

Sex Differences in Metabolic and Adipose Tissue Responses to Juvenile-Onset Obesity in Sheep

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Sex is a major factor determining adipose tissue distribution and the subsequent adverse effects of obesity-related disease including type 2 diabetes. The role of gender on juvenile obesity and the accompanying metabolic and inflammatory responses is not well established. Using an ovine model of juvenile onset obesity induced by reduced physical activity, we examined the effect of gender on metabolic, circulatory, and related inflammatory and energy-sensing profiles of the major adipose tissue depots. Despite a similar increase in fat mass with obesity between genders, males demonstrated a higher storage capacity of lipids within perirenal-abdominal adipocytes and exhibited raised insulin. In contrast, obese females became hypercortisolemic, a response that was positively correlated with central fat mass. Analysis of gene expression in perirenal-abdominal adipose tissue demonstrated the stimulation of inflammatory markers in males, but not females, with obesity. Obese females displayed increased expression of genes involved in the glucocorticoid axis and energy sensing in perirenal-abdominal, but not omental, adipose tissue, indicating a depot-specific mechanism that may be protective from the adverse effects of metabolic dysfunction and inflammation. In conclusion, young males are at a greater risk than females to the onset of comorbidities associated with juvenile-onset obesity. These sex-specific differences in cortisol and adipose tissue could explain the earlier onset of the metabolic-related diseases in males compared with females after obesity. (*Endocrinology* 154: 3622–3631, 2013)

Obesity and associated comorbidities are now the leading preventable causes of mortality, and approximately 3 million adults die annually as a result of being overweight (1). Excess weight gain and obesity can be attributed to an increase in the ratio of calorie consumption to expenditure, and this balance may be reset by a combination of lifestyle and environmental and genetic factors (2). The recent increased prevalence of obesity implies that behavioral and socioeconomic factors, ie, modern sedentary lifestyles and an inadequate diet, especially that associated with social deprivation in Western societies, are involved to a greater extent than genetic constitution (3). There is increasing evidence that

children and/or adolescents who become obese are highly likely to remain so as adults (4). Furthermore, risk factors associated with obesity depend not only on the age at obesity onset but also on the amount of accumulated fat tissue and its anatomical distribution. With central obesity, which is more prevalent in males, fat tissue accumulates in the upper body, ie, within intraabdominal and ectopic regions (5), whereas in premenopausal females, increased sc fat is accumulated in peripheral and lower-body areas, ie, within the gluteal and femoral regions (5). This gender dimorphism in fat distribution is well established, and females tend to have more adipose tissue than males. Nevertheless, body mass

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

Copyright © 2013 by The Endocrine Society
Received March 4, 2013. Accepted July 15, 2013.
First Published Online July 24, 2013

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Abbreviations: a.u., arbitrary unit; AUC, area under the curve; CV, coefficient of variation; GTT, glucose tolerance test; HPA, hypothalamic-pituitary-adrenal.

index-matched males have nearly twice the amount of visceral adipose tissue than females (5). Reduced visceral adiposity in premenopausal females has been linked to the lower prevalence of obesity-related metabolic conditions, such as hypertension, diabetes, hyperlipidemia, and cardiovascular diseases, than those seen in body mass index-matched males, although the incidences converge after the menopause with those of males (6).

Adipose tissue secretes a range of adipokines, which play important local and systemic roles in metabolism, immunity, reproduction, and the cardiovascular system (7). In obesity, adipocytes undergo hypertrophy and hyperplasia, resulting in increased production and secretion of adipokines, which induce macrophage recruitment and infiltration (8). This results in a cumulative state of inflammatory molecule production with additional macrophage recruitment and activation, a mechanism proposed to contribute to the chronic low-grade inflammation associated with obesity (9). Additionally, central obesity promotes activity of the hypothalamic-pituitary-adrenal (HPA) axis, indicating that obesity is a form of systemic stress (10) when the relationship between adipokine signaling hormones such as leptin and adiponectin, together with glucocorticoids and the HPA axis, become dysregulated (10–13). Glucocorticoid status is one contributory factor in the pathologies surrounding obesity, and plasma cortisol has been reported to be raised in females compared with males (14), although the extent to which this may contribute to sex-specific responses remains to be established.

We have previously shown an amplified inflammatory profile within perirenal-abdominal adipose tissue and adverse cardiovascular function in a sheep model of juvenile onset obesity, mediated by a combination of increased food intake and reduced physical activity commencing from the time of weaning (15). Using a more modest model of juvenile obesity in which only physical activity was restricted, we have established that there is a pronounced difference between genders in adverse effects on the renal inflammatory response (14). The extent to which the adipose tissue response to obesity onset in early life differs between depots is not known. We therefore compared the effect of gender and obesity between the 2 major fat depots in the sheep, ie, omental and perirenal-abdominal depots. We wanted to investigate the hypothesis that the development of juvenile-onset obesity promotes metabolic dysfunction and an amplified inflammatory state, mediated by sex and adipose depot-specific adaptations.

Materials and Methods

Animal model

Animal experimentation was conducted in accordance with the UK Home Office and the UK Animals (Scientific Procedures)

Act (1986). All animal work complied with the Principles of Laboratory Animal Care (National Institutes of Health publication number 85–23, revised 1985) and approved by a local research ethics committee at the University of Nottingham. As previously published (14), immediately after weaning at 3 months, nonidentical twin sheep born to Blue-faced Swaledale mothers (except for 2 sets of male-male twins) were separated and randomly allocated into different physical activity groups, ie, rearing in either a restricted obesogenic barn environment with a stocking rate of 6 sheep per 19 m² or in an unrestricted lean pasture environment with a stocking rate of 6 sheep per 1125 m².

The choice of using a mild induction of obesity was motivated by the hypothesis that a supraphysiological induction of obesity could mask the gender specificity of early-onset responses (14). This experimental protocol resulted in lean females (n = 7), obese females (n = 9), lean males (n = 11) and obese males (n = 9). All animals at each stage were fed to fully meet their metabolic requirements (16) with a mix of hay and concentrated pellets (Manor Farm Feeds). At 17 months of age, between July and August when all females were anestrus, each sheep was surgically implanted with temporary jugular vein catheters and an iv glucose tolerance test (GTT) undertaken, 12 hours after food withdrawal, with 5-mL blood samples withdrawn into EDTA tubes at –10-, –5-, 0-, 10-, 20-, 30-, 60-, 90-, and 120-minute time points after 0.5 g/kg^{–1} glucose perfusion, to enable calculation of area under the curve (AUC) (17). A normal feeding regimen was restored after the GTT had been performed and, 5-mL blood samples were withdrawn from overnight fasted animals into lithium heparin tubes for use in determination of plasma leptin and cortisol levels at 0-, 2-, 4-, 8-, and 24-hour time points after being fed to metabolic requirements. Blood samples were centrifuged at 2500 × g for 10 minutes at 4°C to obtain plasma, which was stored at –80°C until analysis. Animals were humanely euthanized: all adipose depots were weighed, frozen in liquid nitrogen, and stored at –80°C, whereas representative samples of the perirenal-abdominal depot were partially dissected and fixed in 10% formalin for histological processing.

Laboratory analyses

Adipocyte histology

Sections (5 μm) were sledge microtomed (Anglia Scientific) at random from perirenal-abdominal adipose tissue samples, mounted on Superfrost Plus slides (Menzel-Gläser Inc), and stained using hematoxylin and eosin. Sections were visualized through a Leica digital resolution microscope (Leica Microsystems) at ×20 magnification and photographed for analysis (Hamamatsu digital camera). Adipocyte perimeters and areas were measured for all complete adipocytes in the field of vision, using one slide for lean (~40 cells per animal) and two slides for obese sheep (~20 cells per animal). Individual adipocyte perimeters (micrometers) and areas (square micrometers) were determined using Volocity version 5.2.0 image software (PerkinElmer).

Gene expression analysis

Each adipose tissue sample (~1.0 g) was treated with 1 mL TRI reagent (Sigma-Aldrich), and total RNA was extracted with an RNeasy Plus Mini extraction kit (QIAGEN) using an adapted version of the single-step acidified phenol-chloroform homogenization method (18). Sample integrity was confirmed by Nanodrop

Table 1. Food Intake and Whole-Body, Lean, Perirenal-Abdominal, and Omental Fat Mass of Lean and Obese Young Adult Sheep

	Lean Female (n = 7)	Obese Female (n = 9)	Lean Male (n = 11)	Obese Male (n = 9)	Effect of Gender Within Body Weight Group
Relative energy intake, MJ/kg ⁻¹ per 24 h ⁻¹	0.35 ± 0.02	0.38 ± 0.02	0.29 ± 0.02	0.36 ± 0.02	NS
Body weight, kg	49.50 ± 1.04	61.56 ± 2.07 ^a	56.38 ± 2.59	83.84 ± 1.82 ^a	<i>P</i> < .005
Lean mass, kg	39.72 ± 0.87	44.03 ± 1.22	49.09 ± 1.89	62.41 ± 1.30	<i>P</i> < .005
Relative fat mass, %	8.42 ± 1.12	14.31 ± 1.26 ^a	6.77 ± 0.66	12.93 ± 1.06 ^a	NS
Perirenal-abdominal fat, kg	0.31 ± 0.04	1.06 ± 0.13 ^b	0.22 ± 0.02	0.96 ± 0.11 ^a	NS
Omental fat, kg	0.33 ± 0.06	1.62 ± 0.17 ^c	0.26 ± 0.05	1.54 ± 0.16 ^b	NS

Abbreviations: NS, no significant difference. Values are mean ± SEM.

^a *P* < .005, effect of obesity within gender group.

^b *P* < .001, effect of obesity within gender group.

^c *P* < .05, effect of obesity within gender group.

ND-1000 spectroscopy (Nanodrop Technologies). RNA samples were normalized by dilution to 1 ng/μL, reverse transcribed using a Superscript II reverse transcriptase enzyme kit (Invitrogen Ltd), and cDNA was amplified on a Touchgene Gradient thermocycler (Techne Inc).

Quantitative PCR was performed using SYBR Green Taq polymerase master mix (Thermo Scientific) with ovine-specific oligonucleotide primers against a cDNA gene standard curve to verify the efficiency of the reaction and with appropriate negative controls. The mRNA abundance for the following genes was determined: 11β-hydroxysteroid dehydrogenase type 1 (*11BHSD1*) forward, GGCCAGATCCCTGTGCGAT, reverse, AGCGGGATACCTTCTTT; 11β-hydroxysteroid dehydrogenase type 2 (*11BHSD2*) (19); adiponectin forward, ATCAAAGTCTGGAACTCCTATCTAC, reverse, TTGCATTGCAGGC TCAAG; adiponectin receptor forward, GGCAAGTGTGACATCTGGTTTC, reverse, GAAACGGAACCTCTGGAGGTT; glucocorticoid receptor type 2 (*GR*) (20); IL-6 (*IL6*) (21); leptin forward, GGGT

CACTGGTTTGGGACTTCA, reverse, ACTGGCGAGGATCTGTTGGTA; leptin receptor forward, TGAAACCACTGCCTCCATCC, reverse, TCCACTTAAACCATAGCGAATC, AMP-related kinase (*AMPK*) (22); Bcl-2-associated X protein (*BAX*); mammalian target of rapamycin (*mTOR*); monocyte chemoattractant protein 1 (*MCP1*) (23); and toll-like receptor 4 (*TLR4*) (21). Gene expression was determined using GeNorm normalization against multiple reference genes L19 ribosomal protein 19 (*RPL19*) (24) and large ribosomal protein (*RPO*) (25), using GeNorm software version 3.5 (Primer Design Ltd).

Plasma metabolite and hormone analysis

Plasma glucose [inter- and intraassay coefficient of variation (CVs) of 3.5% and 2.3%], nonesterified fatty acid (inter- and intraassay CVs of 3.8% and 1.8%), and triglyceride (inter- and intraassay CVs of 4.2% and 2.9%) concentrations were determined colorimetrically, insulin (inter- and intraassay CVs of 5.4% and 4.4%) and cortisol (inter- and intraassay CVs of 4.6% and 3.5%) by ELISA for which the limits of detection were 0.15 ng/mL and 6.9 nM, respectively (17) and plasma leptin (inter- and intraassay CVs of 7.4% and 4.9%) by RIA, which was specific for ovine leptin, and the limit of detection was 0.8 ng/mL (26).

Statistical analysis

For consistency, all data are expressed as mean ± SEM. Data were subjected to the Kolmogorov-Smirnov test to determine their distribution followed by either ANOVA or Kruskal-Wallis statistical tests, respectively, for parametric and non-parametric data, with applied multiple testing post hoc corrections. Exceptions to this were the adipocyte population frequencies, which were treated with an F test for variance distribution (see Figure 3); time course analyses (see Figure 4), which were treated with a 2-way ANOVA with repeated measures; and correlations, which were treated with linear regression

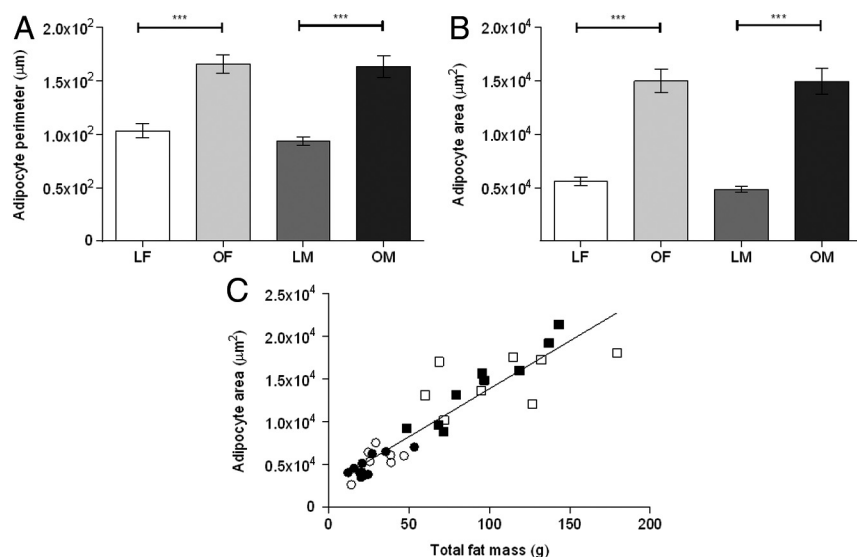


Figure 1. Mean perirenal-abdominal adipocyte perimeter (A) and area (B), for lean (L), obese (O) female (F) and male (M) sheep. Values are mean ± SEM. ***, *P* < .001. C, Positive linear relationship between perirenal-abdominal adipose tissue (PAT) mass and adipocyte area (*r*² = 0.85; *P* < .0001) in lean (open circles) and obese females (open squares) and lean (filled circles) and obese males (filled squares).

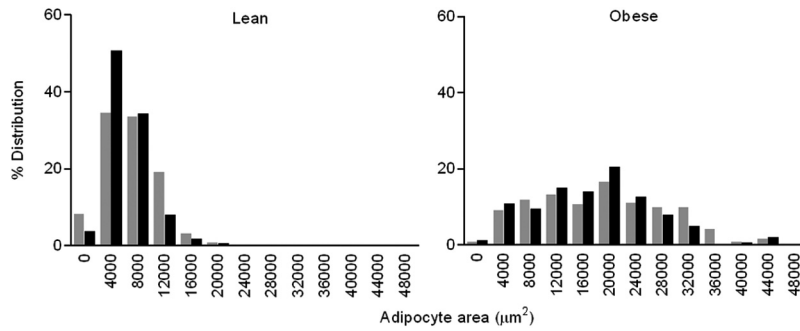


Figure 2. Frequency distribution (percentage) of perirenal-abdominal adipocyte area for lean and obese female (grey bars) and lean male and obese male (black bars) sheep. Variance of distribution between lean groups by F test showed significance ($P = .0002$). Between obese groups there was no statistical significance.

analysis and Pearson's correlation coefficient (see Figure 5). These analyses were performed using PASW version 17.12 statistics software (IBM UK). Statistical significance was accepted with a confidence interval of 95% ($P < .05$).

Results

The effects of sex and juvenile obesity on fat mass and adipose tissue composition

As previously published (14), total body and lean tissues weights were increased by the obesogenic environment, and the increase in both omental and perirenal-abdominal fat mass was similar in both genders, whereas the food intake (per kilogram of body weight) was similar between groups (Table 1). In perirenal-abdominal adipose tissue, this was accompanied by an increase in adipocyte perimeter and area irrespective of sex (Figure 1 and Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>), with the depot size being positively correlated with adipocyte area. Lean males had more

small adipocytes than females, a difference that disappeared with adipocyte enlargement after obesity (Figure 2).

The effect of increased adiposity on basal and stimulated plasma hormone and metabolite concentrations

Fasting insulin increased with obesity in males but not females (Table 2), whereas AUC after the GTT was increased in both genders (Figure 3A). Plasma leptin prior to feeding was unaffected by gender (Table 2), whereas its response to feeding was greater in obese animals (Figure 3B). In contrast, prior to feeding, plasma cortisol was higher in females than males, although this difference was statistically significant only in obese animals (Table 2). The raised plasma cortisol in obese females was maintained throughout the 24-hour sampling period (Figure 3C). There were no significant differences in plasma glucose, nonesterified fatty acids (NEFAs), or triglycerides with obesity or between genders (Table 2). For all animals, the total fat mass was positively correlated with leptin response to feeding, whereas visceral fat mass was positively correlated with cortisol in females only (Figure 4).

Effect of gender and obesity on genes regulating metabolic, inflammatory, apoptotic, and energy-sensing pathways and glucocorticoid action

In perirenal-abdominal fat, gene expression for adiponectin was higher in obese males than females, whereas the abundance of transcripts for its receptor, together with

Table 2. Endocrine and Metabolic Responses Under Fasting and Stimulated Conditions in Lean and Obese Young Adult Sheep

	Lean Female (n = 7)	Obese Female (n = 9)	Lean Male (n = 11)	Obese Male (n = 9)	Effect of Gender Within Body Weight Group
Fasting plasma insulin, ng/mL	0.09 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.14 ± 0.02 ^a	NS
Insulin AUC, ng/mL	15.2 ± 3.7	48.9 ± 13.8	30.6 ± 13.4	71.5 ± 7.3 ^a	NS
Fasting plasma leptin, ng/mL	2.61 ± 0.45	3.95 ± 0.44	2.97 ± 0.17	4.35 ± 0.52	NS
Leptin AUC, ng/mL	67 ± 7	125 ± 12 ^b	63 ± 4	130 ± 16 ^c	NS
Fasting cortisol, nmol/L	31.7 ± 6.3	54.6 ± 9.7	19.2 ± 9.7	22.6 ± 4.2	$P < .005$
Cortisol AUC, nmol/L	579 ± 114	1326 ± 252 ^b	377 ± 48	504 ± 58	$P < .005$
Fasting glucose, mmol/L	3.04 ± 0.18	3.08 ± 0.14	2.94 ± 0.10	3.03 ± 0.11	NS
Fasting NEFAs, mmol/L	0.43 ± 0.06	0.49 ± 0.06	0.40 ± 0.03	0.50 ± 0.06	NS
Fasting triglycerides, mmol/L	0.35 ± 0.06	0.31 ± 0.03	0.40 ± 0.05	0.38 ± 0.07	NS

Abbreviation: NEFA, nonesterified fatty acid; NS, no significant difference. Values are mean ± SEM.

^a $P < .05$, effect of obesity within gender group.

^b $P < .005$, effect of obesity within gender group.

^c $P < .001$, effect of obesity within gender group.

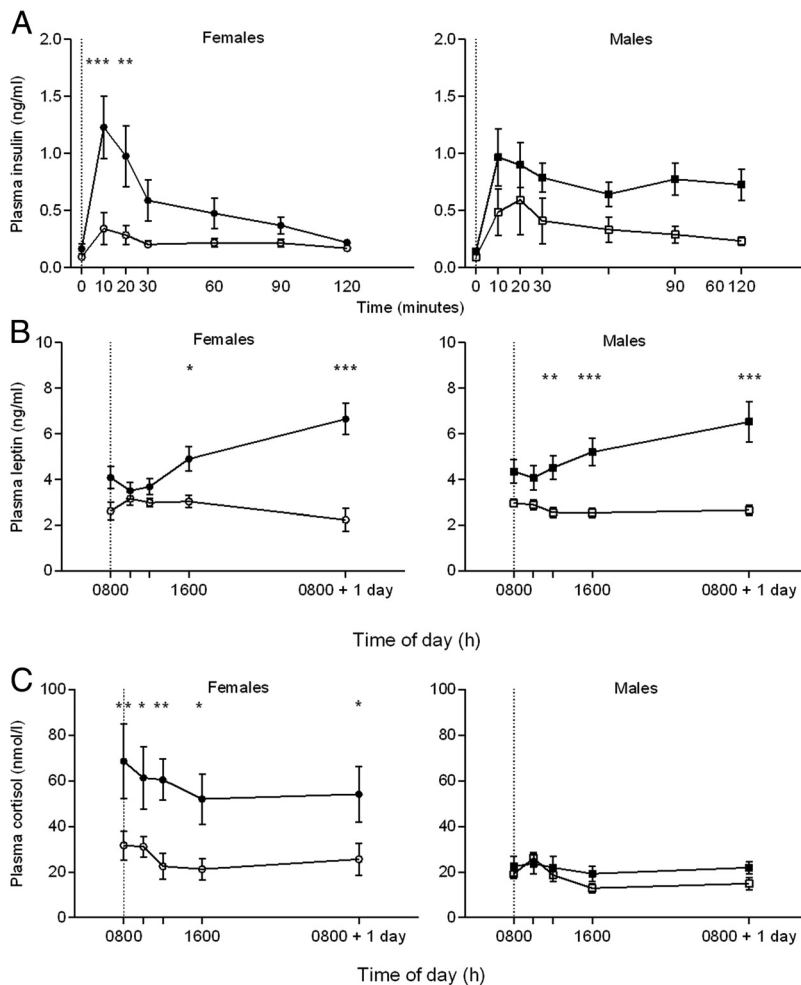


Figure 3. Time course of changes in plasma insulin during a GTT (A), plasma leptin (B), and cortisol (C) in response to feeding in lean (open circles) and obese female (filled circles) and lean (open squares) and obese male (filled squares) sheep. Dotted line represents glucose infusion time or feeding time point. Values are mean \pm SEM with significant differences between lean and obese animals of the same gender indicated. *, $P < .05$; **, $P < .005$; ***, $P < .001$.

those of leptin and its receptor, was unaffected by obesity and gender (Table 3). For omental fat, however, the mRNA abundance for leptin was greater in females than males, a difference that was amplified with obesity [female: lean, 0.07 ± 0.01 ; obese, 0.48 ± 0.01 ($P < .001$); male: lean, 0.05 ± 0.01 ; obese, 0.24 ± 0.04 arbitrary units (a.u.) (male vs female, $P < .05$)]. In contrast, in omental fat, gene expression for adiponectin was raised with obesity but unaffected by gender [female: lean, 0.21 ± 0.03 ; obese, 0.34 ± 0.03 ($P < .05$); male: lean, 0.21 ± 0.04 ; obese, 0.34 ± 0.04 a.u. (lean vs obese $P < .05$)], but mRNA for leptin and adiponectin receptors and *IL6* was below the limits of detection in this depot. When mRNA markers of inflammation within perirenal-abdominal adipose tissue were considered, each gene measured was highly abundant. Furthermore, *IL6* gene expression was greater in lean but not obese males compared with females (Table 3). In contrast, mRNA abundance for both *TLR4*

and *MCP1* were raised with obesity in males but not females in perirenal-abdominal but not omental fat (eg, *TLR4*: female, lean 0.31 ± 0.05 ; obese, 0.42 ± 0.04 ; male: lean, 0.31 ± 0.02 ; obese, 0.39 ± 0.06 a.u.).

The mRNA abundance for the *GR* was similar between genders, irrespective of body weight, in both fat depots examined, although it was raised with obesity in the perirenal-abdominal depot in females (Table 3), and in the omental depot in males [female: lean 0.39 ± 0.05 ; obese 0.51 ± 0.03 ; male: lean 0.37 ± 0.04 ; obese 0.55 ± 0.08 a.u. ($P < .05$)]. In contrast, *11 β HSD1* mRNA abundance was similar between all groups in the omental depot (female: lean 0.46 ± 0.08 ; obese 0.35 ± 0.03 ; male: lean 0.40 ± 0.08 ; obese 0.36 ± 0.04 a.u.) but in the perirenal-abdominal depot was lower in obese males than females (Table 3). Gene expression for *11 β HSD2* was below the limits of detection in the omental fat, but in the perirenal-abdominal, the depot was higher in lean males than females (Table 3).

There were marked differences between genders and fat depots in the genes involved in apoptosis (*BAX*) and energy sensing (*AMPK* and *mTOR*) (Table 4). In perirenal-abdominal fat, the expression of each of these genes was greater only in obese females than lean females, whereas in omental fat, an effect of obesity was found in the males but not females.

Discussion

A major finding from our study is that although obesity promoted comparable increases in total fat mass and adipocyte size in both sexes, females alone were characterized as showing a pronounced increase in plasma cortisol with obesity. Raised cortisol is an established measure of physiological stress (27), suggesting that the higher plasma cortisol previously reported in adolescent females (28) reappears with obesity. This could therefore represent an adaptive response that protects females from the early onset of the adverse effects from obesity (14). Similarly, the

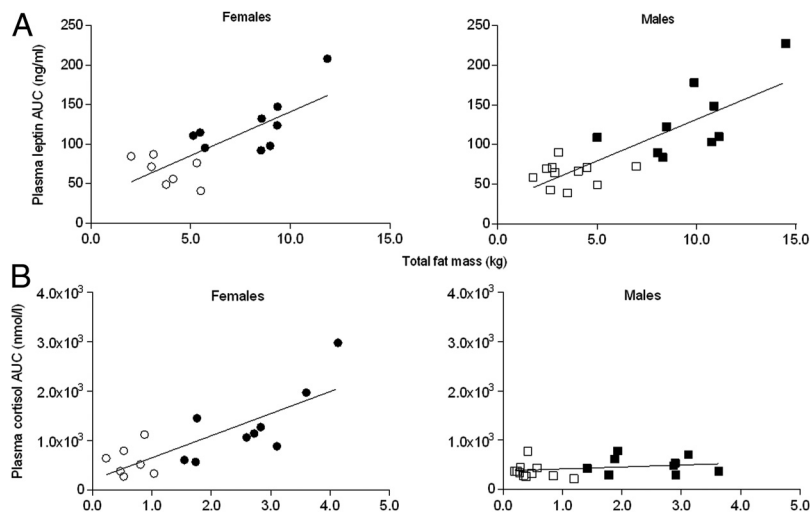


Figure 4. Positive linear relationship between total fat mass and plasma leptin AUC response to feeding in lean and obese females ($r^2 = 0.58$; $P < .0006$) and lean and obese males ($r^2 = 0.68$; $P < .0001$) (A) and total visceral fat mass and plasma cortisol AUC response to feeding in female ($r^2 = 0.63$; $P < .001$) (B) but not in male sheep ($r^2 = 0.07$; not significant). Analyzed groups represented by lean (open circles) and obese females (filled circles) and lean (open squares) and obese males (filled squares).

impaired insulin response to a glucose challenge found in obese males could be related to their failure to increase plasma cortisol with obesity, ultimately placing them at increased risk of diabetes.

Overall, in accord with epidemiological evidence (29), only obese males had elevated plasma insulin, indicating their increased sensitivity to developing insulin resistance. These gender differences in insulin responsiveness have been attributed to sex hormones because, in humans, they disappear after the menopause when estrogen declines at the expense of androgens. This hormonal shift is suspected to be a major determinant behind the subsequent rise in visceral fat

mass in females, which can lead to insulin resistance (30). The exact mechanism behind a sex hormone-mediated retardation in the rate of development of insulin resistance in obesity observed in females is currently unknown but could potentially include a change in cortisol action. The effect of gender in response to obesity on HPA axis activation and the resulting stress response are likely to be due, in part, to differences in the circulating gonadal sex steroid hormone milieu. Estrogen, acting through the central nervous system, can amplify HPA function, whereas T has the opposite effect (31). It is possible therefore that juvenile-onset obesity promotes estrogenic effects on the HPA axis in females. This results in raised cortisol, an outcome not observed in the obese males, which conversely may be due to suppressive effects of T (32).

Changes in adipose tissue deposition and physiology

We have shown that both sexes exhibit an increase in total fat mass after early life exposure to an obesogenic environment. As expected, lean and obese female animals were fatter than the males, results that are consistent between species including humans (33). In obese humans, total fat mass is greater than 32% and greater than 25%

Table 3. Effect of Gender and Juvenile Obesity on Gene Expression Markers of Adipose Tissue Metabolism, Inflammation, and Glucocorticoid Action in Perirenal-Abdominal Adipose Tissue of Young Adult Sheep

Pathway Gene	Accession Number	Lean Female (n = 7)	Obese Female (n = 9)	Lean male (n = 11)	Obese Male (n = 9)	Effect of Gender Within Body Weight Group
Adipose tissue metabolism						
Leptin	NM_173928.2	0.14 ± 0.06	0.27 ± 0.06	0.18 ± 0.10	0.25 ± 0.04	NS
Leptin receptor	NM_001009763.1	0.22 ± 0.02	0.27 ± 0.06	0.24 ± 0.07	0.18 ± 0.05	NS
Adiponectin	XM_004003053	0.10 ± 0.02	0.09 ± 0.03	0.24 ± 0.14	0.46 ± 0.09	$P < .05$
Adiponectin receptor	XM_004007604	0.27 ± 0.05	0.50 ± 0.07	0.41 ± 0.05	0.36 ± 0.06	NS
Inflammation						
<i>IL6</i>	NM_001009392	0.19 ± 0.02	0.34 ± 0.10	0.35 ± 0.05	0.36 ± 0.07	$P < .05$
<i>MCP1</i>	NM_174006	0.08 ± 0.02	0.24 ± 0.10	0.06 ± 0.01	0.26 ± 0.10 ^a	NS
<i>TLR4</i>	NM_001135930.1	0.32 ± 0.03	0.41 ± 0.04	0.22 ± 0.02	0.35 ± 0.04 ^a	NS
Glucocorticoid action						
<i>11BHS1</i>	NM_001009395.1	0.16 ± 0.04	0.14 ± 0.02	0.10 ± 0.02	0.07 ± 0.01	$P < .05$
<i>11BHS2</i>	NM_174642.2	0.29 ± 0.03	0.43 ± 0.03	0.53 ± 0.08	0.70 ± 0.20	$P < .05$
<i>GR</i>	NM_001114186.1	0.38 ± 0.03	0.55 ± 0.06 ^a	0.31 ± 0.03	0.39 ± 0.03	NS

Abbreviations: *GR*, glucocorticoid receptor (type 2); *11BHS1*, 11B hydroxysteroid dehydrogenase type 1; *11BHS2*, 11B hydroxysteroid dehydrogenase type 2; *IL6*, IL-6; *MCP1*, monocyte chemoattractant protein 1; NS, no significant difference; *TLR4*, Toll-like receptor 4. Values are mean ± SEM.

^a $P < .05$, effect of obesity within gender group.

Table 4. Contrasting Effect of Gender and Juvenile Obesity on Gene Expression for Markers of Apoptosis and Energy Sensing Between Perirenal-Abdominal and Omental Adipose Tissue of Young Adult Sheep

Pathway Gene	Accession Number	Lean Female (n = 7)	Obese Female (n = 9)	Lean Male (n = 11)	Obese Male (n = 9)	Effect of Gender Within Body Weight Group
Perirenal-abdominal depot						
Apoptosis						
<i>BAX</i>	XM_004015364.1	0.35 ± 0.03	0.56 ± 0.06 ^b	0.41 ± 0.05	0.57 ± 0.06	NS
Energy sensing						
<i>AMPK</i>	NM_001112816	0.25 ± 0.02	0.55 ± 0.08 ^a	0.23 ± 0.02	0.33 ± 0.04	<i>P</i> < .05
<i>mTOR</i>	NM_001145455	0.37 ± 0.03	0.57 ± 0.06 ^a	0.43 ± 0.03	0.52 ± 0.04	NS
Omental depot						
Apoptosis						
<i>BAX</i>	XM_004015364.1	0.23 ± 0.05	0.33 ± 0.07	0.36 ± 0.07	0.78 ± 0.07 ^a	<i>P</i> < .05
Energy sensing						
<i>AMPK</i>	NM_001112816	0.26 ± 0.05	0.55 ± 0.07 ^b	0.23 ± 0.02	0.41 ± 0.03 ^b	NS
<i>mTOR</i>	NM_001145455	0.28 ± 0.09	0.44 ± 0.08	0.41 ± 0.07	0.80 ± 0.17 ^b	NS

Abbreviations: AMPK, AMP-related kinase; BAX, Bcl-2-associated X protein; mTOR, mammalian target of rapamycin; NS, no significant difference. Values are mean ± SEM.

^a *P* < .01, effect of obesity within gender group.

^b *P* < .05, effect of obesity within gender group.

of body weight for females and males, respectively (34). Yet in the present study, despite the 2-fold increase of total body fat including central depots, obese females had approximately 15% and males had approximately 13% total fat mass. We therefore suggest that our study's model of juvenile-onset obesity, which is mediated only through restricted physical activity, resulted in a moderate obesity, which was still sufficient to cause significant changes in adiposity and metabolic homeostasis.

The 3-fold enlargement in perirenal adipocytes with obesity, irrespective of gender, indicates that depot enlargement was a consequence of adipocyte hypertrophy rather than hyperplasia, a finding also observed in humans (35). Population distribution analysis identified that lean males had smaller perirenal adipocytes than females, but after exposure to an obesogenic environment, this difference was removed. These results reflect gender differences in responsiveness of visceral adipocytes, with males having a larger storage capacity for triglycerides and lipids, potentially encouraging increased central deposition with early-onset obesity. In adult humans, sc adipocytes are larger and have increased triglyceride storage compared with intraabdominal adipocytes, and in females, adipocytes from this depot are more active, playing a greater role in the metabolic profile (36).

Differences in gender response to obesity in metabolic and inflammatory pathways between central fat depots

The adaptive response to raised cortisol in females did not appear to extend to adipose tissue because there were only modest differences in gene expression responsiveness

between genders and the magnitude of overall response to obesity was similar. In human obesity, hypercortisolism is a prominent feature, both systemically and intracellularly (37), when cortisol production and clearance are simultaneously elevated, but plasma concentrations are unaffected (38). A positive relationship between elevated cortisol clearance and increased fat mass is present in females (39), suggesting their adipose tissue has a greater capacity to bind cortisol acting through its receptor. This divergent sensitivity between sexes is supported by the gender-specific relationship we observed between central fat mass and plasma cortisol response to feeding.

Adult onset obesity also affects glucocorticoid metabolism with raised gene expression for both *GR* and *11BHSD1*, which is greater in visceral compared with sc adipose tissue (40) when increased plasma cortisol is positively correlated to visceral obesity (40). Surprisingly, in our study, gene expression for *11BHSD1* was reduced with obesity in perirenal-abdominal, but not omental, fat, and this would be predicted to reduce local cortisol synthesis (42). Chronic exposure to elevated cortisol and concomitant activation of the HPA axis can contribute to the pathogenesis and development of abdominal obesity (43). Despite a divergent relationship between genders found in the present study, the increase in fat mass with obesity was similar as were the metabolic consequences in terms of changes in plasma leptin. Raised plasma and tissue cortisol can, however, suppress inflammation via inhibition of immune cell function (44) and, as a consequence, can be protective against the chronic low-grade inflammation that accompanies obesity (45) as we have previously found in the kidneys of obese females (14).

Although fat mass is a primary determinant of plasma leptin across species (46), positive correlations between hyperleptinemia, hyperlipidemia, insulin resistance, and hypertension have been shown to be independent of total adiposity (47). We saw no relationship between plasma leptin and insulin but a positive correlation between total fat mass and plasma leptin, irrespective of gender. It is possible that a combination of early-onset obesity, together with the relatively moderate nature of the obesogenic challenge, contributed to raised plasma leptin in an attempt to control appetite and energy balance and thus further weight gain. Furthermore, leptin gene transcription and protein synthesis are both affected by gross changes in energy intake (48), although this response can be adipose depot and gender specific (49), with an enhanced leptin mRNA response in sc, compared with visceral adipose tissue (50). In accord with this proposal, we found raised leptin gene expression only in omental, but not perirenal-abdominal, adipose tissue with obesity, and this response was greater in females than males. However, in perirenal-abdominal, but not omental, adipose tissue, gene expression for both *MCP1* and *TLR4* were raised with obesity in males but not females. This is also indicative of an enhanced inflammatory related response (51), which may contribute to insulin resistance (52, 53).

Interestingly, although *11BHSD2* mRNA was undetectable in omental fat, the increased perirenal-abdominal fat mass with age was negatively correlated with *11BHSD2* gene expression, and this could reflect an increase in the size of this depot during juvenile life (54). In the present study, we found higher gene expression in lean males than females with no effect of obesity. One explanation for the different responsiveness between omental and perirenal-abdominal fat depots could reside in their divergent developmental origins, with the perirenal-abdominal depot being the largest in the fetal sheep (55) in which it is primarily characterized as being brown (56). Then during postnatal life, it transforms into a white or beige depot, whereas, in marked contrast, the omental fat depot does not appear until after birth and is purely a white fat depot (57). The extent to which these divergent developmental profiles could contribute to gender-specific changes in glucocorticoid action remains to be established.

Intracellular glucocorticoid binding to the *GR* is a pathway involved in the inhibition of cytokine transcription and production (58), and the increase in both cortisol and *GR* within perirenal-abdominal adipose tissue could have acted together to prevent any change in the expression of inflammatory genes with obesity in females. In accord with this proposal, gene markers for both apoptosis and energy sensing were raised with obesity only in females within their perirenal-abdominal, but not omental, depots. For males, however, the opposite response was seen,

and this may, potentially, reflect differences in epigenetic regulation (41). The extent to which the changes we have observed for gene expression are translated directly into protein remains to be established, although it is well recognized that undertaking this type of analysis on ovine tissues remains a challenge due to the scarcity of reliable and fully validated antibodies (56).

In conclusion, sex has a major influence on both the endocrine and adipocyte responses to juvenile-onset obesity. Consequently, males show a more pronounced response than females, resulting in amplified inflammatory gene expression, which is predicted to place them at greater risk of subsequent metabolic dysfunction. Females appear to be protected from such a response as a consequence of raised cortisol and greater adipocyte sensitivity in a depot-specific manner. These findings further explain the dimorphic gender response to obesity and its comorbidities.

Acknowledgments

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This work was funded and supported by The British Journal of Anaesthesia, the Royal College of Anaesthetists; the European Union Sixth Framework Program for Research and Technical Development of the European Community, The Early Nutrition Programming Project (Grant FOOD-CT-2005-007036); and the Nutricia Research Foundation.

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Disclosure Summary: The authors have no conflicts of interest to disclose.

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