Molecular and Cellular Endocrinology 503 (2020) 110691

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Developmental programming: Adipose depot-specific changes and thermogenic adipocyte distribution in the female sheep

Muraly Puttabyatappa, Joseph N. Ciarelli, Adam G. Chatoff, Kanakadurga Singer, Vasantha Padmanabhan*

Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

Keywords:
Adipose tissue
Brown adipose tissue
Inflammation
Oxidative stress
Insulin sensitivity

ABSTRACT

Prenatal testosterone (T)-treated female sheep exhibit an enhanced inflammatory and oxidative stress state in the visceral adipose tissue (VAT) but not in the subcutaneous (SAT), while surprisingly maintaining insulin sensitivity in both depots. In adult sheep, adipose tissue is predominantly composed of white adipocytes which favor lipid storage. Brown/beige adipocytes that make up the brown adipose tissue (BAT) favor lipid utilization due to thermogenic uncoupled protein 1 expression and are interspersed amidst white adipocytes, more so in epicardiac (ECAT) and perirenal (PRAT) depots. The impact of prenatal T-treatment on ECAT and PRAT depots are unknown. As BAT imparts a metabolically healthy phenotype, the depot-specific impact of prenatal T-treatment on inflammation, oxidative stress, differentiation and insulin sensitivity could be dictated by the distribution of brown adipocytes. This hypothesis was tested by assessing markers of oxidative stress, inflammation, adipocyte differentiation, fibrosis and thermogenesis in adipose depots from control and prenatal T (100 mg T propionate twice a week from days 30–90 of gestation) -treated female sheep at 21 months of age. Our results show prenatal T-treatment induces depot-specific changes in inflammation, oxidative stress state, collagen accumulation, and differentiation with changes being more pronounced in the VAT. Prenatal T-treatment also increased thermogenic gene expression in all depots indicative of increased browning with effects being more prominent in VAT and SAT. Considering that inflammatory and oxidative stress are also elevated, the increased brown adipocyte distribution is likely a compensatory response to maintain insulin sensitivity and function of organs in the proximity of respective depots.

1. Introduction

Insulin resistance (IR) is the major attribute of various metabolic disorders and cardiovascular diseases (DeFronzo and Ferrannini, 1991; Ruige et al., 1998; Sowers, 1992). A key mediator of IR is the adipose tissue (AT) (Zhang et al., 2015; Tai et al., 2000) as excess adiposity like in obesity or deficiency in lipodystrophy can lead to IR (Grundy, 2015). Although AT distribution and function are governed by genetic predisposition and lifestyle factors (Smith and Kahn, 2016; Gastaldelli et al., 2017), recently the contribution of developmental insults are also gaining recognition. One example of such is gestational exposure to steroids, which are powerful programming agents (Padmanabhan et al., 2010). The fetal adaptations resulting from inadvertent developmental exposure to excess native (Navarro et al., 2015) or environmental steroids (Gore et al., 2015) can lead to AT defects during adulthood. The metabolic dysfunctions accompanied by AT defects observed in rodents, sheep and non-human primates following gestational exposure to excess testosterone (T) provides an excellent example of this premise (Keller et al., 2014; Eisner et al., 2003; Veiga-Lopez et al., 2013; Cardoso et al., 2016; Roland et al., 2010). In the sheep model of prenatal T excess that manifest peripheral IR and dyslipidemia in the female (Veiga-Lopez et al., 2013; Cardoso et al., 2016; Cardoso et al., 2015; Puttabyatappa et al., 2017a), adipocyte defects are manifested as reduced adipogenesis, reduced expression of adipocyte differentiation markers in visceral AT (VAT) (Puttabyatappa et al., 2017b), and increased prevalence of small adipocytes in VAT and subcutaneous AT (SAT) depots (Veiga-Lopez et al., 2013; Cardoso et al., 2016). In addition, adipose (VAT vs. SAT) depot-specific changes such as increases in inflammatory cytokine, macrophage marker and antioxidant expression and markers of oxidative stress in the VAT but not SAT (Puttabyatappa et al., 2017a) were also evident in prenatal T-treated female sheep. Despite these changes, surprisingly, both VAT and SAT continue to maintain responsiveness to insulin stimulation (Lu et al., 2016) and increased expression of insulin signaling members in the VAT (Nada

* Corresponding author. Department of Pediatrics, University of Michigan, 7510 MSRB I, 1150 West Medical Center Drive, Ann Arbor, MI, 48109, USA.
E-mail address: vasantha@umich.edu (V. Padmanabhan).

https://doi.org/10.1016/j.mce.2019.110691
Received 19 September 2019; Received in revised form 4 December 2019; Accepted 17 December 2019
Available online 19 December 2019
0303-7207/ © 2019 Elsevier B.V. All rights reserved.

et al., 2010).

Both VAT and SAT are white adipose tissue (WAT) depots of same developmental lineage (Hocking et al., 2013). However, VAT and SAT differ in their gene expression profile (Gesta et al., 2006), adipokine production (Hocking et al., 2010), ability to store fat and regulate insulin sensitivity (Saely et al., 2012). Accumulation of VAT depots, which include omental and mesenteric AT is a risk factor for development of cardiometabolic disorders (Wajchenberg, 2000; Bjorndal et al., 2011). While VAT adipocytes are lipolytically active and favors development of IR in insulin target tissues (Wajchenberg, 2000; Bjorndal et al., 2011; Hajer et al., 2008), SAT is less lipolytically active, promotes lipid storage and prevent development of IR (Frayn, 2002; Kelley et al., 2000; Smith et al., 2001). While most studies focus on VAT and SAT, there are other WAT depots (Gruzdeva et al., 2018) such as epicardiac (ECAT) and perirenal AT (PRAT), which are linked with the development of atherosclerosis and cardiovascular diseases (Lazaros et al., 2018; Liu et al., 2019; Iacobellis, 2014). For instance, the fatty acids and proinflammatory adipokines secreted from the ECAT can directly regulate cardiac vascular cells and cardiomyocytes (Berg et al., 2019). Similarly, association of PRAT depot with renal arterial endothelial dysfunction in pigs fed a high fat/high fructose diet have been reported (Ma et al., 2016). These findings provide support for AT depot-specific effects on organs in close proximity as well as systemic metabolic effects on insulin sensitivity.

In addition to the adipocytes discussed above, another type of adipocyte called the brown adipocytes of the skeletal muscle lineage also exists in mammals. These brown adipocytes differ from WAT in their thermogenic role and the presence of large number of mitochondria with a higher oxidative capacity (Shabalina et al., 2013). They express uncoupling protein 1 (UCP1) (Ricquier and Bouillaud, 2000; Fuller-Jackson and Henry, 2018), which facilitates release of stored energy as heat (Cannon and Nedergaard, 2004). Brown adipocytes previously thought to be only present in newborn where they were important for shivering thermogenesis are now known to be present in the adult as well (Fuller-Jackson and Henry, 2018; Ong et al., 2018; Nedergaard et al., 2007). ECAT and PRAT depots are rich in brown adipocytes (Henry et al., 2017; Ouellet et al., 2011; Symonds, 2013; Symonds et al., 2015) during the neonatal period in both human and sheep and get increasingly replaced by white adipocytes as they age (Symonds, 2013; Symonds et al., 2015). In addition to brown adipocytes, beige adipocytes - which are inducible form of the smooth muscle lineage - have also been identified (Long et al., 2014; Stern and Scherer, 2015). The increased presence of brown/beige adipocytes in WAT helps improve insulin sensitivity because of their thermogenic properties (Symonds, 2013; Guerra et al., 1998; Aldiss et al., 2017; Ishibashi and Seale, 2010). Sex-specific differences in the distribution of brown adipocytes have been identified in humans and rodents with females having more brown AT than males (Karastergiou et al., 2012), thus supportive of a role for sex steroids in regulating their distribution. To what extent developmental exposure to steroids contribute to differential prevalence of brown/beige adipocytes in various AT depots is not known.

Considering that browning/beiging of WAT is associated with a metabolically healthy phenotype by reducing insulin resistance (Aldiss et al., 2017; Ishibashi and Seale, 2010), it is important to determine if developmental insults alter the relative proportion of brown/beige adipocytes in the various adipose depots. Although VAT manifests an inflammatory and high oxidative stress state in prenatal T-treated female sheep (Puttabyatappa et al., 2017a), VAT along with SAT continue to be responsive to insulin stimulation (Lu et al., 2016). Whether this is reflective of change in distribution of thermogenic brown/beige adipocytes in these depots and if differences in their presence is also reflected in the ECAT and PRAT depot-specific state of inflammation, oxidative stress, and adipocyte differentiation remains to be determined. In this study using prenatal T-treated female sheep as a model system, we tested the hypothesis that gestational T-excess programs depot-specific changes in AT differentiation markers, their inflammatory and oxidative stress state and that these adipose depot-specific changes reflect their relative thermogenic potential as determined by changes in expression of markers of thermogenesis.

2. Methods

2.1. Animals

All studies were conducted using multiparous Suffolk sheep at the University of Michigan Sheep Research Facility (Ann Arbor, MI) under the Institutional Animal Care and Use Committee of the University of Michigan approved protocols. Animals were cared for following the National Institutes of Health Guide for Use and Care of Animals. Details of their maintenance, breeding and lambing have been described previously (Manikkam et al., 2004; Veiga-Lopez et al., 2008). All experimental animals from both control and prenatal T-treated group were housed and provided similar diet throughout. Before weaning, a pelleted diet (Shur-Gain, Elma, NY) composed of 3.6 MCal/kg digestible energy and 18% crude protein were provided. Following weaning at 8 weeks of age, all lambs were group housed outdoors and fed ad libitum until they attained 40 kg body weight. The diet was then switched to contain 15% crude protein (Shur-Gain) till they attained 6 months of age. Adult sheep were fed a ration consisting of 2.3 MCal/kg digestible energy and 11.3% crude protein.

2.2. Prenatal treatments

Pregnant ewes were randomly assigned to two prenatal treatment groups: control (C), and prenatal T-treated (T). Gestational T-treatment consisted of twice weekly intramuscular administration of 100 mg T propionate (1.2 mg/kg; Millipore Sigma, St. Louis, MO) suspended in 2 ml corn oil from 30 to day 90 of gestation. Only one female offspring was used in any given treatment group when the dam had more than one offspring in the same pregnancy. The sample sizes for the two treatment groups were C = 6, and T = 5. The effects of prenatal T treatment on reproductive attributes, insulin sensitivity, and adipocyte size distribution from this cohort have been previously published (Cardoso et al., 2016; Puttabyatappa et al., 2017a, 2017b; Lu et al., 2016; Padmanabhan et al., 2015; Abi Salloum, Herkimer, Lee et al., 2012).

2.3. Tissue collection

To prevent confounding effects from differing steroid background, all animals were ovariectomized at the end of the second breeding season and a 1 cm estradiol implant was inserted subcutaneously (Goodman et al., 1981) to maintain early follicular phase levels of estradiol. Twenty-eight days later, an artificial estrus cycle was initiated by placing two controlled internal drug-releasing implants containing progesterone (CIDR-G; InterAg, Hamilton, New Zealand; implanted subcutaneously) to mimic a luteal phase. Progesterone implants were removed 14 days later and four 30-mm estradiol implants were inserted subcutaneously as before to mimic follicular phase. Tissues were harvested 18 h later following euthanization by barbiturate overdose (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI). Animals were subject to fasting for 48 h before collection of the tissue. The different adipose depots: VAT from the mesenteric fat surrounding the ventral sac of the rumen, SAT from the sterna region, PRAT from around the kidney and ECAT from between the myocardium and the serous/visceral layer after breaking open the pericardial sac were collected, flash-frozen and stored at −80 °C until processed.

2.4. Real time RT-PCR

Total RNA was isolated using Trizol reagent (Life Technologies,

Carlsbad, CA), DNAse-treated, purified using the RNAeasy kit (Qiagen, Germantown, MD) and reverse transcribed into cDNA using the SuperScript Vilo cDNA synthesis kit (Life Technologies) as per the manufacturer's guidelines. Markers of inflammation (proinflammatory cytokines: interleukin 1 beta [IL1B], IL6, tumor necrosis factor alpha [TNF], chemokine ligand 2 [CCL2] and macrophage marker [CD68]), antioxidants (glutathione reductase [GSR], superoxide dismutase [SOD] 1 and 2), adipocyte differentiation (adiponectin [ADIPOQ], peroxisome proliferator activated receptor gamma [PPARG] and fatty acid synthase [FASN]), insulin signaling members (insulin receptor substrate 2 [IRS2], mammalian target of rapamycin complex 1 [MTOR], phosphatidylinositol 3-kinase [PI3K] and protein kinase B [AKT]) and thermogenic adipocytes (UCP1, UCP2, UCP3, PPARG coactivator 1 beta [PPARGC1B], cell death inducing DFFA like effector a [CIDEA] and activating transcription factor 2 [ATF2]) gene expression analysis was carried out using a SYBR green based real time RT-PCR assay on a myiQ iCycler real time PCR instrument (BioRad, Herculus, CA). Oligonucleotide primers for the genes under study were used from either previously published reports or designed using Primer Express software (Life Technologies). The sequences for the primers used are shown in Table 1. The relative amount of each transcript was calculated using the ΔΔCT method and normalized to the endogenous reference gene ribosomal protein L19 (RPL19).

2.5. Assessment of oxidative stress markers

Oxidative stress markers, Thiobarbituric Acid Reactive Substances (TBARS), were quantified utilizing commercially available colorimetric assay kit (Cayman Chemicals, Ann Arbor, MI) as per manufacturer's recommendation. The intra- and inter-assay coefficients for TBARS assay were 5.6% and 10.2%, respectively and the measurable range is 0.0625–50 μM. Oxidized protein tyrosine moieties, 3-nitrotyrosine (NY) and o, o'-dityrosine (DY) were quantified using isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) as described (Vivekanandan-Giri et al., 2011). Intra-assay coefficients of variation for NY and DY were 8.47% and 8.16%, respectively.

2.6. Sircol collagen assay

The acid-soluble collagen content from the different adipose depots were measured using a sirius red dye binding assay following the protocol described before (Coentro et al., 2017). Briefly, the acid-soluble collagen was extracted from 100 mg adipose tissues by homogenization and incubation with 0.5 M acetic acid for 48 h at 4 °C. The soluble collagen was obtained by centrifugation and incubated with 1 mL 0.1% Direct Red 80 (Millipore Sigma) in saturated picric acid dye reagent solution for 1 h at room temperature. The collagen–dye complex was precipitated by centrifugation at 10,000 g for 10 min, and the supernatant was removed. The resulting pellets were dissolved in 1 mL 0.5 M sodium hydroxide, and the relative absorbance was measured in a 96-well plate at 540 nm using a microplate reader (Molecular Devices SpectraMax, San Jose, CA). Collagen content was normalized to total protein content determined as described before (Puttabyatappa et al., 2017a). The inter and intra-assay coefficients of variance for Sircol assay was 1.43 ± 0.32 and 1.71 ± 0.94. The measurable range for this assay is 31.25–500 μg/mL.

2.7. Statistical analysis

The effect of prenatal T-treatment on inflammatory, oxidative, adipogenic and thermogenic markers in each of the 4 adipose depots was assessed by Student's t-test. The expression of thermogenic markers between the different adipose depots were analyzed using one-way ANOVA followed by Tukey's post hoc test. Both analyses were carried out using the Prism 7 software (GraphPad, La Jolle, CA) and the threshold for significance was set as $p \leq 0.05$. As lack of significance may not be sufficient to reject the null hypothesis with studies involving small sample sizes (Amrhein et al., 2019), Cohen's effect size analysis (Cohen, 1992; Nakagawa and Cuthill, 2007) was also used. Using this the magnitude of differences between control and prenatal T-treated group were determined. The computed Cohen's d value of 0.8 and above indicates large effect size differences (Cohen, 1992; Nakagawa and Cuthill, 2007).

3. Results

3.1. The effects of prenatal T excess on adipose depot-specific inflammatory status

To determine the adipose depot specific programming in inflammatory tone from prenatal T excess gene expression analysis for macrophage markers and proinflammatory cytokines was conducted (Fig. 1). Prenatal T-treatment induced non-significant large magnitude

Table 1
Oligonucleotide primers used for RT-PCR.

Gene ID	Forward Primer	Reverse Primer	Accession Number
TNF	ACACCATGAGCACCAAAAGC	AGGCACAAGCAACTTCTGGA	XM_005223596.3
CCL2	CCAGCAGCAAGTGTCCTAAAG	GGCTTTGGAGTTTGGTTTTTC	XM_004012471.2
CD68	CAGGGGACAGGGAATGACT	CCAAGTGGTGGTTCTGTGG	NM_001045902.1
IL6	ACATCGTCGACAAAATCTCTGCAA	GCCAGTGTCTCCTTGCTGTTT	NM_001009392.1
GSR	CTGGAAGAGTTGCCTCGCC	TCATTATTGATGTCTTAGAACCCAGG	XM_015104590.1
SOD1	ATCATGGGTTCCACGTCCA	CATGCCTCTCTTCATCCTTTGG	NM_001145185.1
SOD2	CGCTGGAGAAGGGTGATGTC	CAGATTTGTCCAGAAGATGCTGTG	NM_001280703.1
ADIPOQ	CTCCGTGCTCCTCTATCTGG	TTTCACTTTGCACTGCTTGG	XM_012097442.2
PPARG	CCTTCACCACCGTTGACTTCT	GATACAGGCTCCACTTTGATTGC	HQ589347.1
FASN	CCCAGCTCAACGAAACCA	GACGAGGTCAACACCCTTCC	AF479289.1
IRS2	CCCGAGAAGGTGGCCCGCATCA	AGCAACACGCCCGAGTCCATC	NM_003749.2
MTOR	ATCACCCTTGCTCTCCGAACTCTC	CCAGCTCCCGGATCTCAAACACCT	NM_001145455
PI3K	TTGTCCAGCCACCATGATGT	TGAGCAAGAGGCTTTGGAATATT	M93252.1
AKT	CGGCTCCCTCTCCTGTTAGG	GGGATTTTCCAGCCAAGAGTACT	AF207873
UCP1	GAGTTCTTCACCACAGGGAAA	TCTGACCTTGACCACCTCTGT	NM_001280694.1
UCP2	AAGGCCCACCTAATGACAGA	CCCAGGGCAGAGTTCATGT	NM_001280682.1
UCP3	ACCTGCTCACCGACAACTTC	CATATACCGCGTCTTCACCA	NM_001308581.1
PPARGC1B	CAGTGGTGCCCAGAGAACT	AGGGCCTCATTCTCACTGTC	XM_027970395.1
CIDEA	AAGGCCACCATGTACGAGAT	GGTGCCCATGTGGATAAGACA	XM_012103597.3
ATF2	TCCCACTTGTTCGACCAGTCA	TTGACAGTATCGCCGTTGGT	XM_004004570.4
RPL19	CCTTGGCTCGCCGGA	CATGTGGCGGTCAATCTTCTTA	XM_012186026.1

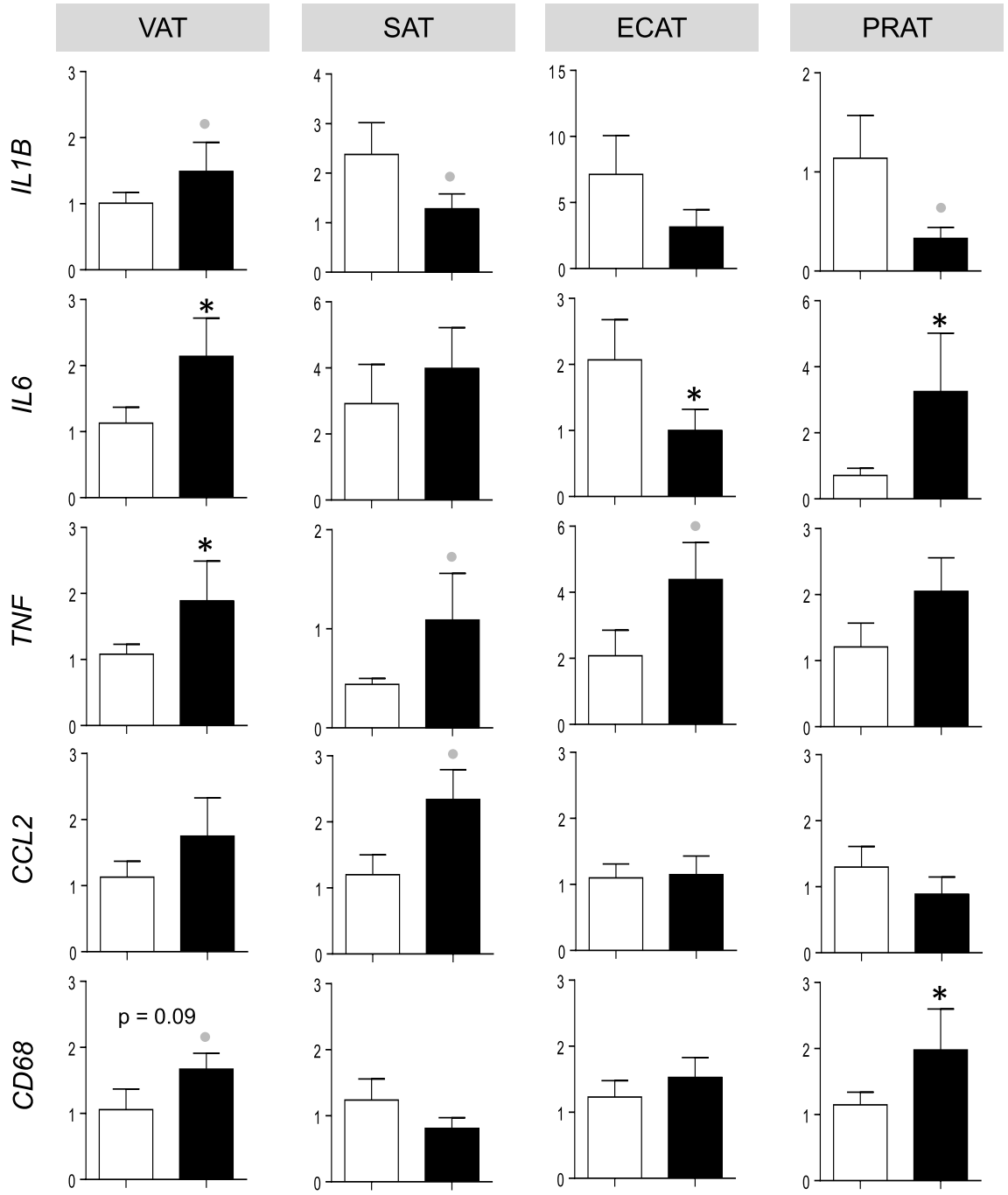


Fig. 1. Mean ± SEM of fold change in mRNA expression of inflammatory cytokines and macrophage marker genes in the different adipose depots from control (C) and prenatal T-treated (T) animals. * = p < 0.05 by Student's t-test and solid grey circle = large effect size by Cohen's effect size analysis.

increase in *IL1B* in the VAT (d = 0.8) and non-significant large magnitude decrease in SAT (d = 1.0) and PRAT (d = 1.2) with no effect in ECAT depot. Prenatal T excess induced a significant large magnitude increase in *IL6* in the VAT (d = 1.0) and PRAT (d = 0.9) but a significant large magnitude decrease in ECAT (d = 1.1), with no change in SAT. With *TNF*, prenatal T excess also resulted in a significant increase in the VAT (d = 0.9), a large magnitude non-significant increase in SAT (d = 0.8) and ECAT (d = 1.0) with no effect in PRAT. Within the SAT alone the chemokine *CCL2* was enhanced with a significant large magnitude effect of prenatal T excess (d = 0.9). The effect of prenatal T excess on the macrophage maker *CD68* was evident as a trend for a large magnitude increase in VAT (d = 0.9; $p = 0.09$) and a significant large magnitude increase in PRAT (d = 0.8) but not in the other two depots.

3.2. The effects of prenatal T excess on adipose depot-specific oxidative stress

The status of oxidative stress in adipose depots was assessed by examining the oxidative stress markers TBARS, a byproduct of lipid peroxidation, oxidized tyrosine products, and expression of antioxidants. Among the different adipose depots, a large magnitude increase (d = 0.8) in TBARS was evident only by effect size analysis in the VAT (Fig. 2). With NY, prenatal T excess induced a significant large magnitude increase in both ECAT (d = 1.4) and PRAT (d = 1.9) (Fig. 2). We had previously found significant large magnitude increase

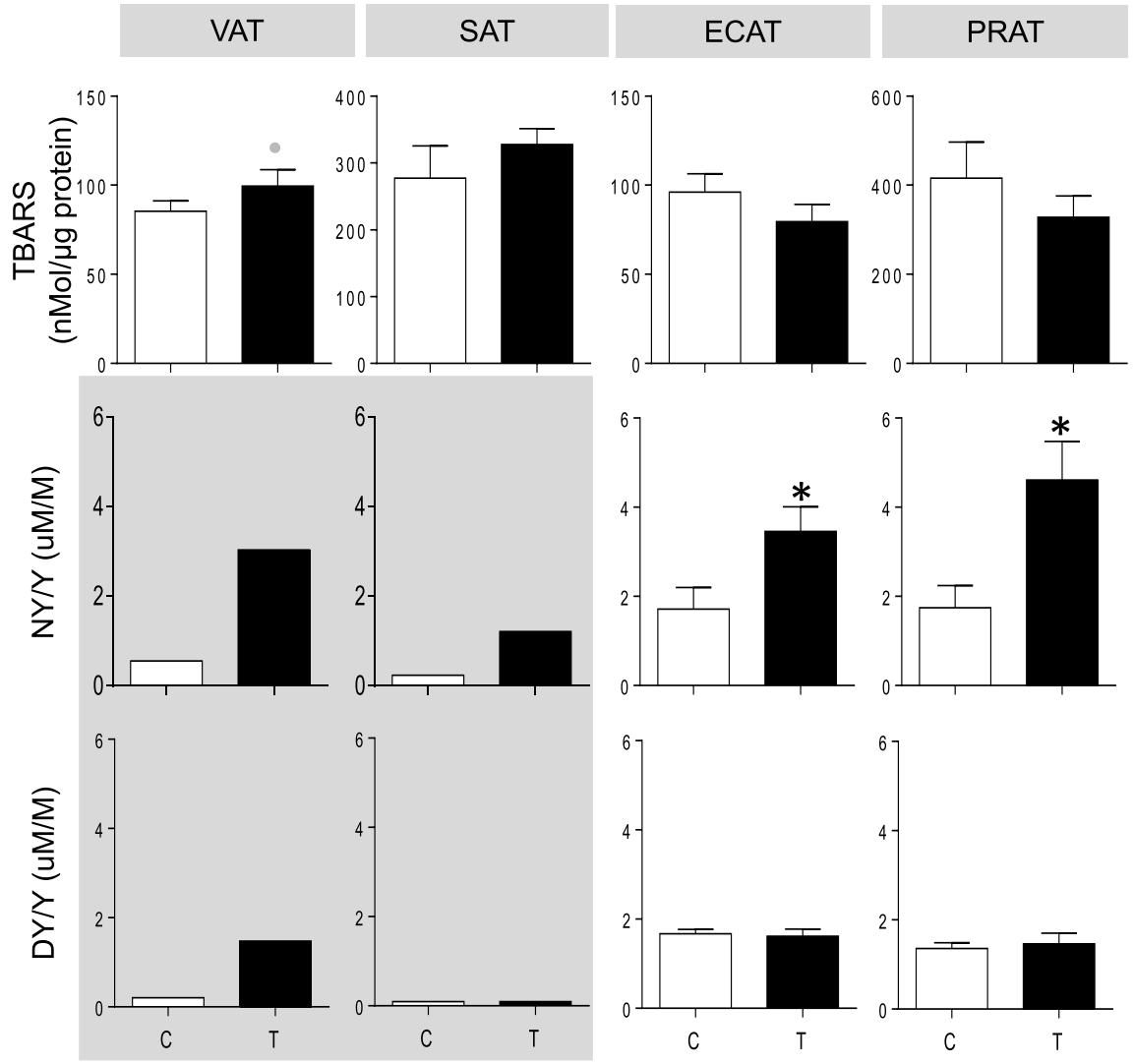


Fig. 2. Mean ± SEM of levels of oxidative stress markers in the different adipose depots from control (C) and prenatal T-treated (T) animals. Mean values of oxidized tyrosine concentrations in VAT and SAT depots have been previously published (Puttabyatappa et al., 2017a) and means are shown in the grey area for comparison. * = p < 0.05 by Student's t-test and solid grey circle = large effect size by Cohen's effect size analysis.

in NY in the VAT and a trend for a large magnitude increase in NY in SAT and DY in VAT as well (Puttabyatappa et al., 2017a). In contrast, no changes in DY were evident in the ECAT and PRAT.

Among the antioxidants, prenatal T-treatment induced significant large magnitude increase (d = 1.2) in *GSR* and a large magnitude increase in *SOD1* (d = 1.0; $p = 0.07$) in VAT (Fig. 3) but not in the other 3 depots. In contrast, a large magnitude decrease in *SOD2* (d = 1.2; $p = 0.09$) was evident in the ECAT but not the other 3 depots (Fig. 3).

3.3. The effects of prenatal T excess on adipose depot-specific adipocyte differentiation markers

Among the markers of adipocyte differentiation, prenatal T-treatment induced a large magnitude increase in *ADIPOQ* in the VAT (p = 0.07; d = 1.4), a significant large magnitude increase in the SAT (d = 0.8), a large magnitude increase in ECAT (d = 1.2) with no effect in PRAT (Fig. 4). Prenatal T only led to a significant large magnitude increase in *PPARG* in SAT (d = 1.2) alone. In contrast, prenatal T had no effect on the expression levels of *FASN* in VAT and SAT, but induced a significant increase in ECAT (d = 1.5) and PRAT (d = 1.2).

3.4. The effects of prenatal T excess on adipose depot-specific collagen content

We tested to see if prenatal T excess induces changes in AT collagen content as metabolic dysfunction in AT is accompanied by increased accumulation of extracellular matrix proteins (Khan et al., 2009). Prenatal T excess induced a non-significant large magnitude increase in collagen content, in the VAT (d = 0.9) and ECAT (d = 1.5) depots but not in the other two depots (Fig. 5).

3.5. Adipose depot-specific expression of insulin signaling genes

Previously we have found prenatal T-treatment increased the expression of members of the insulin signaling pathway in VAT (Nada et al., 2010). The effects of prenatal T-treatment on insulin signaling pathway in SAT, ECAT and PRAT were not investigated. Consistent with the earlier findings, prenatal T-treatment induced large magnitude albeit non-significant increase in *IRS2* (d = 1.2), *MTOR* (d = 0.9) and *PI3K* (d = 1.2; p = 0.06) expression in VAT. However, in the SAT, prenatal T-treatment induced a non-significant large magnitude decrease *IRS2* expression (d = 1.5) but significantly reduced the expression of *PI3K* expression in the SAT depot (Fig. 6). Similarly, a non-

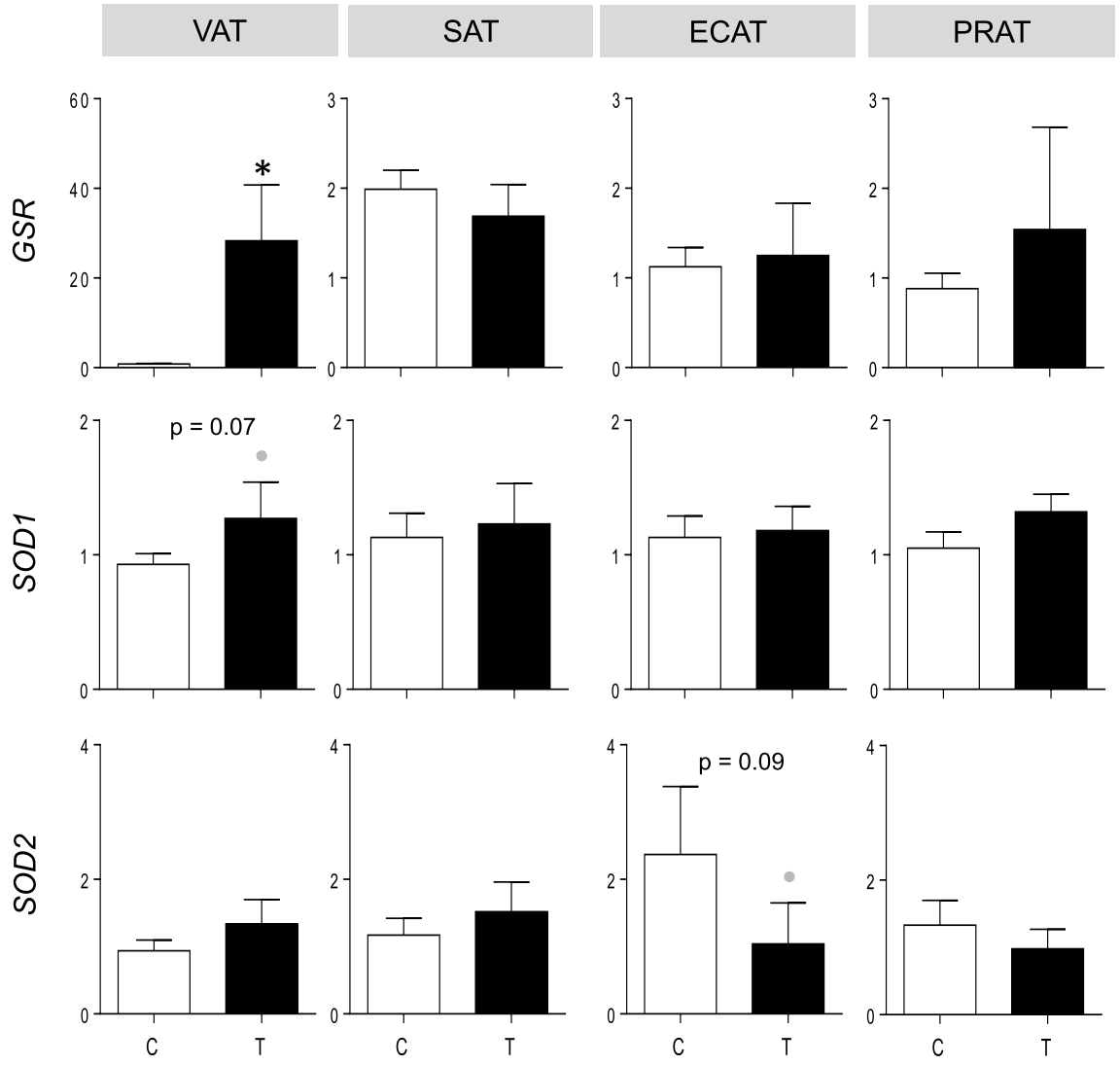


Fig. 3. Mean ± SEM of fold change in mRNA expression of antioxidant genes in the different adipose depots from control (C) and prenatal T-treated (T) animals. * = $p < 0.05$ by Student's t-test and solid grey circle = large effect size by Cohen's effect size analysis.

significant large magnitude decrease in *IRS2* (d = 1.4) and *MTOR* (d = 0.9) were evident in the ECAT depot of prenatal T-treated animals (Fig. 6). None of the depots showed any change in *AKT* expression and prenatal T-treatment did not seem to have any effect on the expression of any insulin signaling genes examined in the PRAT depot.

3.6. Adipose depot-specific expression of thermogenic genes

The expression of different thermogenic gene markers UCP1, UCP2, UCP3, PPARGC1B, CIDEA and ATF2 in the different adipose depots of control animals are summarized in Fig. 7. The expression of *UCP1* was lowest in the VAT depot, highest in the ECAT and PRAT depots and intermediate but significantly higher in the SAT compared to VAT depot (Fig. 7). *UCP2* expression was significantly higher in ECAT depot compared to the other three depots. In contrast, there were no differences in *UCP3* expression among the AT depots. The expression of *PPARGC1B* was significantly higher in the ECAT depot compared to the other 3 depots. *PPARGC1B* levels were also significantly lower in the SAT and PRAT compared to VAT depot. The *CIDEA* expression was significantly higher in SAT and ECAT depots relative to VAT and PRAT. *ATF2* levels were also higher in ECAT compared to other 3 depots (Fig. 7).

3.7. The effects of prenatal T excess on adipose depot-specific expression of thermogenic gene markers

In the VAT, prenatal T-treatment induced a significant large magnitude increase in the levels of *UCP1* (d = 1.1), *UCP2* (d = 1.0) and *PPARGC1B* (d = 1.0) with no change in *CIDEA* (Fig. 8). In contrast, in the SAT prenatal T excess resulted in a significant large magnitude increase in *CIDEA* (d = 1.5) with increase in *UCP2* and *PPARGC1B* evident as large magnitude increases by effect size analysis only (*UCP2*: d = 0.8; *PPARGC1B*: d = 1.2). In ECAT, there was a significant large magnitude increase in *UCP1* (d = 0.8) only. Similarly, in PRAT, prenatal T induced a large magnitude increase in *UCP1* (d = 0.9) but not in other markers.

4. Discussion

Findings from this study demonstrate depot-specific impact of prenatal T-treatment on markers of inflammatory and oxidative stress state, adipocyte differentiation, collagen accumulation, and insulin sensitivity (Fig. 9). Further, gestational T treatment programs depot-specific changes in thermogenic potential of adipocytes, which may have contributed to the maintenance of responsiveness of VAT and SAT to insulin, as reported previously (Lu et al., 2016). The significance of

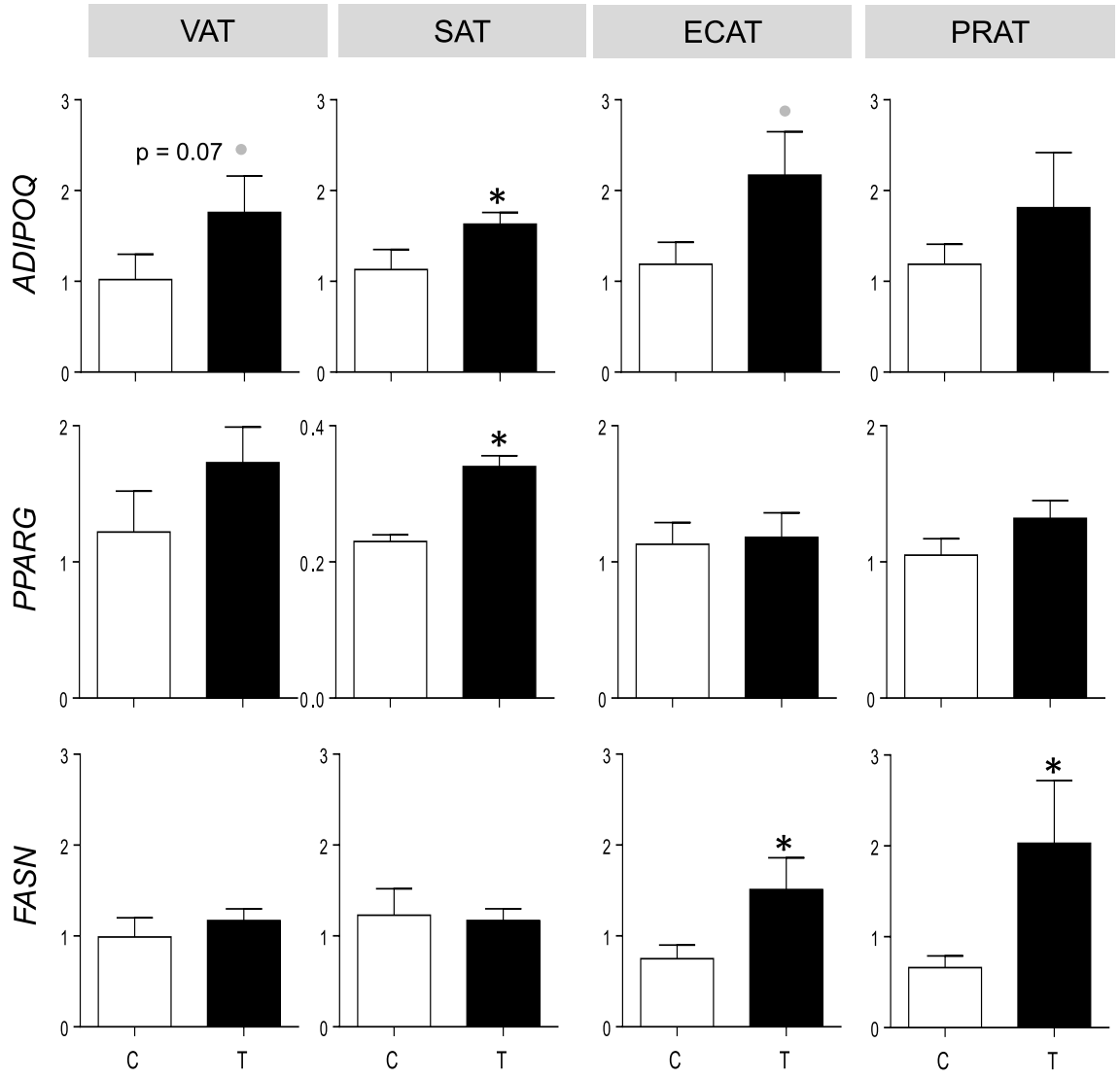


Fig. 4. Mean ± SEM of fold change in mRNA expression of adipocyte differentiation marker genes in the different adipose depots from control (C) and prenatal T-treated (T) animals. * = p < 0.05 by Student's t-test and solid grey circle = large effect size by Cohen's effect size analysis.

these findings and their relationships are discussed below.

Influence of gestational T excess on depot-specific inflammatory and oxidative stress state and collagen accumulation: The findings of depot-specific changes in pro-inflammatory cytokines and macrophage marker *CD68* in the 4 AT depots extends our previous observation that focused only on VAT and SAT (Puttabyatappa et al., 2017a). Consistent with this prior study, of the 4 AT depots studied, VAT appears to be the most severely affected with 4 of the 5 inflammatory markers studied manifesting large magnitude increases in prenatal T offspring suggestive of an increased inflammatory status. SAT and PRAT had a more

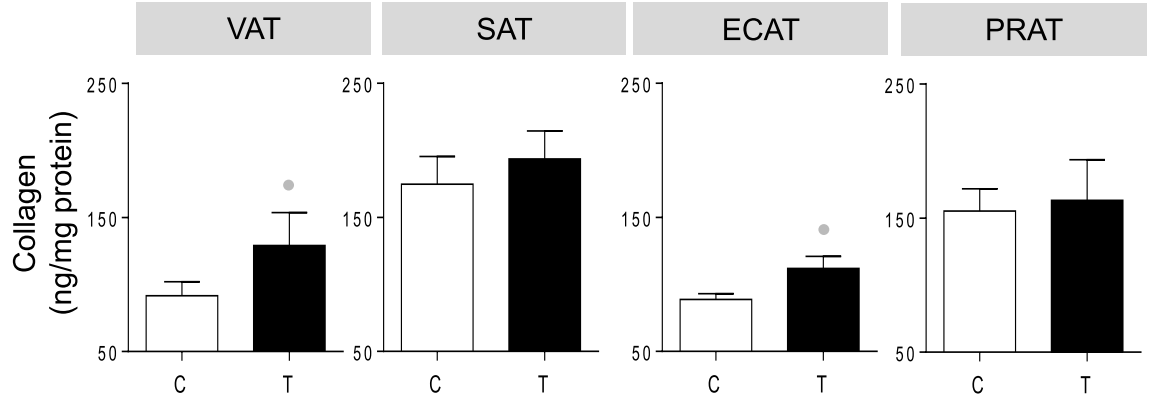


Fig. 5. Mean ± SEM of collagen content normalized to total protein in the different adipose depots from control (C) and prenatal T-treated (T) animals. Solid grey circle = large effect size by Cohen's effect size analysis.

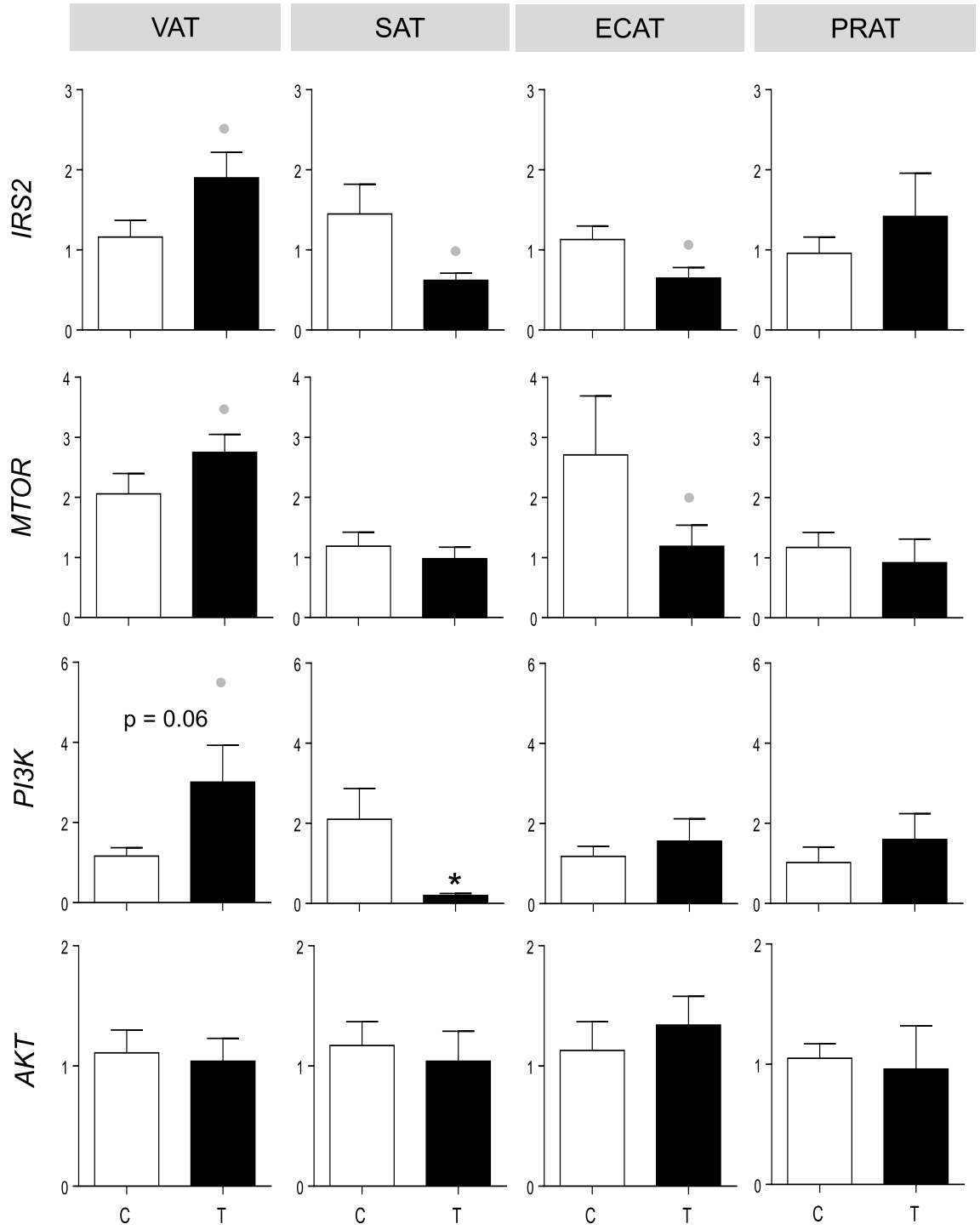


Fig. 6. Mean ± SEM of fold change in mRNA expression of insulin signaling genes in the different adipose depots from control (C) and prenatal T-treated (T) animals. Solid grey circle = large effect size by Cohen's effect size analysis.

intermediate inflammatory activation with 2 of the 5 markers elevated. In contrast, ECAT appears to have some protection from inflammation due to 1) a decrease in *IL6* that likely offsets the possible increase in *TNF* and 2) lack of change in macrophage marker *CD68*. Such differences in the 5 inflammatory markers studied point to the four AT depots manifesting different degrees of inflammation that are regulated by different mediators. Considering AT inflammation is shown to play a role in the development of insulin resistance (Esser et al., 2014), the activated inflammatory profile in 3 of the 4 depots in the prenatal T-treated animals would be suggestive of insulin resistant state of these tissue depots and potential contribution to peripheral insulin resistance (Puttabyatappa and Padmanabhan, 2017).

The increased expression of pro-inflammatory cytokine expression may suggest increased macrophage accumulation in VAT compared to SAT. Such changes are also noted in other insulin resistant states stemming from obesity (Hocking et al., 2010; O'Rourke et al., 2009). Since these prenatal T-treated sheep are not obese (Veiga-Lopez et al., 2013), the increase in proinflammatory cytokines may be a function of prenatal T-programming. Additionally, as prenatal T-treated animals are born with low birth weight and exhibit post-natal catch-up growth

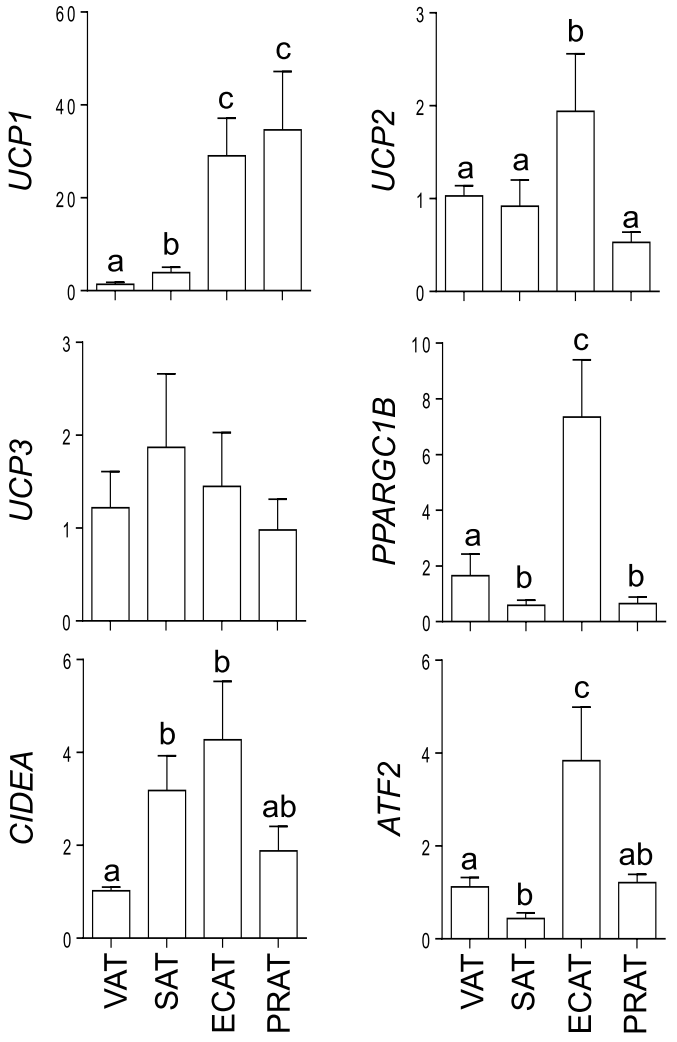


Fig. 7. Mean ± SEM of fold change in mRNA expression of thermogenic genes in the different adipose depots with VAT depot set as baseline. Gene expression was examined in the adipose depots from control animals. Differing alphabet superscripts indicate statistically significant differences by ANOVA (a vs. b vs. c: $p < 0.05$; ab not different from a or b).

(Manikkam et al., 2004), there is a possibility that these outcomes may have served as an intermediary in programming adipose defects.

The elevated inflammatory cytokine expression seen in the PRAT of prenatal T-treated sheep may contribute to the cardiovascular defects exhibited in these animals (King et al., 2007; Vyas et al., 2016). Increased TNF secretion from the PRAT depot has been found to be associated with vascular dysfunction in pigs fed high fat/high fructose diet (Ma et al., 2016). While the association of elevated proinflammatory adipokines in ECAT of humans with cardiovascular defects (Berg et al., 2019) has been noted, the opposing expression pattern of *IL6* and *TNF* observed in this study suggests that this depot is not likely contributing to the cardiovascular defects seen in prenatal T-treated sheep through inflammation. On the contrary these changes may reflect a compensatory protective mechanism at the cardiac level to overcome cardiac defects.

In addition to inflammation, oxidative stress within tissues is associated with tissue dysfunction. The finding that nitrosative oxidative stress was evident in all the depots examined while lipid peroxidation was elevated only in VAT suggests that, as was the case with the inflammatory status, oxidative stress status was more severely impaired in the VAT compared to other AT depots. Such AT depot-specific changes in oxidative stress markers has not been examined in humans or other animal models. Since inflammation and oxidative stress go hand in hand (Chatterjee, 2016), the increase in NY levels in the VAT, SAT and PRAT depots are in agreement with the increased proinflammatory cytokine expression. Adipocyte depot-specific regulation also extended to antioxidant expression with *GSR* and *SOD1* being elevated only in VAT and *SOD2* decreased in ECAT. To what extent these changes in antioxidant expression at different depots reflect different degrees of compensatory response to overcome the oxidative stress is unclear.

Both inflammation and oxidative stress has been shown to induce fibrosis, which is manifested by collagen accumulation in other systems (Kershenobich Stalnikowitz and Weissbrod, 2003). In addition, metabolic dysfunction with AT defects are accompanied by increased accumulation of extracellular matrix proteins (Khan et al., 2009). The increase in collagen accumulation observed in the VAT depot with prenatal T is consistent with this premise. In the ECAT depot, the increase in *TNF* and nitrative stress coupled with decreased *SOD2*, may have contributed to the elevated collagen accumulation. As AT fibrosis plays an important role in metabolic dysregulation (Khan et al., 2009), it is possible that collagen accumulation in VAT and ECAT may play a role in the development of prenatal T-programmed development of peripheral insulin resistance and cardiac defects (Cardoso et al., 2015), respectively. The lack of increase in collagen accumulation in SAT and PRAT depots suggests some inherent protective mechanism may be in place to prevent fibrosis even with gestational T excess.

Influence of gestational T excess on depot-specific adipocyte differentiation: Lack of changes in the transcription factor involved in adipogenesis and adipocyte differentiation, *PPARG* (Lowell, 1999), and genes involved in adipocyte function *FASN* with only a modest increase in *ADIPOQ* expression in VAT are consistent with our previous findings that adipocyte differentiation is impaired in this depot (Puttabyatappa et al., 2017b). However, the findings of increased *ADIPOQ* and *PPARG* in SAT of prenatal T-treated sheep is not consistent with increased distribution of small adipocytes (Cardoso et al., 2016) seen in these animals. The increased expression of *ADIPOQ* may explain why SAT maintains a lesser inflammatory state compared to VAT and retain its insulin responsiveness (Lu et al., 2016) in prenatal T-treated sheep. The findings of increased *ADIPOQ* and *FASN* in the ECAT and PRAT depots are suggestive that prenatal T-treatment does not impair adipocyte differentiation in these depots.

Influence of gestational T excess on depot-specific insulin signaling: A trend in increase in *PI3K* and non-significant large magnitude increase in *IRS2* and *MTOR* in the VAT are in agreement with the previous report that prenatal T-treatment increases the expression of genes involved in insulin signaling (Nada et al., 2010). However, we did not identify an increase in *AKT* expression as has been previously reported (Nada et al., 2010). The increased expression of insulin signaling genes in the VAT from both the current and previous study (Nada et al., 2010) are consistent with the maintenance of response to insulin stimulation in the VAT from prenatal T-treated animals (Lu et al., 2016). However, the reduced levels of *PI3K* and *IRS2* observed in the SAT depot is not consistent with the maintenance of responsiveness to insulin stimulation reflected as increase in phosphoAKT, described previously (Lu et al., 2016). The reason for this difference may be that the mRNA changes observed in this study do not reflect the protein changes or alternatively that the reduced expression may be a compensatory response to overcome the increased protein levels or insulin signaling. While the insulin responsiveness in ECAT and PRAT depots remains to be examined, the non-significant but large magnitude decreases in *IRS2* and *MTOR* is suggestive of compromised insulin sensitivity in the ECAT. Further studies are required to determine if reduced mRNA expression of markers of insulin signaling measured at basal state are also reflective of reduced responsiveness to insulin. For instance, reduced mRNA levels of markers of insulin signaling in SAT is not consistent with maintenance of phosphoAKT responsiveness to insulin stimulation.

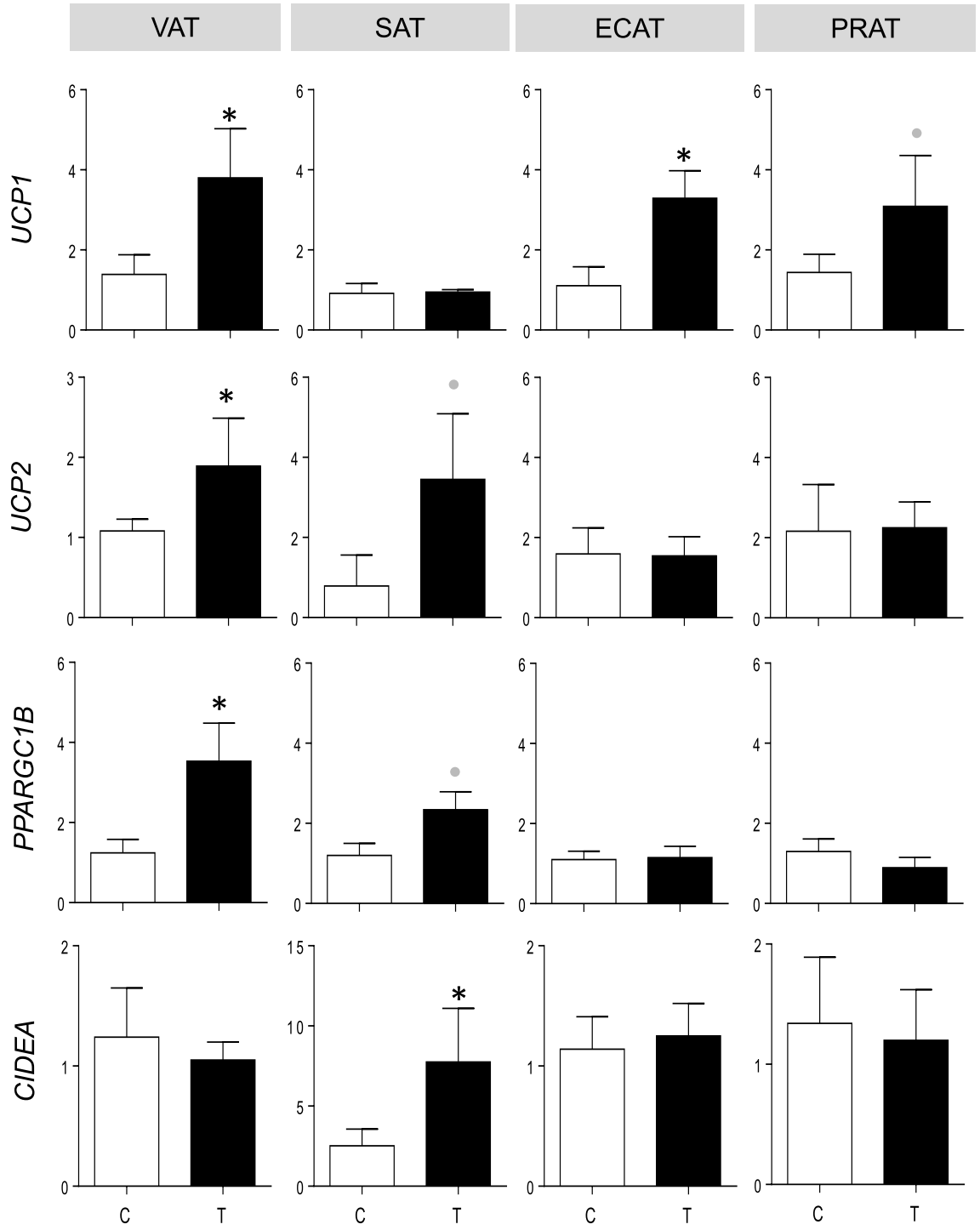


Fig. 8. Mean ± SEM of fold change in mRNA expression of thermogenic gene markers in the different adipose depots from control (C) and prenatal T-treated (T) animals. * = $p < 0.05$ by Student's t-test and solid grey circle = large effect size by Cohen's effect size analysis.

Distribution of thermogenic adipocytes in the different adipose depots of the female sheep: The detection of thermogenic gene markers in all the 4 depots examined is consistent with previous studies, which have found dispersed brown adipocytes within WAT depots in sheep (Symonds et al., 2015). Interestingly the distribution of thermogenic adipocytes as surmised by expression level of thermogenic genes appear to vary amongst the 4 depots. All thermogenic markers showed higher expression in the ECAT implying that this depot in adult female sheep has a higher distribution of brown/beige adipocytes. This is followed by PRAT and SAT depots with VAT having the lowest thermogenic potential. These findings are consistent with the reported distribution of brown/beige adipocytes in the sheep (Fuller-Jackson and Henry, 2018; Henry et al., 2017; Symonds et al., 2015).

Influence of gestational T excess on depot-specific distribution of thermogenic adipocytes: The distribution of thermogenic adipocytes have been found to be developmentally programmed by nutrition (Bispham et al., 2005). That steroids can also play a role in this process is supported by our findings of prenatal T-induced increase in thermogenic genes in the 4 AT depots studied. While 3 of the 4 thermogenic markers were increased by prenatal T excess in the VAT and SAT, only an

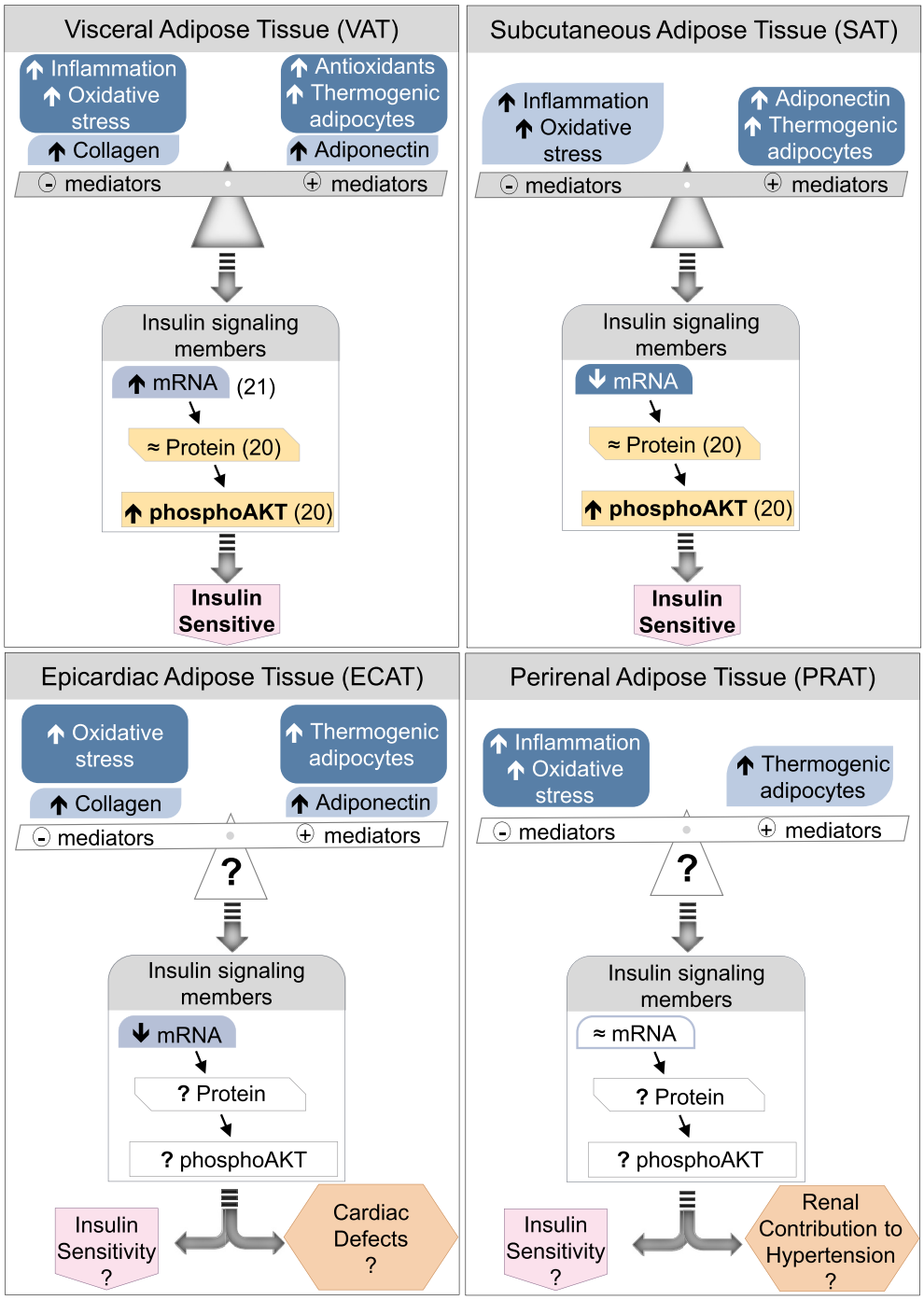


Fig. 9. Schematic showing the depot-specific changes in negative and positive mediators of insulin sensitivity and members of the insulin signaling pathway and their potential contribution to insulin sensitivity of the various AT depots in the prenatal T-treated female sheep. The mediators in the dark blue box represent statistically significant changes while those in the light blue boxes are evident by Cohen's effect size analysis only. Among the insulin signaling members those in the box shaded yellow represent previously published data Lu et al., 2016. Note the mRNA expression of members of insulin signaling pathway in VAT examined in this study is consistent with the previous report Nada et al., 2010. The balance between negative (eg. inflammatory and oxidative state) and positive (eg. antioxidants and distribution of thermogenic adipocytes) mediators may have contributed to the maintenance of the insulin sensitive state in VAT and SAT depots (top 2 panels) as evident from insulin-stimulated increase in phosphoAKT described previously Lu et al., 2016. Bottom 2 panels show prenatal T-treatment induced changes in both positive and negative mediators and members of insulin signaling pathway in the ECAT and PRAT depots. Whether the balance between the negative and positive mediators of insulin sensitivity is altered to affect insulin sensitivity, and cardiac defects and renal contribution to hypertension, respectively, in ECAT and PRAT depots remains to be determined. Similarly, whether the large magnitude decrease in ECAT and lack of changes in PRAT in the expression of insulin signaling members reflect reduced/unchanged insulin stimulated phosphoAKT/insulin sensitivity in them also needs to be assessed.

increase in *UCP1* was evident in ECAT and PRAT depots. While the reason for this is not clear, whether these depot-specific changes in thermogenic gene expression govern the unique function and manifestation of adipose tissue defects in prenatal T-treated sheep needs to be determined.

Tying the depot-specific thermogenic changes with insulin sensitivity: Our previous studies have shown that prenatal T-treatment leads to depot-specific changes in VAT and SAT with increased inflammatory and oxidative state in VAT than SAT (Puttabyatappa et al., 2017a). Despite these changes both VAT and SAT maintained their phosphoAKT responsiveness to insulin stimulation (Lu et al., 2016). While increased expression of antioxidants may have aided in maintaining the insulin sensitivity status of the VAT (Puttabyatappa et al., 2017a; Puttabyatappa and Padmanabhan, 2017), the findings from this study of increased expression of thermogenic adipocytes offers another means through which insulin sensitivity is maintained not only in the VAT but also the SAT (Fig. 9). To what extent the non-significant large magnitude decrease in expression of genes involved in insulin signaling in ECAT and lack of change in the PRAT depot will be reflected under insulin stimulated condition and any such compromise is countered by the increase in thermogenic gene expression in these two depots (Fig. 9) remains to be ascertained. Likewise, if changes in AT browning/beiging in ECAT and PRAT depots through paracrine action influences cardiac and renal functions, respectively, and play a role in the development of cardiac and hypertensive phenotype observed in prenatal T-treated sheep (King et al., 2007; Vyas et al., 2016) needs to be explored further. While it is possible that gestational exposure to excess T may have directly programmed these compensatory changes, the prevailing

steroidal and metabolic milieu of the adult animals may have played an activational role in facilitating these defects.

4.1. Translational relevance

The findings from this study may be of translational relevance with metabolic defects associated with polycystic ovary syndrome (PCOS) and metabolic syndrome as prenatal T-treated sheep mirror many of the defects seen clinically (Cardoso et al., 2015; Puttabyatappa and Padmanabhan, 2017). The findings in VAT and SAT from prenatal T-treated sheep compare well with what has been seen in human studies that have examined VAT and SAT depots in conditions associated with insulin resistance such as PCOS, obesity and cardiovascular disorders (Gruzdeva et al., 2018; Martinez-Garcia et al., 2013; Montes-Nieto et al., 2013). These studies report a strong association between VAT inflammation and oxidative stress and metabolic abnormalities compared to that of SAT (Pou et al., 2007; Fox et al., 2007). Considering 1) the recent focus on brown/beige AT as a regulator and therapeutic target to overcome metabolic abnormalities associated with obesity (Abdullahi and Jeschke, 2017), and 2) the finding that lean individuals can also exhibit metabolic derangements including insulin resistance (Yki-Jarvinen, 2005), the thermogenic profile of prenatal T-treated sheep that parallel brown AT distribution in humans (Symonds et al., 2015), are likely to be of translational value.

A major strength of this study is the assessment of depot-specific changes in inflammation, oxidative stress, collagen accumulation and adipocyte differentiation in relation to the thermogenic potential in a well characterized model that manifest a metabolic disease phenotype. To date no studies have investigated AT depot-specific changes programmed by prenatal T exposure in such detail. A limitation of the study is that for the most part, impact of prenatal T excess was made at the gene expression level. Difficulties in identifying specific antibodies for use in sheep precluded our extension of these findings to the protein level. Another limitation was the small sample size which limited achievement of statistical significance in spite of Cohen's effect size analysis revealing large magnitude effects. The stringent study design restricting use of only one offspring from each dam and the inability to control numbers of dams giving birth to female offspring in each group contributed to this limitation. Nonetheless, the finding of increased inflammatory cytokine gene expression in 3 of the 4 depots that are consistent with the increase in well characterized markers of oxidative stress (Cipak Gasparovic, Zarkovic, Zarkovic et al., 2017) lend support that gene expression changes likely reflect functional depot-specific changes. Additional studies to confirm these findings in a larger cohort of animals are required.

In conclusion, the increase in thermogenic potential of VAT and SAT depots failed to prevent the prenatal T-induced increase in inflammatory and oxidative stress status of these depots. On the contrary the increased thermogenic potential may serve as a compensatory mechanism to preserve the insulin sensitive status of at least these two depots (Lu et al., 2016). If the same holds true at the ECAT and PRAT depots remains to be determined.

Funding source

Research reported in this publication was supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) grant P01 HD44232.

Declaration of competing interest

Authors have nothing to disclose.

Acknowledgments

We thank Mr. Douglas Doop and Gary McCalla for their valuable assistance in breeding, lambing, and careful animal care; Dr. Almudena Veiga-Lopez, Dr. Bachir Abi Salloum, Mr. Evan Beckett, Mrs. Carol Herkimer and students supported through the Undergraduate Research Opportunity Program (University of Michigan) for the help provided with administration of treatments and tissue collection; Drs Lixia Zeng and Subramaniam Pennathur for measurement of oxidized tyrosine moieties.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2019.110691.

References

Abdullahi, A., Jeschke, M.G., 2017. Taming the flames: targeting white adipose tissue browning in hypermetabolic conditions. Endocr. Rev. 38, 538–549.
Abi Salloum, B., Herkimer, C., Lee, J.S., Veiga-Lopez, A., Padmanabhan, V., 2012. Developmental programming: prenatal and postnatal contribution of androgens and insulin in the reprogramming of estradiol positive feedback disruptions in prenatal testosterone-treated sheep. Endocrinology 153, 2813–2822.
Aldiss, P., Davies, G., Woods, R., Budge, H., Sacks, H.S., Symonds, M.E., 2017. 'Browning' the cardiac and peri-vascular adipose tissues to modulate cardiovascular risk. Int. J. Cardiol. 228, 265–274.
Amrhein, V., Greenland, S., McShane, B., 2019. Scientists rise up against statistical significance. Nature 567, 305–307.
Berg, G., Miksztowicz, V., Morales, C., Barchuk, M., 2019. Epicardial adipose tissue in cardiovascular disease. Adv. Exp. Med. Biol. 1127, 131–143.
Bispham, J., Gardner, D.S., Gnanalingham, M.G., Stephenson, T., Symonds, M.E., Budge, H., 2005. Maternal nutritional programming of fetal adipose tissue development: differential effects on messenger ribonucleic acid abundance for uncoupling proteins and peroxisome proliferator-activated and prolactin receptors. Endocrinology 146, 3943–3949.
Bjorndal, B., Burri, L., Staalesen, V., Skorve, J., Berge, R.K., 2011. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. J Obes 2011, 490650.
Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev. 84, 277–359.
Cardoso, R.C., Puttabyatappa, M., Padmanabhan, V., 2015. Steroidogenic versus metabolic programming of reproductive neuroendocrine, ovarian and metabolic dysfunctions. Neuroendocrinology 102, 226–237.
Cardoso, R.C., Veiga-Lopez, A., Moeller, J., Beckett, E., Pease, A., Keller, E., Madrigal, V., Chazenbalk, G., Dumesic, D., Padmanabhan, V., 2016. Developmental programming: impact of gestational steroid and metabolic milieus on adiposity and insulin sensitivity in prenatal testosterone-treated female sheep. Endocrinology 157, 522–535.
Chatterjee, S., 2016. Oxidative Stress, Inflammation, and Disease, Oxidative Stress and Biomaterials. Elsevier, pp. 35–58.
Cipak Gasparovic, A., Zarkovic, N., Zarkovic, K., Semen, K., Kaminskyy, D., Yelisyeyeva, O., Bottari, S.P., 2017. Biomarkers of oxidative and nitro-oxidative stress: conventional and novel approaches. Br. J. Pharmacol. 174, 1771–1783.
Coentro, J.Q., Capella-Monsonis, H., Graceffa, V., Wu, Z., Mullen, A.M., Raghunath, M., Zeugolis, D.I., 2017. Collagen quantification in tissue specimens. Methods Mol. Biol. 1627, 341–350.
Cohen, J., 1992. A power primer. Psychol. Bull. 112, 155–159.
DeFronzo, R.A., Ferrannini, E., 1991. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14, 173–194.
Eisner, J.R., Dumesic, D.A., Kemnitz, J.W., Colman, R.J., Abbott, D.H., 2003. Increased adiposity in female rhesus monkeys exposed to androgen excess during early gestation. Obes. Res. 11, 279–286.
Esser, N., Legrand-Poels, S., Piette, J., Scheen, A.J., Paquot, N., 2014. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res. Clin. Pract. 105, 141–150.
Fox, C.S., Massaro, J.M., Hoffmann, U., Pou, K.M., Maurovich-Horvat, P., Liu, C.Y., Vasan, R.S., Murabito, J.M., Meigs, J.B., Cupples, L.A., D'Agostino, R.B., Sr, O'Donnell, C.J., 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 116, 39–48.
Frayn, K.N., 2002. Adipose tissue as a buffer for daily lipid flux. Diabetologia 45, 1201–1210.
Fuller-Jackson, J.P., Henry, B.A., 2018. Adipose and skeletal muscle thermogenesis: studies from large animals. J. Endocrinol. 237, R99–R115.
Gastaldelli, A., Gaggini, M., DeFronzo, R.A., 2017. Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the san antonio metabolism study. Diabetes 66, 815–822.
Gesta, S., Bluher, M., Yamamoto, Y., Norris, A.W., Berndt, J., Kralisch, S., Boucher, J., Lewis, C., Kahn, C.R., 2006. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc. Natl. Acad. Sci. U. S. A. 103, 6676–6681.
Goodman, R.L., Legan, S.J., Ryan, K.D., Foster, D.L., Karsch, F.J., 1981. Importance of variations in behavioural and feedback actions of oestradiol to the control of seasonal breeding in the Ewe. J. Endocrinol. 89, 229–240.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: the Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev, er20151010.
Grundy, S.M., 2015. Adipose tissue and metabolic syndrome: too much, too little or neither. Eur. J. Clin. Investig. 45, 1209–1217.
Gruzdeva, O., Borodkina, D., Uchasova, E., Dyleva, Y., Barbarash, O., 2018. Localization of fat depots and cardiovascular risk. Lipids Health Dis. 17, 218.
Guerra, C., Koza, R.A., Yamashita, H., Walsh, K., Kozak, L.P., 1998. Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. J. Clin. Investig. 102, 412–420.
Hajer, G.R., van Haeften, T.W., Visseren, F.L., 2008. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur. Heart J. 29, 2959–2971.
Henry, B.A., Pope, M., Birtwistle, M., Loughnan, R., Alagal, R., Fuller-Jackson, J.P., Perry, V., Budge, H., Clarke, I.J., Symonds, M.E., 2017. Ontogeny and thermogenic role for sternal fat in female sheep. Endocrinology 158, 2212–2225.
Hocking, S.L., Wu, L.E., Guilhaus, M., Chisholm, D.J., James, D.E., 2010. Intrinsic depot-specific differences in the secretome of adipose tissue, preadipocytes, and adipose tissue-derived microvascular endothelial cells. Diabetes 59, 3008–3016.
Hocking, S., Samocha-Bonet, D., Milner, K.L., Greenfield, J.R., Chisholm, D.J., 2013. Adiposity and insulin resistance in humans: the role of the different tissue and cellular lipid depots. Endocr. Rev. 34, 463–500.
Iacobellis, G., 2014. Epicardial adipose tissue in endocrine and metabolic diseases. Endocrine 46, 8–15.
Ishibashi, J., Seale, P., 2010. Medicine. Beige can be slimming. Science 328, 1113–1114.
Karastergiou, K., Smith, S.R., Greenberg, A.S., Fried, S.K., 2012. Sex differences in human adipose tissues - the biology of pear shape. Biol. Sex Differ. 3, 13.
Keller, E., Chazenbalk, G.D., Aguilera, P., Madrigal, V., Grogan, T., Elashoff, D., Dumesic, D.A., Abbott, D.H., 2014. Impaired preadipocyte differentiation into adipocytes in subcutaneous abdominal adipose of PCOS-like female rhesus monkeys. Endocrinology 155, 2696–2703.
Kelley, D.E., Thaete, F.L., Troost, F., Huwe, T., Goodpaster, B.H., 2000. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. Am. J. Physiol. Endocrinol. Metab. 278, E941–E948.
Kershenobich Stalnikowitz, D., Weissbrod, A.B., 2003. Liver fibrosis and inflammation. A review. Ann. Hepatol. 2, 159–163.
Khan, T., Muise, E.S., Iyengar, P., Wang, Z.V., Chandalia, M., Abate, N., Zhang, B.B., Bonaldo, P., Chua, S., Scherer, P.E., 2009. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. Mol. Cell. Biol. 29, 1575–1591.
King, A.J., Olivier, N.B., Mohankumar, P.S., Lee, J.S., Padmanabhan, V., Fink, G.D., 2007. Hypertension caused by prenatal testosterone excess in female sheep. Am. J. Physiol. Endocrinol. Metab. 292, E1837–E1841.
Lazaros, G., Antonopoulos, A., Antoniades, C., Tousoulis, D., 2018. The role of epicardial fat in pericardial diseases. Curr. Cardiol. Rep. 20, 40.
Liu, B.X., Sun, W., Kong, X.Q., 2019. Perirenal fat: a unique fat pad and potential target for cardiovascular disease. Angiology 70, 584–593. https://doi.org/10.1177/0003319718799967.
Long, J.Z., Svensson, K.J., Tsai, L., Zeng, X., Roh, H.C., Kong, X., Rao, R.R., Lou, J., Lokurkar, I., Baur, W., Castellot Jr., J.J., Rosen, E.D., Spiegelman, B.M., 2014. A smooth muscle-like origin for beige adipocytes. Cell Metabol. 19, 810–820.
Lowell, B.B., 1999. PPARgamma: an essential regulator of adipogenesis and modulator of fat cell function. Cell 99, 239–242.
Lu, C., Cardoso, R.C., Puttabyatappa, M., Padmanabhan, V., 2016. Developmental programming: prenatal testosterone excess and insulin signaling disruptions in female sheep. Biol. Reprod. 94, 113.
Ma, S., Zhu, X.Y., Eirin, A., Woollard, J.R., Jordan, K.L., Tang, H., Lerman, A., Lerman, L.O., 2016. Perirenal fat promotes renal arterial endothelial dysfunction in obese swine through tumor necrosis factor-alpha. J. Urol. 195, 1152–1159.
Manikkam, M., Crespi, E.J., Doop, D.D., Herkimer, C., Lee, J.S., Yu, S., Brown, M.B., Foster, D.L., Padmanabhan, V., 2004. Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. Endocrinology 145, 790–798.
Martinez-Garcia, M.A., Montes-Nieto, R., Fernandez-Duran, E., Insenser, M., Luque-Ramirez, M., Escobar-Morreale, H.F., 2013. Evidence for masculinization of adipokine gene expression in visceral and subcutaneous adipose tissue of obese women with polycystic ovary syndrome (PCOS). J. Clin. Endocrinol. Metab. 98, E388–E396.
Montes-Nieto, R., Insenser, M., Martinez-Garcia, M.A., Escobar-Morreale, H.F., 2013. A nontargeted proteomic study of the influence of androgen excess on human visceral and subcutaneous adipose tissue proteomes. J. Clin. Endocrinol. Metab. 98, E576–E585.
Nada, S.E., Thompson, R.C., Padmanabhan, V., 2010. Developmental programming: differential effects of prenatal testosterone excess on insulin target tissues. Endocrinology 151, 5165–5173.
Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol. Rev. Camb. Philos. Soc. 82, 591–605.
Navarro, G., Allard, C., Xu, W., Mauvais-Jarvis, F., 2015. The role of androgens in metabolism, obesity, and diabetes in males and females. Obesity 23, 713–719.
Nedergaard, J., Bengtsson, T., Cannon, B., 2007. Unexpected evidence for active brown adipose tissue in adult humans. Am. J. Physiol. Endocrinol. Metab. 293, E444–E452.
O'Rourke, R.W., Metcalf, M.D., White, A.E., Madala, A., Winters, B.R., Maizlin II, , Jobe, B.A., Roberts Jr., C.T., Slifka, M.K., Marks, D.L., 2009. Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. Int. J. Obes. 33, 978–990.
Ong, F.J., Ahmed, B.A., Oreskovich, S.M., Blondin, D.P., Haq, T., Konyer, N.B., Noseworthy, M.D., Haman, F., Carpentier, A.C., Morrison, K.M., Steinberg, G.R., 2018. Recent advances in the detection of brown adipose tissue in adult humans: a review. Clin. Sci. (Lond.) 132, 1039–1054.
Ouellet, V., Routhier-Labadie, A., Bellemare, W., Lakhal-Chaieb, L., Turcotte, E., Carpentier, A.C., Richard, D., 2011. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J. Clin. Endocrinol. Metab. 96, 192–199.
Padmanabhan, V., Sarma, H.N., Savabieasfahani, M., Steckler, T.L., Veiga-Lopez, A., 2010. Developmental reprogramming of reproductive and metabolic dysfunction in sheep: native steroids vs. environmental steroid receptor modulators. Int. J. Androl. 33, 394–404.
Padmanabhan, V., Veiga-Lopez, A., Herkimer, C., Abi Salloum, B., Moeller, J., Beckett, E., Sreedharan, R., 2015. Developmental programming: prenatal and postnatal androgen antagonist and insulin sensitizer interventions prevent advancement of puberty and improve LH surge dynamics in prenatal testosterone-treated sheep. Endocrinology 156, 2678–2692.
Pou, K.M., Massaro, J.M., Hoffmann, U., Vasan, R.S., Maurovich-Horvat, P., Larson, M.G., Keaney Jr., J.F., Meigs, J.B., Lipinska, I., Kathiresan, S., Murabito, J.M., O'Donnell, C.J., Benjamin, E.J., Fox, C.S., 2007. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 116, 1234–1241.
Puttabyatappa, M., Lu, C., Martin, J.D., Chazenbalk, G., Dumesic, D., Padmanabhan, V., 2017b. Developmental programming: impact of prenatal testosterone excess on steroidal machinery and cell differentiation markers in visceral adipocytes of female sheep. Reprod. Sci. 25, 1010–1023 1933719117746767.
Puttabyatappa, M., Padmanabhan, V., 2017. Prenatal testosterone programming of insulin resistance in the female sheep. Adv. Exp. Med. Biol. 1043, 575–596.
Puttabyatappa, M., Andriessen, V., Mesquitta, M., Zeng, L., Pennathur, S., Padmanabhan, V., 2017a. Developmental programming: impact of gestational steroid and metabolic milieus on mediators of insulin sensitivity in prenatal testosterone-treated female sheep. Endocrinology 158, 2783–2798.
Ricquier, D., Bouillaud, F., 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. J. Physiol. 529 Pt 1, 3–10.
Roland, A.V., Nunemaker, C.S., Keller, S.R., Moenter, S.M., 2010. Prenatal androgen exposure programs metabolic dysfunction in female mice. J. Endocrinol. 207, 213–223.
Ruige, J.B., Assendelft, W.J., Dekker, J.M., Kostense, P.J., Heine, R.J., Bouter, L.M., 1998. Insulin and risk of cardiovascular disease: a meta-analysis. Circulation 97, 996–1001.
Saely, C.H., Geiger, K., Drexel, H., 2012. Brown versus white adipose tissue: a mini-review. Gerontology 58, 15–23.
Shabalina, I.G., Petrovic, N., de Jong, J.M., Kalinovich, A.V., Cannon, B., Nedergaard, J., 2013. UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep. 5, 1196–1203.
Smith, U., Kahn, B.B., 2016. Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J. Intern. Med. 280, 465–475.
Smith, S.R., Lovejoy, J.C., Greenway, F., Ryan, D., deJonge, L., de la Bretonne, J., Volafova, J., Bray, G.A., 2001. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism 50, 425–435.
Sowers, J.R., 1992. Insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and accelerated atherosclerosis. J. Clin. Pharmacol. 32, 529–535.
Stern, J.H., Scherer, P.E., 2015. Adipose tissue biology in 2014: advances in our understanding of adipose tissue homeostasis. Nat. Rev. Endocrinol. 11, 71–72.
Symonds, M.E., 2013. Brown adipose tissue growth and development. Scientifica 305763 (Cairo). 2013.
Symonds, M.E., Pope, M., Budge, H., 2015. The ontogeny of Brown adipose tissue. Annu. Rev. Nutr. 35, 295–320.
Tai, E.S., Lau, T.N., Ho, S.C., Fok, A.C., Tan, C.E., 2000. Body fat distribution and cardiovascular risk in normal weight women. Associations with insulin resistance, lipids and plasma leptin. Int. J. Obes. Relat. Metab. Disord. 24, 751–757.
Veiga-Lopez, A., Ye, W., Phillips, D.J., Herkimer, C., Knight, P.G., Padmanabhan, V., 2008. Developmental programming: deficits in reproductive hormone dynamics and ovulatory outcomes in prenatal, testosterone-treated sheep. Biol. Reprod. 78, 636–647.
Veiga-Lopez, A., Moeller, J., Patel, D., Ye, W., Pease, A., Kinns, J., Padmanabhan, V., 2013. Developmental programming: impact of prenatal testosterone excess on insulin sensitivity, adiposity, and free fatty acid profile in postpubertal female sheep. Endocrinology 154, 1731–1742.
Vivekanandan-Giri, A., Byun, J., Pennathur, S., 2011. Quantitative analysis of amino Acid oxidation markers by tandem mass spectrometry. Methods Enzymol. 491, 73–89.
Vyas, A.K., Hoang, V., Padmanabhan, V., Gilbreath, E., Mietelka, K.A., 2016. Prenatal programming: adverse cardiac programming by gestational testosterone excess. Sci. Rep. 6, 28335.
Wajchenberg, B.L., 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. 21, 697–738.
Yki-Jarvinen, H., 2005. Fat in the liver and insulin resistance. Ann. Med. 37, 347–356.
Zhang, M., Hu, T., Zhang, S., Zhou, L., 2015. Associations of different adipose tissue depots with insulin resistance: a systematic review and meta-analysis of observational studies. Sci. Rep. 5, 18495.