls (412.3±14.3 vs 278.7±12.6 grams), and increased with chronic DHT treatment in both MC4R<sup>-/-</sup> and WT rats. DHT in WT rats increased body weights by approximately 42% (394.6±31.3 grams), but in MC4R<sup>-/-</sup> rats, body weights only increased by 13% with DHT (466.5±15.9 grams) (Figure 5A). As shown in Figure 5B, MAP was higher in control MC4R<sup>-/-</sup> than control WT rats (106±1 vs. 97±1 mm Hg, *p*<0.01). DHT significantly increased MAP in female WT rats (105±1 mm Hg, *p*<0.05), whereas DHT had no effect on MAP in female MC4R<sup>-/-</sup> rats (105±1 mm Hg; *P*=NS vs untreated MC4R<sup>-/-</sup>).

**Discussion**

In the present studies we found that: 1) HAF rats have increased subcutaneous abdominal fat compared to control females; 2) adrenergic blockade normalizes MAP in HAF rats; 3) renal denervation normalizes MAP in HAF rats but has no effect on MAP in controls; 4) hypothalamic MC4R expression is increased in HAF rats compared to controls, and the MC4R antagonist, SHU-9119, reduces MAP and heart rate in HAF rats; and 5) in the absence of an active MC4R in MC4R null rats, DHT does not increase blood pressure. Taken together, these data suggest that sympathetic activation, renal nerve activation, and MC4R contribute to the elevation in blood pressure that occurs in HAF rats.
We previously showed that DHT in females causes an increase in food intake of approximately 25 grams per day and leads to an increase in body weight by 14-16 weeks of age (26). In the present study we determined the distribution of adipose tissue in HAF rats using computed tomography. Body weight was increased by 40%, but femur length was not different in HAF; only the subcutaneous adipose fat levels were different from controls, not the visceral fat. This is one difference between our rat model and women with PCOS who have an increase in abdominal fat (2,18). It is also possible that skeletal muscle may be increased in the HAF rats due to the androgen influence, but we did not measured this.

The fact that adrenergic blockade and renal denervation normalized MAP in HAF rats suggests that the sympathetic and renal nervous systems contribute to the increase in MAP in HAF rats. As noted in preliminary reports by Schlaich and colleagues, renal nerve ablation in women with PCOS also reduced their blood pressure (21), suggesting that the renal nerves contribute to the elevated blood pressure in women with PCOS as well.

In the present study we found that blockade of MC4R with SHU-9119 in female rats reduced MAP. In addition, we found that DHT treatment in MC4R−/− rats failed to increase blood pressure as found in DHT-treated WT rats. These data suggest that the elevated blood pressure in DHT-treated rats is mediated at least in part by the MC4R. MC4Rs are activated by increases in α-MSH, the neurotransmitter released by activation of pro-opiomelanocortin (POMC) neurons. Hall and colleagues reported that hypertension is mediated, at least in part, by sympathetic activation that is due to MC4R in two distinctive models, male obese rats and male spontaneously hypertensive rats (SHR) that are not obese (9,10,12). MC4R antagonism, however, did not decrease blood pressure in lean female SHR, regardless of age, whereas MAP in aged male SHR was significantly decreased with SHU-9119 just as in young males (20). The fact that SHU-9119 attenuated the modest hypertension that occurs with androgens in our
HAF model suggests that the MC4R may mediate the sympathetic activation. Perhaps the
difference between the SHR and the HAF rats is that the HAF rats exhibit an increase in MC4R
expression in the hypothalamus and perhaps the SHR females do not although this hypothesis
remains to be tested in female SHR. Elevated androgens have been shown previously to
increase gene expression of pro-opiomelanocortin, the precursor for α-melanocyte stimulating
hormone (α-MSH, the ligand for MC4R) in monkeys and rats (1,6), which would in turn activate
MC4R.

However, the effect of androgens on POMC-MC4R system is controversial. Adams and
colleagues reported that testosterone upregulated POMC gene expression in non-human
primates, and that castration reduced POMC mRNA levels in the arcuate nucleus (1). Earlier,
these investigators showed that the testosterone-mediated increase in POMC mRNA in rats
affected very few of the POMC neurons and only in the more caudal portion of the arcuate
nucleus and not the dorsal portion (6). However, in a later study, Fodor and Delemarre-van de
Waal found androgen receptors on only 3% of POMC neurons in the arcuate nucleus (11).
Lindblom and colleagues reported that the anabolic androgenic steroid, nandrolone decanoate,
actually reduced expression of POMC mRNA expression in the hypothalamus (17). However,
whether and how POMC precursor actually contributes to changes in α-MSH is not clear. Cakir
and colleagues reported that with diet induced obesity, α-MSH levels are reduced, while POMC
levels are unchanged (4), due to endoplasmic reticulum stress that inhibits post-translational
processing of POMC to α-MSH. Another caveat for all of these studies is that they were
performed in male animals; thus whether similar effects of androgens would occur in females as
in males is not clear. For example, we found previously in obese male Zucker rats, that are
androgen deficient, androgen supplementation caused weight loss, improvement in insulin
resistance, and reductions in plasma cytokines (7). In the HAF rats, androgens cause an
increase in food intake, weight increase, insulin resistance, and increase in plasma cytokines (26). The only effect that we have found that is common to both males and females treated with androgens is an increase in blood pressure (7,26). In addition, whether androgens increase hypothalamic MC4R expression in obese male Zucker rats, as we found in the HAF rats, has not, to our knowledge, been reported. Thus, whether MC4R and POMC neurons are similarly affected in males and females in response to androgens needs further study.

Another finding of our studies is that female MC4R−/− rats had higher blood pressure than did wild type females. Spradley and colleagues reported that blood pressure as measured by carotid arterial catheter in conscious, restrained rats was not significantly different between female MC4R+/− and WT rats (22). These investigators did not evaluate blood pressure in female MC4R−/− rats, however. However, our present data are different from those published by Stepp and colleagues who found that there was no difference in blood pressure between male MC4R−/− and WT (23), as measured by radiotelemetry, thus suggesting a sex difference in blood pressure in these genetically modified rats.

The hyperandrogenemic female rat has limitations as a model for PCOS in women and not all the characteristics present in women with PCOS are present in our model, as noted above with the lack of visceral adiposity in our model. However, most of the characteristics of PCOS that are important for the present study, such as insulin resistance, hyperlipidemia, and abnormal estrous cycling, do occur in our model. In addition, while the model is developed using exogenous DHT because DHT cannot be converted to estradiol, the DHT levels are low enough that endogenous steroid production is intact and estradiol levels remain normal (26), as in women with PCOS. The HAF rats do exhibit an abnormal estrous cycle, however, with a 6 day cycle rather than a typical 4 day cycle (21). Increased androgen levels in women with PCOS are directly associated with increased sympathetic activity (21,24), and likely hypertension, just
as in our model. Thus the model is relevant for studies into potential mechanisms for the elevated blood pressure.

**Perspectives**

PCOS is a syndrome in which reproductive age women have increased androgens, fertility difficulty, and increased risk factors for cardiovascular disease, including elevated blood pressure. The mechanisms responsible for the elevated blood pressure are unclear, but sympathetic activation has been implicated, although the mechanism(s) for sympathetic activation in women with PCOS is not clear. In the present study we found that MC4R antagonism or genetic MC4R deficiency attenuates the elevated blood pressure in our novel rat model of hyperandrogenemia. In addition to the implications of our study for women with PCOS, the model may also be important in evaluating potential mechanisms of cardiovascular disease in transgender individuals who take high doses of androgens for masculinization, and also have a high incidence of PCOS (3). In addition, androgens are elevated in many postmenopausal women (23), and hypertension in postmenopausal women is often associated with increased sympathetic activity (13,14,25). The model thus provides a means to study potential therapeutic targets that may be useful in treatment of hypertension in hyperandrogenemic women in general.

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References


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17. *Lindblom J, Kindlundh AM, Nyberg F, Berstron L, Wikberg JE.* Anabolic androgenic steroid nandrolone decanoate reduces hypothalamic proopiomelanocortin mRNA levels. *Brain*


**Figure legends:**

**Figure 1**: Adrenergic blockade reduced MAP in HAF rats. HAF rats were treated with $\alpha_1$, $\beta_1,2$-adrenergic blockers, terazosin and propranolol, as described in Methods, and MAP was measured by telemetry. *, p<0.05 compared with baseline MAP.

**Figure 2**: Renal denervation decreased MAP in HAF rats. HAF and control rats were renally denervated and implanted with telemetry probes as described in Methods. After 2 weeks recovery, MAP was measured in both groups. *, p<0.05 HAF compared to control rats; §, denervated HAF rats compared to sham HAF rats.

**Figure 3**: HAF rats exhibited higher protein levels of MC4R in the hypothalamus than control rats. Western blot analyses were performed as described in Methods. *, p<0.05, HAF rats compared with control rats.

**Figure 4**: SHU-9119, MC4R antagonist, reduced MAP in HAF rats. HAF and control rats were implanted with telemetry and icv cannulae. After two weeks recovery, baseline MAP was measured for 5 days, and then SHU-9119 was given icv via osmotic minipump in both HAF and
control rats for 5 days, and MAP measured, as described in Methods. *, p<0.05 compared with controls; §, p<0.05, compared with baseline period in the same group.

**Figure 5: DHT increased MAP in wild type (WT) but not MC4R⁻/⁻ rats.** WT and MC4R⁻/⁻ rats were treated with DHT, as described in Methods, beginning at 5-6 weeks of age. Controls received placebo pellets. At 14 weeks of age, rats were implanted with radiotelemeters. After two weeks, MAP was measured continuously for 8 days, and the data averaged. **Panel A.** Body weights. **Panel B.** MAP averaged over 8 days. *, p<0.05, compared to WT control; §, p<0.05, compared to DHT-treated WT; ¶, p<0.05, compared to untreated MC4R⁻/⁻.
Figure 1

Baseline $\alpha_1, \beta_{1,2}$-Blockade

Days

MAP (mmHg)

0 1 2 3 4 5 6 7 8 9 10 11 12

80 90 100 110 120

* * *
Figure 2
Figure 3

MC4R

Control (no DHT)  HAF

GAPDH

Control            HAF

AU

Control          HAF

*
Figure 4
Figure 5A

Body weight (grams)

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>MC4R(^{-/-})</th>
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* \includegraphics[width=0.1\textwidth]{asterisk.png}  
§ \includegraphics[width=0.1\textwidth]{section.png}  
¶ \includegraphics[width=0.1\textwidth]{section.png}
Figure 5B.
Table 1: Body weights, total abdominal fat (TAF), subcutaneous abdominal fat (SAF), visceral abdominal fat (VAF), measured by computed tomography and factored for body weight or femur length in hyperandrogenemic female (HAF) rats and placebo controls, aged 12 weeks.

Fat masses factored for body weight:

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>TAF (mm$^3$/g)</th>
<th>SAF (mm$^3$/g)</th>
<th>VAF (mm$^3$/g)</th>
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<tr>
<td>Placebo (n=4/grp)</td>
<td>230.6±4.0</td>
<td>4.33±0.38</td>
<td>1.52±0.11</td>
<td>2.69±0.31 §</td>
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<tr>
<td>HAF (n=4/grp)</td>
<td>329.0±11.3*</td>
<td>4.47±0.27</td>
<td>2.16±0.16*</td>
<td>2.21±0.11</td>
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Fat masses factored for femur length:

<table>
<thead>
<tr>
<th></th>
<th>Femur length (cm)</th>
<th>TAF (mm$^3$/cm)</th>
<th>SAF (mm$^3$/cm)</th>
<th>VAF (mm$^3$/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=4/grp)</td>
<td>3.47±0.11</td>
<td>288.5±26.8</td>
<td>101.1±7.86</td>
<td>179.1±21.0 §</td>
</tr>
<tr>
<td>HAF (n=4/grp)</td>
<td>3.58±0.09</td>
<td>409.4±23.5*</td>
<td>197.3±9.78*</td>
<td>203.4±17.1</td>
</tr>
</tbody>
</table>

Abbreviations: BW, body weight; g, grams. *, p<0.05, HAF vs placebo; §, p<0.05, VAF vs SAF in the same group.