

Maternal tobacco smoke increased visceral adiposity and serum corticosterone levels in adult male rat offspring

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BACKGROUND: Maternal tobacco smoke (MTS) predisposes human and rat offspring to visceral obesity in early adulthood. Glucocorticoid excess also causes visceral obesity. We hypothesized that *in utero* MTS would increase visceral adiposity and alter the glucocorticoid pathway in young adult rats.

METHODS: We developed a novel model of *in utero* MTS exposure in pregnant rats by exposing them to cigarette smoke from E11.5 to term. Neonatal rats were cross-fostered to control dams and weaned to standard rat chow through young adulthood (postnatal day 60).

RESULTS: We demonstrated increased visceral adiposity (193%)*, increased visceral adipose 11- β hydroxysteroid dehydrogenase 1 mRNA (204%)*, increased serum corticosterone (147%)*, and no change in glucocorticoid receptor protein in adult male MTS rat offspring. Female rats exposed to MTS *in utero* demonstrated no change in visceral or subcutaneous adiposity, decreased serum corticosterone (60%)*, and decreased adipose glucocorticoid receptor protein (66%)*. * $P < 0.05$.

CONCLUSION: We conclude that *in utero* MTS exposure increased visceral adiposity and altered in the glucocorticoid pathway in a sex-specific manner. We speculate that *in utero* MTS exposure programs adipose dysfunction in adult male rat offspring via alteration in the glucocorticoid pathway.

Maternal tobacco smoke (MTS) impacts more than 13% of pregnancies despite aggressive antismoking campaigns (1). In adult human studies, MTS exposure *in utero* predisposes to the development of metabolic syndrome, including increased visceral adiposity (2). The increased adiposity seen after gestating in an adverse intrauterine environment, such as maternal tobacco or nicotine exposure, is often adipose depot specific, with the visceral adipose tissue (VAT) increasing in excess to the subcutaneous adipose tissue (SAT) (3,4).

VAT seems to have a detrimental role in the development of metabolic syndrome (5,6). Even when considering dietary and lifestyle choices, MTS exposure *in utero* independently increases the risk of developing visceral obesity (7,8). Children whose mothers smoked during pregnancy had decreased birth

weights yet an increased obesity and BMI index as early as 9–10 y of age, independent of dietary intake and activity levels (8). Furthermore, the effect of MTS on body weight was stronger when mothers smoked during pregnancy compared with that of isolated smoke exposure in childhood (7). Taken together, these findings suggest that MTS exposure during this developmentally sensitive time period both causes intrauterine growth restriction (IUGR) and adversely programs obesity later in life.

Little insight exists on how MTS programs increased visceral adiposity. Rodent studies examining the effect of *in utero* MTS exposure on the offspring have not examined the etiology of increased visceral adiposity. Rodent studies of tobacco smoke exposure only during lactation demonstrate increased adiposity (9). Furthermore, rodent studies of maternal nicotine, one of the many compounds found in tobacco smoke, demonstrate increased obesity in adult offspring when exposed during gestation or lactation (10–12). One potential mechanism through which MTS and maternal nicotine may program increased visceral adiposity includes alteration of glucocorticoid action on adipocytes. One function of glucocorticoids is to increase fat deposition in the body. Syndromes that increase glucocorticoid levels demonstrate increased visceral adiposity. Tissue response to glucocorticoids involves both depot-specific changes in levels of glucocorticoid receptors (GRs) and local formation of active glucocorticoids. Glucocorticoid signaling occurs through activation of the GR variants, including GR- α , which induce transcription of an array of proadipogenic factors. The conversion of inactive glucocorticoid (cortisone in humans, 11-dihydrocorticosterone in rodents) to active glucocorticoid (cortisol in humans, corticosterone in rodents) is catalyzed by the adipocyte enzyme 11- β hydroxysteroid dehydrogenase 1 (11- β HSD 1). Thus, determination of relative amounts of 11- β HSD 1 and GR in adipose depots may provide insight into the mechanism through which MTS programs increased visceral adiposity. However, despite the importance of MTS programming increasing visceral adiposity in the offspring, to date relative amounts of GR and 11- β HSD 1 in rats exposed to MTS *in utero* have not been studied.

Using a novel model of MTS exposure during pregnancy, we hypothesized that *in utero* MTS exposure would program

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adipose tissue dysregulation in young adult rat offspring. Specifically, MTS would increase visceral adiposity, serum corticosterone levels, and VAT GR and 11- β HSD 1 levels.

RESULTS

Weight Gain, Food Intake, and Cotinine Levels

No MTS-exposed dams died nor had to be removed from MTS exposure because of illness during these experiments. MTS-exposed rat dams gained less weight and ate less food during smoke exposure compared with control dams (Table 1). Litter sizes and pup male-to-female ratios were same in all groups. MTS increased maternal serum cotinine levels compared with control dams. Correspondingly, MTS increased fetal serum cotinine levels compared with control fetuses (not detected in the control neonatal rats, and 306 ± 18 ng/ml in the neonatal MTS rats, $P < 0.05$, pooled serum from the whole litter).

All MTS-exposed pups weighed 12% less than their gender-matched control pups at birth (Table 2). In addition, placentae from MTS-exposed rat dams weighed 6% less compared with control placentae. By postnatal day 21, male and female MTS rats continue to weigh less than control male and female rats, respectively (MTS male: 57.3 g, control male: 60.9 g, $P < 0.001$; MTS female 55.4 g, control female: 59.3 g, $P < 0.001$). However, by postnatal day 60, MTS males and females caught up to the body weight of respective control rats. MTS decreased food and caloric intake in males at postnatal day 60.

Table 1. Effect of tobacco smoke exposure on pregnant dams

	Controls (n = 28 dams)	MTS (n = 32 dams)
Maternal weight gain (E11–E21) (g)	122.3 \pm 10.4	45.3 \pm 17.3*
Maternal food intake (E11–E21) (g/d)	26.2 \pm 7.7	19.2 \pm 4.8*
Litter sizes	11.6 \pm 2.6	10.3 \pm 2.1
Litter male-to-female ratio	1:0.94	1:0.96
Maternal serum cotinine (ng/ml)	Not detected	119 \pm 27*

Tobacco smoke exposure decreased maternal weight gain and food intake. MTS increased maternal cotinine levels. Twenty-eight to thirty-two rat dams were analyzed per tobacco smoke exposure group; data represented as mean \pm SEM.

MTS, maternal tobacco smoke.

* $P \leq 0.05$ MTS dam compared with control dam.

Table 2. Effect of maternal tobacco smoke exposure on offspring

	Control male	MTS male	Control female	MTS female
Body weight at birth (g) (n = 94–258)	4.26 \pm 0.446	3.77 \pm 0.400*	4.07 \pm 0.409	3.57 \pm 0.448
Placental weight (g) (n = 64–170)	0.609 \pm 0.111	0.566 \pm 0.110*	0.580 \pm 0.093	0.544 \pm 0.129*
Body weight at day 60 (g) (n = 12)	373.8 \pm 31.4	366.1 \pm 26.0	230.8 \pm 16.7	225.6 \pm 18.1
kcal consumed/day (n = 8)	91 \pm 2	76 \pm 3*	44 \pm 3	46 \pm 3

MTS decreased fetal and placental weights at birth compared with sex-matched controls. MTS males and females caught up to their respective controls by postnatal day 60. MTS males consumed less food compared with control males. The number of rats per sex per group analyzed is represented above; data represented as mean \pm SEM.

MTS, maternal tobacco smoke.

* $P \leq 0.05$ MTS compared with sex-matched control.

Adipose Depot Quantification, Adipocyte Size, and Total Adipocyte Number

MTS increased VAT and the ratio of VAT to SAT in males compared with control males (Figure 1). MTS did not alter female VAT, SAT, or ratio of VAT:SAT.

MTS did not change adipocyte size in either VAT or SAT in males or in females in the representative subset VAT and SAT depots (Figure 2). MTS did not change the number of adipocytes per tissue points in either VAT or SAT in males or in females.

Serum Corticosterone and Adipokines

MTS increased serum corticosterone in males but decreased serum corticosterone in females (Figure 3). MTS did not change serum leptin or adiponectin levels in either sex.

VAT and SAT mRNA and Protein

With respect to VAT, MTS did not change leptin or adiponectin mRNA in either gender (Figure 4). MTS increased 11- β HSD 1 mRNA in males and females. MTS decreased total GR and GR α protein in females, with no change in males (Figure 5).

With respect to SAT, MTS increased leptin and 11- β HSD 1 mRNA in males, and adiponectin, and 11- β HSD 1 mRNA in females (Figure 4). MTS decreased GR α protein in females, with no change in males (Figure 5).

DISCUSSION

The primary finding of this study is that MTS exposure *in utero* increased visceral adiposity and serum corticosterone but not GR protein levels in the adult male rat. Moreover, increased visceral adiposity in the adult male MTS rat occurred despite decreased food intake. Increased visceral adiposity and increased serum corticosterone without a change in VAT GR protein levels in male rats suggest that increased visceral adiposity may be a result of increased corticosterone.

In humans, multiple studies demonstrate that maternal smoking decreases birth weight (13,14). Greater variability in humans exposed to MTS compared with other forms of IUGR may be found from the variation in duration and quantity of tobacco smoke or nicotine intake during gestation. The mechanism through which maternal smoking induces growth restriction is unknown, although likely occurs through multiple mechanisms. These potential mechanisms include

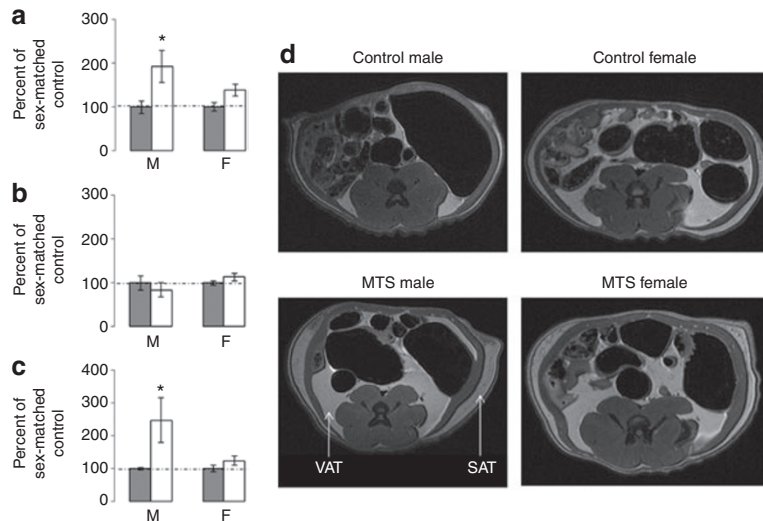


Figure 1. (a) Maternal tobacco smoke (MTS) increased visceral adiposity in male rats. (b) MTS did not change subcutaneous adiposity. (c) However, MTS increased the ratio of VAT to SAT in young adult males. (d) Magnetic resonance images of representative adipose depots in male and female control and MTS rats. *n* = 4, data represented as mean ± SEM. Control data are shown in gray bars, and MTS data in white bars. **P* ≤ 0.05 MTS compared with sex-matched control. SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

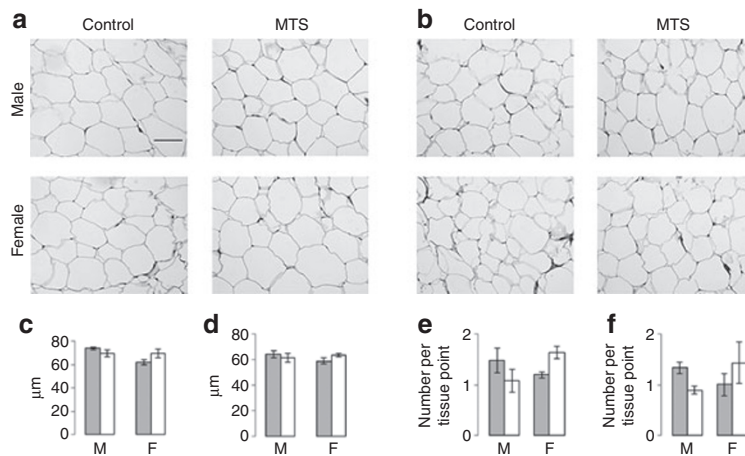


Figure 2. Representative adipose depot images for (a) visceral adipose tissue (VAT) and (b) subcutaneous adipose tissue (SAT); scale bar in the upper left panel of a represents 50 µm. MTS did not alter adipocyte size in (c) VAT or (d) SAT or adipocyte number per tissue point in (e) VAT or (f) SAT. *n* = 4, data represented as mean ± SEM. Control data are shown in gray bars, and MTS data in white bars. MTS, maternal tobacco smoke.

carbon monoxide and catecholamine-induced vasoconstriction, hypoxia, toxins from the large array of chemicals in tobacco smoke, and decreased nutrient consumption (15,16). Furthermore, unlike other forms of IUGR, MTS exposes the fetus to greater than 4,800 chemicals found in cigarette smoke during a time of sensitivity to developmental programming. One such chemical is nicotine, which induces vasoconstriction, decreasing blood supply to the uterus and developing fetus. The standard measurement used for quantifying nicotine levels is cotinine, a stable metabolite of nicotine. Neonatal rat cotinine levels fell well within the range of 200 to 800 ng/ml, indicating an “active smoker” according to ARUP laboratories.

Not only does MTS predispose the fetus to IUGR, but it also increases the risk of obesity in childhood (2,7,8,17,18). Certainly, social factors interplay with MTS exposure during pregnancy, such as decreased physical activity, parental

education, and socioeconomic status associated with postnatal cigarette exposure. Furthermore, catch-up growth, or growth that allows an IUGR fetus to “catch up” in weight to a normally grown peer, may also contribute to the formation of obesity in childhood. Low birth weight humans who had subsequent catch-up growth demonstrated an increased incidence of obesity (19). Similar results have been seen in animal studies. Several rat studies of IUGR induced by nutrient restriction and subsequently allowed *ad libitum* access to nursing and rat chow showed evidence of increased adiposity (20,21). Similarly, rats in this study demonstrate catch-up growth and visceral adiposity by postnatal day 60. While catch-up growth likely impacts the results of increased adiposity seen in this study as well as many other human and animal studies, catch-up growth is necessary for proper neurodevelopmental function and thus critical for the IUGR offspring. Furthermore, when correcting

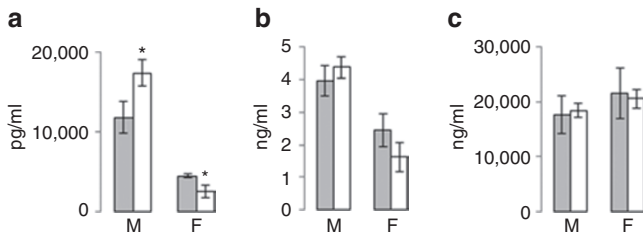


Figure 3. Maternal tobacco smoke (MTS) increased serum corticosterone levels in males (a). MTS decreased serum corticosterone levels in females (a). MTS did not change serum leptin (b) or adiponectin levels (c) in males or females compared with sex-matched controls. $n = 6$, data represented as mean \pm SEM. Control data are shown in gray bars, and MTS data in white bars. $*P \leq 0.05$ MTS compared with sex-matched control.

for these factors, MTS remains an independent risk factor for childhood obesity (2,7,8,17,18). The independent risk of obesity with maternal smoking suggests that MTS programs altered adipocyte physiology, leading to increased adiposity in the progeny.

One way through which MTS may increase childhood obesity is through increased caloric consumption in the offspring. In humans, MTS increased highly palatable food consumption and BMI by age 14 y (22). Limited information is available in animal models about caloric consumption in the offspring exposed to MTS *in utero*, however, maternal nicotine exposure leading to increased caloric consumption has been examined. Prenatal intravenous nicotine exposure in pregnant Sprague-Dawley rats increased sucrose seeking behavior in offspring without altering locomotor activity (23). Interestingly, in our study of MTS exposure, male offspring consumed fewer total calories as tobacco-naive rats. Increased caloric consumption seen in other studies may be programmed by increased consumption of highly palatable food, but not necessarily typical rat chow. Importantly, MTS increased visceral adiposity despite decreased food intake in adult male rats, suggesting that *in utero* MTS exposure alters VAT energy storage homeostasis.

Examination of adipose tissue in obese humans demonstrates altered expression of genes involved in glucocorticoid metabolism, such as 11- β HSD 1, the enzyme that converts inactive to active glucocorticoid. In obese humans, 11- β HSD 1 mRNA and its activity are increased in SAT (24). Furthermore, increased 11- β HSD 1 in adipose tissue predicts increased adiposity 2.5 y later (25). Animal models of increased adiposity have shown similar findings. Animals with increased adiposity have increased adipose tissue 11- β HSD 1 (26). Importantly, increased 11- β HSD 1 seems to be causal for increased visceral adiposity. Transgenic mice that overexpress 11- β HSD 1 develop increased visceral adiposity (27) while 11- β HSD 1 knockout mice are resistant to developing increased adiposity when fed a high-fat diet (28). Increased 11- β HSD 1 in VAT suggests increased glucocorticoid reactivation within the VAT tissue itself, which can lead to increased serum corticosterone as seen in our study. There is also evidence that perinatal stress programs increased adipose tissue 11- β HSD 1 mRNA and subsequent increased adiposity in the marmoset offspring (29). Furthermore, maternal nicotine during lactation increases

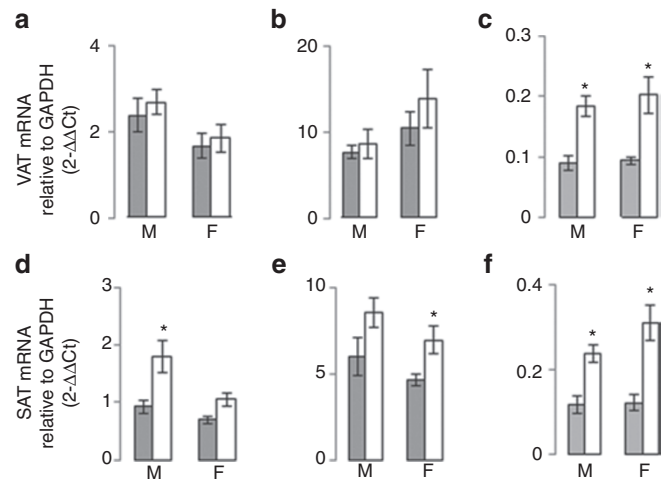


Figure 4. In visceral adipose tissue, maternal tobacco smoke (MTS) did not change leptin (a) or adiponectin mRNA levels (b), but increased 11- β hydroxysteroid dehydrogenase 1 (11- β HSD 1) mRNA in both males and females (c). In subcutaneous adipose tissue, MTS increased leptin (d) and 11- β HSD 1 mRNA in males (f), and adiponectin (e) and 11- β HSD 1 mRNA (f) in females. $n = 6$, data represented as mean \pm SEM. Control data are shown in gray bars, and MTS data in white bars. $*P \leq 0.05$ MTS compared with sex-matched control. GAPDH, glyceraldehyde-3 phosphate dehydrogenase; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

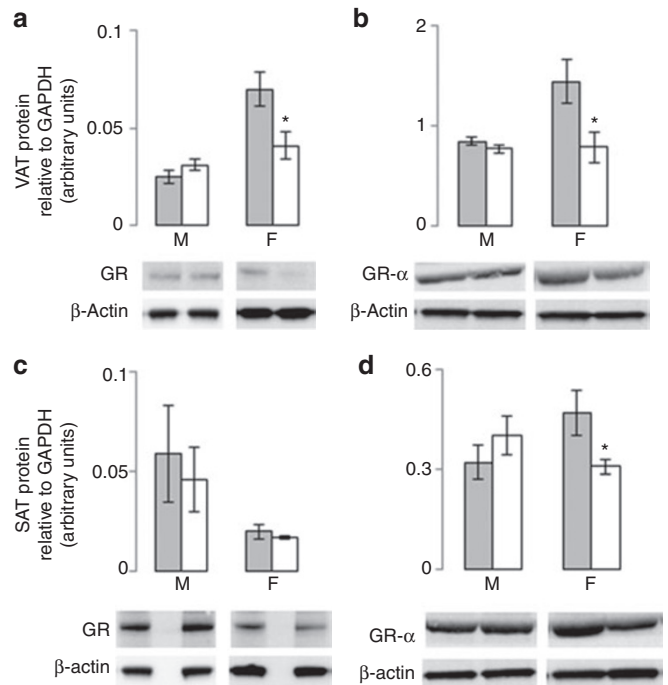


Figure 5. Maternal tobacco smoke (MTS) exposure decreased total GR protein (a) and GR- α protein (b) in female VAT. MTS did not change GR or GR- α protein in either adipose depot in males (a,b). MTS did not change either GR or GR- α protein in SAT in either sex (c). MTS decreased GR- α protein in females in SAT (d). Representative western blots of GR and GR- α relative to control gene β -actin are shown below the graphical representation of the data. $n = 6$, data represented as mean \pm SEM. Control data are shown in gray bars, and MTS data in white bars. $*P \leq 0.05$ MTS compared with sex-matched control. GAPDH, glyceraldehyde-3 phosphate dehydrogenase; GR, glucocorticoid receptor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

male offspring adiposity and serum corticosterone levels in rats (30). MTS, another type of perinatal stress, may program increased 11- β HSD 1, providing local tissue level and serum increases in glucocorticoid levels and leading to increased visceral adiposity.

Increased visceral adiposity and unchanged visceral adipocyte size indicate that the VAT depot increased by increasing the total number of adipocytes. In animal models of chronic glucocorticoid exposure, chronic glucocorticoid exposure increased the total number of adipocytes in VAT when the whole VAT depot was examined (31). However, chronic glucocorticoid exposure did not change adipocyte size and decreased VAT GR in male rats with increased adiposity (31). Interestingly, despite increased total VAT in MTS males on magnetic resonance imaging, MTS did not change adipocyte size or number of adipocytes in the small subset of representative adipose depot sections. This finding suggests that MTS programs increased the total number of adipocytes throughout the whole VAT depot, but not adipocyte size in the male rat offspring.

Adverse intrauterine environments, such as maternal smoking, often alter metabolic outcomes in a sex-specific manner. In humans, MTS exposure increases visceral adiposity in males earlier than in females (32,33). Animal studies also demonstrate sex specificity in metabolic outcomes from adverse intrauterine environments. Perinatal insults, including MTS, often affect male offspring earlier than female offspring (34–36). Consistent with these findings, male MTS rats demonstrated increased VAT in our study, while female VAT remained unaffected. MTS increased 11- β HSD 1 mRNA in both sexes. Consistent with increased 11- β HSD 1 mRNA, MTS male rats had increased serum glucocorticoid levels without changes in GR protein levels. MTS may disrupt the mechanisms regulating 11- β HSD 1 and GR expression in male rats, and may represent one mechanism through which MTS leads to the formation of increased adiposity in males. Conversely, MTS decreased serum corticosterone and adipose GRs in females. Elevated 11- β HSD 1 mRNA can increase local tissue corticosterone without affecting systemic circulating levels (27). Irrespective of the local adipose tissue corticosterone levels, the response to adipose tissue corticosterone may be blunted by decreased adipose tissue GRs in MTS females.

A limitation of this study is that pregnant dams are exposed to tobacco smoke only during the latter half of gestation. This timeframe was chosen because the detrimental health effects of MTS on offspring are largely seen in the second and third trimesters (8,37). Furthermore, this MTS regimen allowed us to ask a specific perinatal question of how prenatal MTS increases visceral adiposity in the adult offspring (8,37). Second, the neonatal MTS rats were cross-fostered to tobacco-naive mothers for rearing consistency. Therefore, neonatal rats were not exposed to postnatal tobacco smoke as many human babies born to tobacco-smoking mothers would be. While we cannot exclude the possibility of cross-fostering affecting the results, we were able to isolate the effects of MTS exposure *in utero* from extrauterine exposure, which provides valuable insight into a potential mechanism of increased visceral adiposity.

In conclusion, MTS exposure programs adipose dysfunction in adult male rats. Specifically, MTS programs increased visceral adiposity, visceral adipose 11- β HSD 1 mRNA, and serum glucocorticoids without changing GR protein in male rats. These findings suggest that MTS adversely programs sex-specific glucocorticoid signaling in VAT of adult rats.

METHODS

Animal Model

All animal procedures were carried out according to the US National Institutes of Health (Bethesda, MD) (NIH) *Guide for the Care and Use of Laboratory Animals*, and the University of Utah Animal Care Committee approved all procedures. Timed pregnant female Sprague-Dawley rats were obtained from Charles River (Wilmington, MA). Rats were exposed to 80 University of Kentucky 3R4F Research Reference Cigarettes (Lexington, KY) per day in a TE-10 Smoke Exposure System (Teague Enterprises, Davis, CA). Rats were exposed to both mainstream and side stream cigarette smoke in four sessions of 35 min cigarette smoke followed by 35 min fresh air. Mainstream cigarette smoke was obtained by pulling air through the cigarette. Side stream cigarette smoke was obtained by collecting smoke off the end of the burning cigarette. Providing both mainstream and side stream cigarette smoke to the pregnant dams was chosen because human mothers who smoke cigarettes breathe both mainstream and side stream cigarette smoke. Rats were exposed daily Monday through Friday from E11.5 to term (E21.5) (38). This gestation range was selected because birth weight is most significantly decreased by smoke exposure in the latter half of gestation (8). Tobacco-naive control rats were housed similarly but were not exposed to tobacco smoke. At delivery, all pups were cross-fostered to control dams to isolate prenatal MTS exposure. Litters were culled to six for rearing consistency. Pups were weaned on postnatal day 21, the standard time for weaning, and allowed *ad libitum* access to food and water for the remainder of the study. Food was weighed weekly for the latter 2 wk of the study (2 wk before postnatal day 60) to determine food intake. Postnatal day 60 was examined because this time point represents a pubertal or young adult rat, and puberty in the MTS-exposed human is associated with the first signs of obesity (8). A total of 46 MTS-exposed pregnant dams, 42 control dams, and offspring from these dams were used in this study. There were no differences in body weight between subgroups used for the following analyses and the population as a whole.

Measurement of Serum Cotinine Levels

Cotinine, a stable metabolite of nicotine, was measured to determine serum levels of nicotine. Maternal and fetal serum cotinine levels were measured using a solid phase competitive enzyme-linked immunosorbent assay kit (BQ Kits, San Diego, CA) following manufacturer's protocol. A total of 32 pregnant dams exposed to MTS and 28 pregnant dams as controls were used for measuring maternal cotinine levels, and their respective pooled litters obtained via Cesarean section at E21.5 were used for measuring fetal cotinine levels.

Abdominal Magnetic Resonance Imaging for Fat Quantification

Abdominal magnetic resonance images were performed, and data were analyzed as previously described (39). In brief, magnetic resonance imaging was performed on 60-day-old rats at the small animal imaging facility at the University of Utah. Paired images of water and fat plus water were obtained from diaphragm to pelvis. Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MA). Magnetic resonance images were analyzed from kidney to pelvis for visceral fat, using retroperitoneal fat as a representative visceral fat depot, and from diaphragm to pelvis for subcutaneous fat. Total subcutaneous and visceral fat depots were analyzed for four unrelated rats per sex per group, and these rats were not used for other analyses in this study.

Adipocyte Size and Number

A small subset of left retroperitoneal adipose was used as a representative VAT depot, and a small subset left flank adipose was used as a

representative SAT depot. Adipose tissue was fixed in 10% formalin, embedded in paraffin, sliced to 5 μm thick, and stained with hematoxylin and eosin at the University of Utah Dermatopathology laboratory. Adipocyte diameter was quantified using Bioquant True Color Windows Image Analysis System (R&M Biometrics, Nashville, TN) measuring five adipocytes per high powered field for 15 high powered fields per adipose depot per rat. Adipocyte number was determined using Bioquant by counting all adipocytes in a high powered field for 15 high powered fields per adipose depot per rat. Adipocyte size and adipocyte number were analyzed from four unrelated rats per sex per group, and these rats were not used for other analyses in this study.

Serum Corticosterone and Adipokine Quantification

Mixed arterial and venous serum was collected at necropsy after a 6-h fast in serum separator tubes (BD Vacutainer, BD, Franklin Lakes, NJ) and placed on ice. Serum was separated from cellular components within 30 min of collection, and serum aliquots were frozen at -80°C . Serum corticosterone was quantified using an ELISA kit (Cayman Chemical Company, Ann Arbor, MI), with a kit detection limit of 30 pg/ml. Serum adiponectin and leptin were quantified using an ELISA kit (Millipore, St Charles, MI), with kit detection limits of 0.08 ng/ml for leptin and 0.155 ng/ml for adiponectin. Serum from six unrelated rats per sex per group was analyzed, and these rats were used for adipose tissue mRNA and protein studies.

Adipose Tissue mRNA Quantification

VAT was obtained from the retroperitoneum as a representative VAT depot. SAT was obtained from the flank. Visceral and SAT was flash frozen in liquid nitrogen and stored at -80°C . Total RNA was extracted as described previously using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) for visceral and subcutaneous fat (39). Total RNA was quantified spectrophotometrically. The cDNA was synthesized from 1 μg RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) per manufacturer's protocol.

Real-Time Reverse Transcriptase PCR

Semiquantitative real-time reverse transcription-PCR quantification was performed using glyceraldehyde-3 phosphate dehydrogenase as an internal control because Ct values of glyceraldehyde-3 phosphate dehydrogenase did not differ between control and MTS animals. Relative quantification of PCR products was based on differences between glyceraldehyde-3 phosphate dehydrogenase and the target using the comparative Ct method (TaqMan Gold RT-PCR manual; PE Biosystems, Foster City, CA) (40). Assays were performed with Taqman Gene Expression Assays (Applied Biosystems, Carlsbad, CA) for 11- β HSD 1, leptin, and adiponectin.

Adipose Tissue Protein Quantification

Visceral and SAT protein was isolated using whole-cell lysates in a buffer containing 150 mM NaCl, 50 mM Tris pH 7.4, 1 mM EDTA, 0.25% Na-deoxycholate, 1% Igepal CA-630. Total protein was quantified using a bovine serum albumin standard curve. Fifty micrograms protein was run on a 4–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA). Protein was transferred to a polyvinylidene difluoride membrane. Membranes were blocked with 5% milk. GR and GR- α (ab3578 and ab3580, Abcam, Cambridge, UK) antibodies were used to identify and quantify specific protein content. β -Actin (A1978, Sigma-Aldrich, St Louis, MO) was used as a loading control. Protein was detected with Western Lightning enhanced chemiluminescence (PerkinElmer Life Sciences, Waltham, MA) using goat anti-rabbit horseradish peroxidase secondary antibody from Cell Signaling Technology. Blots were quantified using a Kodak Image Station 2000R (Eastman Kodak/SIS, Rochester, NY).

Statistics

Tables and figures were expressed as mean \pm SEM. Data analysis was completed with ANOVA (Fisher's protected least-significant difference) and Student's unpaired *t*-test. A *P* value ≤ 0.05 was considered to be statistically significant.

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