Life Sciences 337 (2024) 122344

Contents lists available at ScienceDirect

Life Sciences

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

Maternal obesity impacts fetal liver androgen signalling in a sex-specific manner

Ashley S. Meakin [a,*], Peter W. Nathanielsz [b], Cun Li [b], Vicki L. Clifton [c], Michael D. Wiese [d], Janna L. Morrison [a,*]

[a] Early Origins of Adult Health Research Group, Health and Biomedical Innovation, Clinical & Health Sciences, University of South Australia, Adelaide, SA, Australia
[b] Department of Animal Science, University of Wyoming, Laramie, WY, USA
[c] Mater Medical Research Institute, The University of Queensland, Brisbane, QLD, Australia
[d] Centre for Pharmaceutical Innovation, Clinical & Health Sciences, University of South Australia, Adelaide, SA, Australia

ARTICLE INFO

Keywords:
Androgens
Maternal obesity
Fetal liver
Cytochrome P450

ABSTRACT

Background: Maternal obesity (MO) increases fetal androgen concentrations, the prevalence of macrosomia, and predisposes offspring to metabolic dysfunction in later life, especially males. These risks may be, in part, the result of increased liver-specific androgen signalling pathway activity in utero. Androgen signalling activity can be suppressed by androgen metabolism via cytochrome P450 (CYP) isoenzymes (CYP2B6, CYP3A) or through inhibition of the full-length androgen receptor (AR-FL) via the antagonistic isoform, AR-45. We hypothesised MO impairs CYP enzyme activity and AR-45 expression in male fetal livers, thereby enhancing activity of androgen signalling pathways.

Methods: Nine months prior to pregnancy, nulliparous female baboons were assigned to either ad libitum control or high fat diet. At 165 day (d) gestation (term, 180 d) fetal liver was collected (n = 6/sex/group). CYP activity was quantified using functional assays; subcellular AR expression was measured using Western blot.

Results: CYP2B6 and CYP3A activity, and nuclear expression of AR-45, was reduced in MO males only. Nuclear AR-45 expression was inversely related with fetal body weight of MO males only.

Conclusions: Reduced CYP2B6 and CYP3A activity in conjunction with decreased nuclear AR-45 expression may enhance liver androgen signalling in males from MO pregnancies, thereby increasing the risk of macrosomia, as well as metabolic dysfunction in later life.

1. Introduction

Obesity during pregnancy (maternal obesity (MO)) is a global health burden [1] that has consequences for both pregnant individuals and the developing fetus, as well as increasing offspring risk of liver-specific dysfunction and disease in later life [2]. Interestingly, a sexually dimorphic response to MO has been reported such that the risk of programmed liver-specific diseases is greater in males than females [3,4]. In adult males liver disease is associated with lower circulating androgen (testosterone, dihydrotestosterone (DHT)) concentrations, whereas the opposite is observed in adult females [5]; however, increased androgen concentrations in utero can contribute to programmed liver dysfunction and disease in later life in both sexes [6–8]. Thus, alterations to fetal liver-specific androgen signalling may be an important factor that contributes to the sexual dimorphism of programmed liver disease in offspring of MO pregnancies. Indeed, MO is associated with increased maternal and fetal plasma androgen concentrations, but only in male-bearing pregnancies [9]; however, it is unclear how MO impacts fetal liver-specific androgen signalling between the sexes.

Androgen signalling is a complex process that is initiated by the interaction of bioactive androgen ligands (testosterone and DHT) with androgen receptors (ARs): through this interaction, the androgen-mediated transcriptome is tightly regulated [10]. Higher testosterone and DHT concentrations can enhance AR activity and subsequent downstream signalling pathways primarily involved in growth. For example, reduced metabolism of testosterone to inactive metabolites via cytochrome P450 (CYP) 2B6 and CYP3A enzymes increases testosterone concentration, increases AR activity, and thus enhances cellular growth

* Corresponding authors at: Early Origins of Adult Health Research Group, Health and Biomedical Innovation, UniSA: Clinical and Health Sciences, University of South Australia, GPO Box 2471, Adelaide, SA 5001, Australia.
E-mail addresses: ashley.meakin@unisa.edu.au (A.S. Meakin), Janna.Morrison@unisa.edu.au (J.L. Morrison).

https://doi.org/10.1016/j.lfs.2023.122344
Received 12 September 2023; Received in revised form 20 November 2023; Accepted 6 December 2023
Available online 10 December 2023

and proliferation [11,12]. Although increased activity of these CYPs may negatively impact the activity of androgen signalling, testosterone is also metabolised by 5α-reductase type 1 (SRD5A1) to DHT, a metabolite with higher binding affinity to AR and increased half-life compared with testosterone, which therefore increases the activity of the androgen signalling axis [13]. Expression of CYP2B6, CYP3A, and SRD5A1 has previously been reported in the human fetal liver [14,15], but it is unclear how complications of pregnancy such as MO affects their activity between the sexes and how this impacts androgen signalling pathways.

Canonical genomic androgen signalling is mediated via the full-length AR (AR-FL) protein; however, through alternative splicing of the AR gene, multiple isoforms can be produced [10]. This phenomenon is an important biological process that modulates androgen signalling in response to intracellular homeostatic shifts, such as those observed in the fetal liver in response to MO. Thus, a changing landscape of liver AR protein isoforms may modulate androgen signalling in the fetal liver, which could have consequences for organ development and function. For example, the C-terminally truncated isoform (AR-v) transcriptionally regulates AR-FL-mediated target genes and regulates a distinct transcriptomic profile in the absence of androgens [16]. In contrast, the N-terminally truncated isoform AR-45 forms a non-responsive heterodimer with AR-FL resulting in suppression of androgen signalling [17,18]. Sex differences in androgen signalling are reported [19–22]; however, no study to date has examined the impact of MO on sex-specific fetal liver androgen signalling.

There are several established animal models of MO during pregnancy and its impact on fetal metabolic outcomes. In the current study, we have used a well-characterised baboon model to investigate the impact of MO on fetal liver androgen signalling and whether sex impacts these responses [23]. Indeed, previous work on this cohort reported an upregulation of *AR* and altered expression of several AR-mediated transcripts in response to MO; however, this previous study did not examine the impact fetal sex has on these molecular responses. We hypothesised that MO would result in males upregulating factors that favour a pro-androgenic environment within the liver, whereas females would suppress liver-specific androgen signalling.

2. Methods

All animal procedures were approved by the Texas Biomedical Research Institute (TBRI) Institutional Animal Care and Use Committee (IACUC) and conducted in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) international-approved facility. The experimental design was informed by the ARRIVE guidelines [24] and the 3Rs (https://www.nhmrc.gov.au/research-policy/ethics/animal-ethics/3rs).

2.1. Animal model

Baboons were maintained in groups of up to 16 in custom-built outdoor facilities allowing full socialisation and free movement. Healthy, non-pregnant female nulliparous baboons of similar weight, body dimensions and age were randomly assigned to either an ad libitum regular/normal diet (CD, total energy content 3.07 kcal/g; n = 12) or an ad libitum combination of the CD and high-fat/high-fructose diet (HFHED, total energy content 4.03 kcal/g; n = 12) with free access to a sugar-containing drink (Kool Aid) at least 9 months pre-pregnancy [25].

2.2. Caesarean sections and tissue collection

Pregnant baboons underwent Caesarean section at 165 day (d) gestation (term ~180 d) using standard sterile surgical techniques [26]. Briefly, baboons were pre-medicated with ketamine hydrochloride (Fort Dodge Animal Health, Fort Dodge, USA; 10 mg/kg intramuscularly), and maintained at a surgical plane of anaesthesia with isoflurane (2 %). After hysterectomy, the fetuses were exsanguinated under general anaesthesia as approved by the American Veterinary Medical Association Panel on Euthanasia [23]. Morphometric measurements were collected, and tissue samples obtained immediately (CD, male n = 6, female n = 6; HFHED, male n = 6, female n = 6). The right lobe of the fetal liver was snap frozen in liquid nitrogen and stored at −80 °C. Buprenorphine hydrochloride (Hospira, Inc., Lake Forest, USA; 0.015 mg/kg/day, 2 daily doses for 3 d) was administered for postoperative maternal analgesia [26]. After recovery from anaesthesia, baboons were individually caged for the initial post-operative period and then group-housed for 90 d with a vasectomised male to prevent pregnancy before the surgical site was completely healed.

2.3. Microsome extraction

Fetal liver microsites were extracted using differential centrifugation as previously described [27–29]. Briefly, frozen tissue samples of ~250 mg were homogenised (Tissue Lyser, QIAGEN, Germany) in 600 μl of homogenising buffer (1.15 % KCl, 1 mM EDTA, pH 7.4). The homogenate was centrifuged at 9000*g* for 20 min at 4 °C. The supernatant was then transferred and centrifuged at 16,000*g* for 60 min at 4 °C. Supernatant was then discarded and the microsomal pellet was resuspended in buffer (100 mM potassium phosphate buffer, 20 % glycerol, pH 7.4). The amount of protein in each extraction was determined using a Micro Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce, Thermo Fisher Scientific Inc., Rockford, USA) with bovine serum albumin (2 mg/ml) to generate a standard curve. Microsomal extractions were stored at −80 °C until enzymatic assays.

CYP activity assay.

To quantify the activity of androgen metabolising enzymes, the rate of testosterone to 16β-hydroxytestosterone (CYP2B6), 6β-hydroxytestosterone (CYP3A), estradiol (CYP19A1), or dihydrotestosterone (SRD5A1) was determined by adding 350 μM testosterone to 70 μg of microsomal protein, 10 mM NADPH, and assay buffer (50 mM phosphate buffer, 2 mM magnesium chloride, pH 7.4) to a total reaction volume of 100 μl. Reactions were stopped by adding 100 μl methanol containing 100 ng/ml 6β-hydroxytestosterone-d3 (Toronto Research Chemicals, Canada). Incubation mixtures were centrifuged at 12,000*g* for 10 min at 4 °C and the supernatant transferred to a liquid chromatography tandem mass spectrometry (LC-MS/MS) vial for subsequent analysis.

2.4. Liquid chromatography tandem mass spectrometry (LC-MS/MS)

Quantitation of testosterone metabolites were determined using LC-MS/MS (SCIEX 6500 Triple-Quad (SCIEX, US) with Shimadzu Nexera XR (Shimadzu, Japan)). Incubation mixtures were transferred to a 0.4 ml autosampler vial and then 2 μl of sample was injected onto an ACQUITY BEH C18 Column (130 Å, 1.7 μm, 2.1 mm × 100 mm (Waters Corp., US)). Mobile phases were 0.2 % formic acid in water (A) and 0.2 % formic acid in 100 % acetonitrile (B). Flow rate was 0.2 ml/min and mobile phase B was initially 15 % for 1 min, increased to 40 % over 1.5 min, then to 50 % over 6 min, and then to 98 % over 3.5 min. The gradient was held at 98 % for 0.8 min, after which it returned to 15 % over 0.2 min held at 15 % for 2 min prior to injection of the next sample.

2.5. Quantification of fetal liver protein abundance

Tissue subcellular fractionation and Western blotting was performed as previously described [19,30–32]. Subcellular lysates (containing 50 μg protein) were electrophoresed via SDS-PAGE [33] and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Hybond ECL, GE Healthcare, Australia). Membranes were blocked in 5 % BSA in TBS with 1 % Tween (TBS-T) for 1 h at room temperature, and then incubated with affinity purified polyclonal rabbit anti-AR (1:1000, Cell Signalling, USA Cat no. 54653; 1:100, Abcam, UK Cat no. ab74272) antibodies. The appropriate secondary antibody (goat anti-rabbit;

1:20,000) was applied for 1 h. Membranes were subsequently probed with anti-β actin (1:4000, Bethyl laboratories, USA Cat no. A300-491 A) as loading control. SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) was used to detect reactive bands by enhanced chemiluminescence. Western blots were imaged using ImageQuant LAS 4000 (GE Healthcare) and protein abundance was determined by densitometry using Image Quant software (GE Healthcare) and initially normalised to their respective loading control. As per previously published work examining steroid receptor isoform protein data [34], protein expression of each AR isoform was normalised to matched AR isoform expression in a pooled sample, which allowed for comparisons between membranes.

2.6. Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc., USA). Initial normality testing was conducted on data within each study group. For data where at least one of the groups was not normally distributed (Shapiro-Wilk P-value < 0.05), non-parametric tests including Mann-Whitney U test or Kruskal-Wallis with Dunn's multiple comparisons post-hoc analysis was performed. For data where all groups were normally distributed (Shapiro-Wilk P-value > 0.05), two-way ANOVA (factors = diet and sex) with Tukey's post-hoc analysis was performed. Potential outliers were detected using Grubbs' test. Data are presented as mean ± SEM, unless stated otherwise. The alpha level was 0.05.

3. Results

3.1. Maternal and fetal morphometric measurements

Maternal weight was higher in MO compared to controls 30 d preconception and at tissue collection (Table 1). Maternal weight change across pregnancy was unaffected by diet or fetal sex. Maternal glucose concentrations were increased in MO compared to control animals irrespective of fetal sex (Table 1).

Diet did not affect placental weight (PW), fetal body weight (BW), BW:PW, fetal brain weight or liver weight (Table 1). In addition, diet did not affect relative brain or liver weights. Male fetal body weight was increased compared with females irrespective of diet ($P = 0.049$; Table 1). MO increased female relative liver weight compared to males ($P = 0.0024$). Fetal glucose concentrations were unaffected by diet or fetal sex (Table 1).

3.2. MO impacts fetal liver testosterone metabolism in a sex-specific manner

CYP2B6 and CYP3A activity were lower in males from MO pregnancies (59 % and 55 % reduction, respectively), but there was no difference in females (Fig. 1A). SRD5A1 and placental-specific CYP19A1 activity was not affected by diet or sex (Fig. 1A & B).

3.3. Liver AR isoform expression is altered by MO and sex

We identified AR-FL (110 kDa), AR-v (~80 kDa) and AR-45 (45 kDa) in cytoplasmic and nuclear fractions of female and male fetal liver samples (Fig. 2A). Additional immunoreactive protein bands were identified in both subcellular fractions but have not been confirmed as AR isoforms and thus were not analysed.

Cytoplasmic AR-FL was increased in females when compared to males, irrespective of diet ($P = 0.0002$; Fig. 2B), but nuclear AR-FL expression was not impacted by diet or sex. In males only, cytoplasmic AR-v was reduced in response to diet (Tukey's post-hoc $P = 0.0035$; Fig. 2B). Nuclear AR-v expression was reduced in response to diet independent of sex ($P = 0.0021$; Fig. 2C). AR-45 expression was reduced in MO males when compared with control males in the cytoplasm (Tukey's post-hoc $P = 0035$; Fig. 2B) and nucleus (Tukey's post-hoc $P = 0.0162$; Fig. 2C). In addition, AR-45 expression was lower in MO males when compared with MO females in the nucleus only (Tukey's post-hoc $P = 0.0002$; Fig. 2C).

3.4. Subcellular AR isoform expression is impacted by diet in a sex-specific manner

In males only, AR-FL was increased in the nucleus when compared with the cytoplasm ($P < 0.0001$; Fig. 3A). No change in AR-FL expression was reported between subcellular compartments in females. Both AR-v and AR-45 nuclear expression was higher than cytoplasmic expression in males, independent of diet (Fig. 3A); however, MO males had reduced cytoplasmic and nuclear AR-v and AR-45 expression when compared with control males ($P < 0.0001$; Fig. 3A). In females, nuclear AR-v and AR-45 expression was higher than cytoplasmic expression independent of diet ($P < 0.0001$; Fig. 3B).

3.5. Sex-specific differences in subcellular isoform expression

Given the known antagonistic role of AR-45, we wanted to compare the distribution of AR-45 to AR-FL and determine whether changes in their expression was impacted by diet and/or sex. In males, cytoplasmic AR-45 was significantly higher than cytoplasmic AR-FL ($P < 0.0001$;

Table 1
Maternal and fetal morphometric measurements.

	Male		Female		P_{Diet}	P_{Sex}	P_{Intx}
	CD	HFHED	CD	HFHED			
Maternal measurements							
Maternal weight (kg; 30 d preconception)	17.8 ± 0.6	20.1 ± 0.6	17.1 ± 0.4	19.6 ± 1.0	0.0026	0.3954	0.8961
Maternal weight (kg; 160 d GA weight)	19.1 ± 1.2	20.3 ± 0.9	18.3 ± 0.5	21.3 ± 0.7	0.0229	0.8838	0.3071
Maternal weight change (kg)	1.7 ± 0.9	0.2 ± 0.7	1.2 ± 0.5	1.7 ± 0.4	0.4493	0.4069	0.1197
Maternal glucose (mg/dl)	59.67 ± 12.49	137.33 ± 32.90	57.00 ± 9.74	85.50 ± 18.54	0.0174	0.1983	0.2440
Fetal measurements							
Fetal body weight (BW; g)	802.93 ± 40.90	855.50 ± 15.22	776.5 ± 41.4	746.4 ± 23.9	0.7319	0.0492	0.2161
Fetal heart weight (g)	5.28 ± 0.30	5.42 ± 0.23	4.52 ± 0.44	4.98 ± 0.30	0.3669	0.0808	0.6239
Fetal liver weight (g)	24.09 ± 1.37	24.87 ± 0.98	23.89 ± 1.52	26.35 ± 0.77	0.2003	0.6091	0.5017
Fetal brain weight (g)	86.06 ± 4.24	84.70 ± 2.99	83.61 ± 3.39	76.87 ± 2.24	0.2333	0.1346	0.4234
Placental weight (PW; g)	213.91 ± 13.79	229.78 ± 6.27	193.12 ± 16.19	209.13 ± 13.48	0.2532	0.1423	0.9960
Relative brain weight	0.11 ± 0.01	0.10 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.0871	0.5404	0.6304
BW:PW	3.78 ± 0.24	3.72 ± 0.14	4.12 ± 0.29	3.64 ± 0.23	0.2603	0.5964	0.3940
Relative liver weight	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.0937	0.0032	0.0136
Brain:liver	3.60 ± 0.16	3.43 ± 0.07	3.54 ± 0.14	2.93 ± 0.11	0.0464	0.0081	0.1069
Fetal glucose (mg/dl)	50.33 ± 4.87	35.33 ± 15.60	49.40 ± 13.16	50.67 ± 7.77	0.5040	0.4838	0.4300

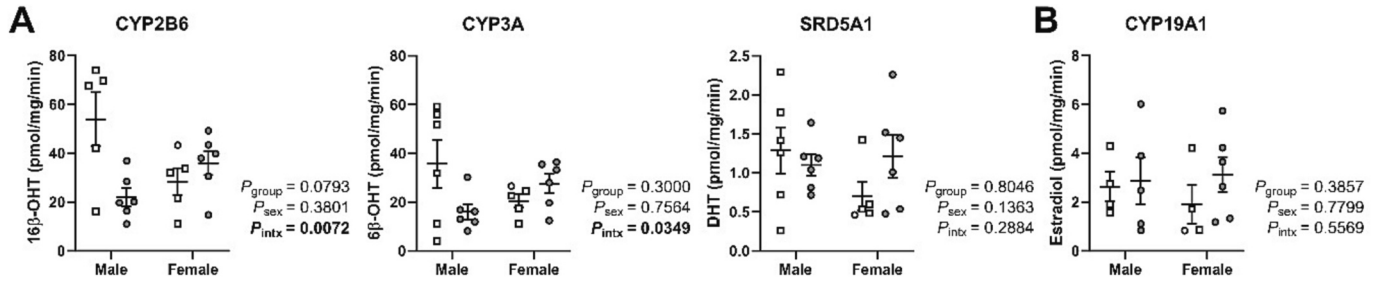

Fig. 1. Maternal obesity reduces activity of testosterone-metabolising enzymes in the male fetal liver only. Activity of A) liver-specific CYP2B6, CYP3A and SRD5A1 and B) placental-specific CYP19A1 was measured by quantifying the amount of metabolite produced per mg microsomal protein per minute of incubation (pmol/mg/min). Functional activity was measured in female and male fetal liver samples from control diet (open data points) and maternal obesity (MO; closed data points) pregnancies. Statistical analysis: two-way ANOVA (factors: diet and sex; Intx = interaction effect) with Tukey's post-hoc analysis. Data is presented as mean ± SEM. $P \leq 0.05$.

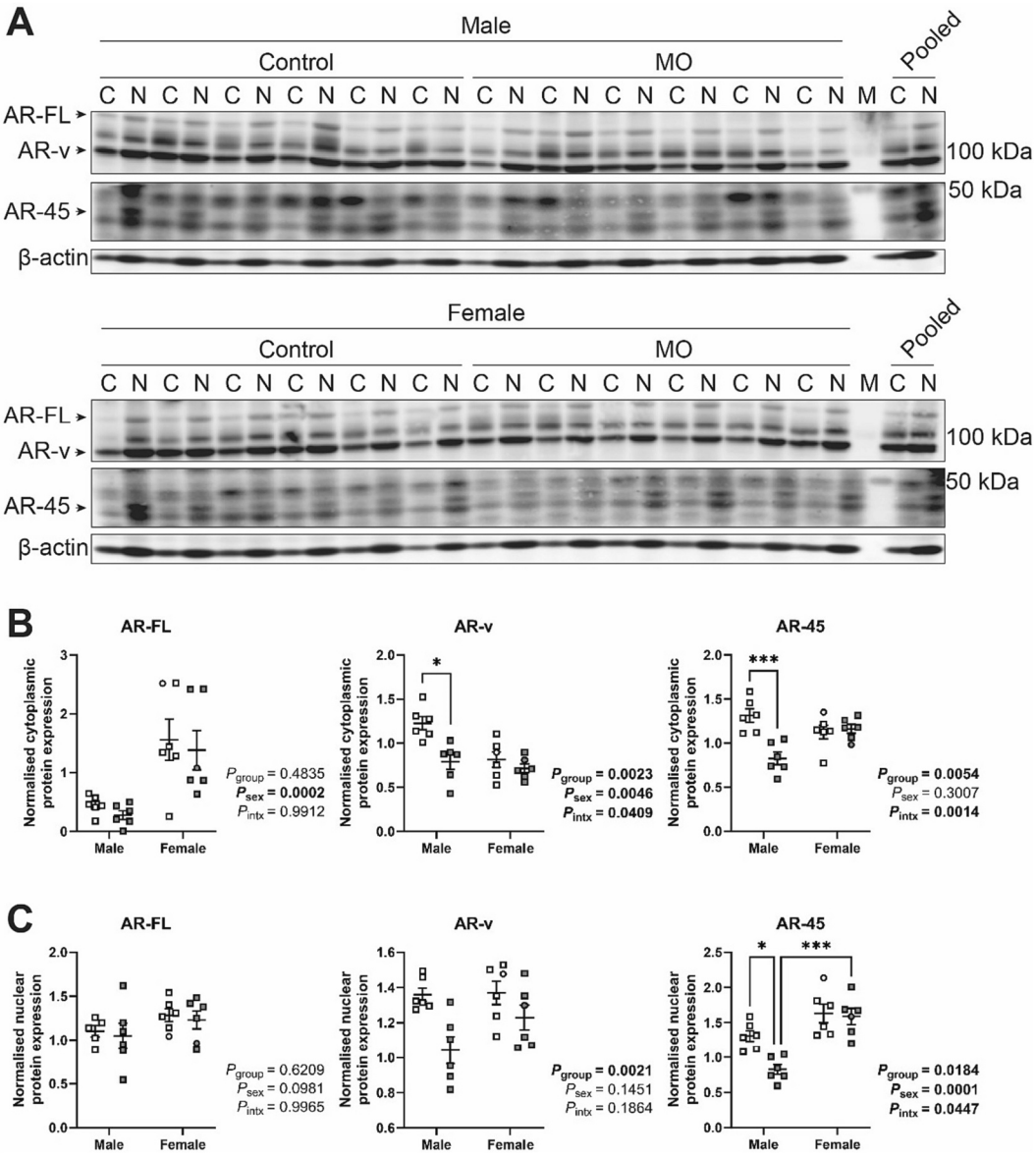

Fig. 2. Androgen receptor (AR) protein isoforms are present in the cytoplasm and nucleus of the baboon fetal liver and vary by sex and diet. A) Cytoplasmic (C) and nuclear (N) protein extracts of female and male liver samples from control and maternal obesity (MO) pregnancies. AR-FL, AR-v and AR-45 were detected in all samples. Membranes were probed with β-actin as a loading control. B) Cytoplasmic and C) nuclear expression of AR-FL, AR-v, and AR-45 in female and male fetal livers from control (open data points) and MO (closed data points) pregnancies. Statistical analysis: two-way ANOVA (factors = diet and sex; Intx = interaction effect) with Tukey's post-hoc analysis. M = molecular marker. Data is presented as mean ± SEM. $P \leq 0.05$; statistical significance: *<0.05.

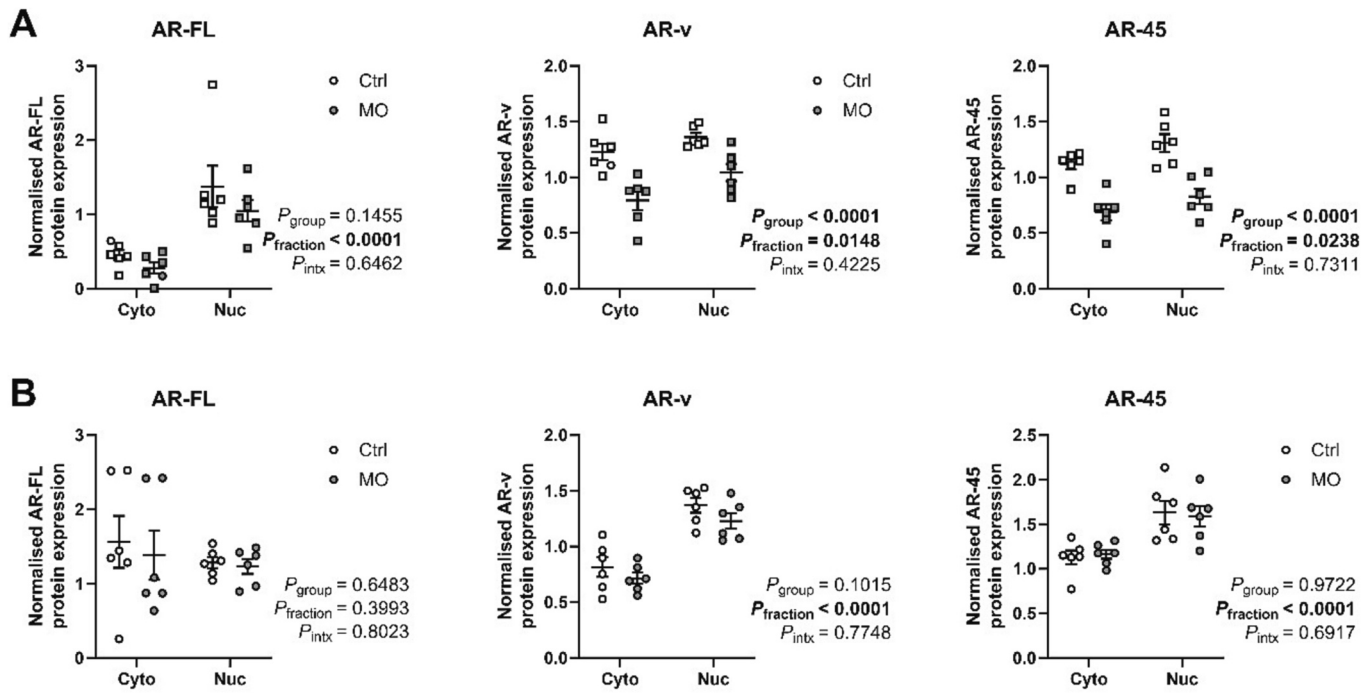

Fig. 3. Subcellular androgen receptor (AR) isoform expression is impacted by diet in a sex-specific manner. Normalised cytoplasmic and nuclear AR isoform expression from A) male and B) female fetal liver samples from control (open data points) and maternal obesity (MO; closed data points) pregnancies. Statistical analysis: two-way ANOVA (factors = diet and fraction; Intx = interaction effect) with Tukey's post-hoc analysis. Data is presented as mean ± SEM.

Fig. 4A), whereas no change was reported in the cytoplasm of female liver samples. However, nuclear AR-45 expression was higher than nuclear AR-FL in female but not male liver samples ($P = 0.0037$; Fig. 4B). There was no change in isoform expression in the nucleus of male samples, but as previously reported nuclear AR-45 expression was reduced in response to diet (Tukey's post-hoc $P = 0.0162$; Fig. 2C and Fig. 4B). The relative nuclear expression of AR-45 was reduced in males ($P < 0.0001$) and increased in females ($P = 0.0038$) when compared with the relative nuclear expression of AR-FL, independent of diet (Fig. 4C).

3.6. Nuclear AR isoform expression is associated with fetal body and liver weight in a sex-specific manner

Nuclear AR-45 expression was negatively associated with MO male fetal body weight only (Fig. 5A). Nuclear AR-FL was not associated with male or female fetal body weight in either control or MO groups (Fig. 5B). Nuclear AR-FL expression was positively associated with relative liver weight in MO males only (Fig. 5D). Nuclear AR-v was not associated with fetal body weight or relative liver weight in any of the study groups (Data not shown). In addition, there were no associations reported between cytoplasmic AR protein isoforms and either fetal body weight or relative liver weight in any of the study groups (Data not shown).

4. Discussion

The data herein highlights novel, sexually dimorphic changes to fetal liver-specific androgen signalling that are further altered in response to MO in baboons. Specifically, we show that livers of male fetuses favour a pro-androgenic environment in response to MO by suppressing activity of testosterone-metabolising CYP enzymes and reducing cytoplasmic and nuclear AR-45 expression. Comparatively, there were minimal changes to hepatic androgen signalling in females in response to MO; however, overall females appear to suppress androgen signalling via increased relative AR-45 nuclear expression when compared with AR-FL. These findings support growing evidence that females and males elicit distinct adaptations to similar intrauterine environments [35], such as those complicated by MO [36]. Our findings highlight changes to the molecular regulation of hepatic androgen signalling in response to MO results in a male-specific pro-androgenic environment that may contribute to sexually dimorphic growth outcomes, increased neonatal risks, and programmed liver dysfunction and disease in later life.

Our finding that MO reduces CYP2B6 and CYP3A activity in male livers from MO pregnancies may have implications for pre- and post-natal androgen mediated signalling activity. Indeed, previous work has demonstrated reductions to CYP activity in androgen-dependent tissue (i.e., prostate) result in increased AR activity [11]. In adult males, increased serum testosterone concentrations are generally associated with reduced hepatic steatosis [5,37]. Similarly, testosterone therapy reduces hepatic steatosis in adult males with type 2 diabetes [38]. Evidently, elevated testosterone concentration in adults is protective against hepatic steatosis; however, when compared with females, male offspring of MO pregnancies have a greater incidence of liver disease that is associated with hepatic steatosis [2]. Indeed, prenatal androgen excess has been linked to liver dysfunction in male adolescent offspring [6]. Thus, reduced CYP2B6 and CYP3A activity in male livers from MO pregnancies may contribute to the increased burden of liver dysfunction and/or disease in male offspring via AR-mediated pathways.

When examining the expression and subcellular localisation of AR protein isoforms, we found that males, but not females, had increased nuclear translocation of AR-FL, as determined by an increase in nuclear relative to cytoplasmic expression. These data suggest that independent of MO, males prioritise androgen-mediated signalling pathways within the liver; a finding that is unsurprising given the importance of androgen signalling in utero for the developing male fetus. However, nuclear expression of AR-45 was reduced in response to MO in male livers only. AR-45 is an N-terminally truncated isoform that forms a non-responsive heterodimer with AR-FL, thereby suppressing canonical, androgen-mediated signalling pathways in multiple tissues including the liver [17,18,39]. Previous studies have shown AR-45 gene therapy can ameliorate disease outcomes associated with aberrant androgen

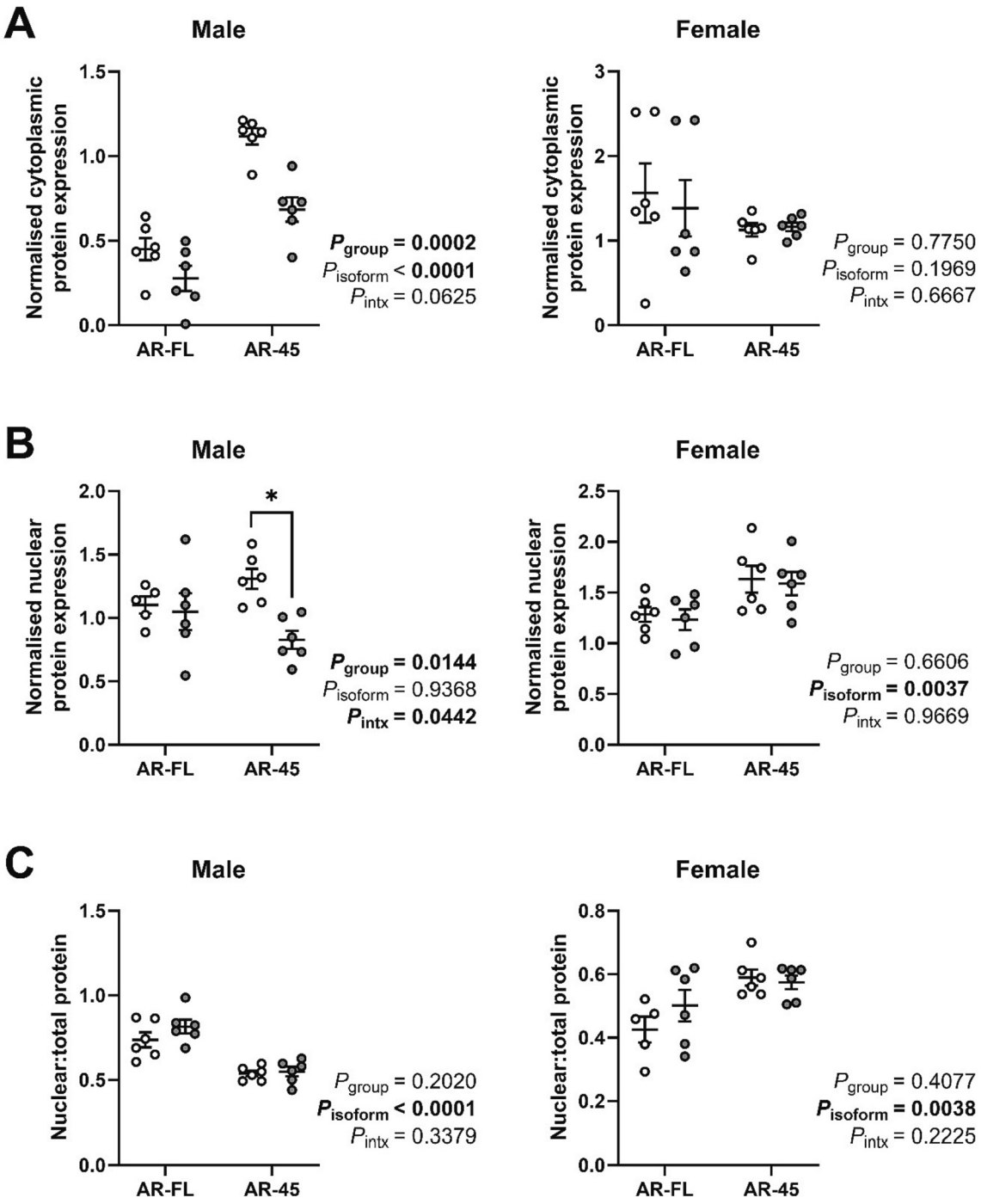

Fig. 4. Sex differences in subcellular isoform expression. A) Cytoplasmic, B) nuclear and C) relative nuclear expression of AR-FL and AR-45 from female and male fetuses exposed to control (open data points) or maternal obesity (MO; closed data points) pregnancies. Statistical analysis: two-way ANOVA (factors = diet and isoform; Intx = interaction effect) with Tukey's post-hoc analysis. Data is presented as mean ± SEM; statistical significance: *<0.05.

signalling [18]. In the placenta, AR-45 expression is downregulated in response to increased androgen concentrations in vitro [19]. Thus, in response to MO pregnancies the male liver undergoes a switch that impairs metabolism of testosterone to inactive metabolites: this switch may impact the developing liver's AR isoform profile by suppressing AR-45 function, thereby upregulating the activity of androgen mediated signalling and exacerbating liver dysfunction and/or disease risk in later life. It is evident that future studies that comprehensively characterise sex-specific androgen signalling in the developing fetus are needed to understand the physiological role of AR isoforms in different fetal tissues including the liver.

Unlike males, females from MO pregnancies had no change in factors involved in testosterone metabolism. Previous work has reported associations between maternal BMI and circulating maternal testosterone concentrations regardless of fetal sex [40]; however, Maliqueo et al. [9] showed MO increases maternal circulating testosterone concentrations in male-bearing pregnancies only. These previously reported sex biases may be due to changes in placental-specific aromatase (CYP19A1) function. Indeed, the same study showed female placentae from MO pregnancies have increased CYP19A1 expression [9]; however, our study found no change in placental CYP19A1 activity in response to MO or fetal sex. Therefore, we are unable to conclude whether female fetuses

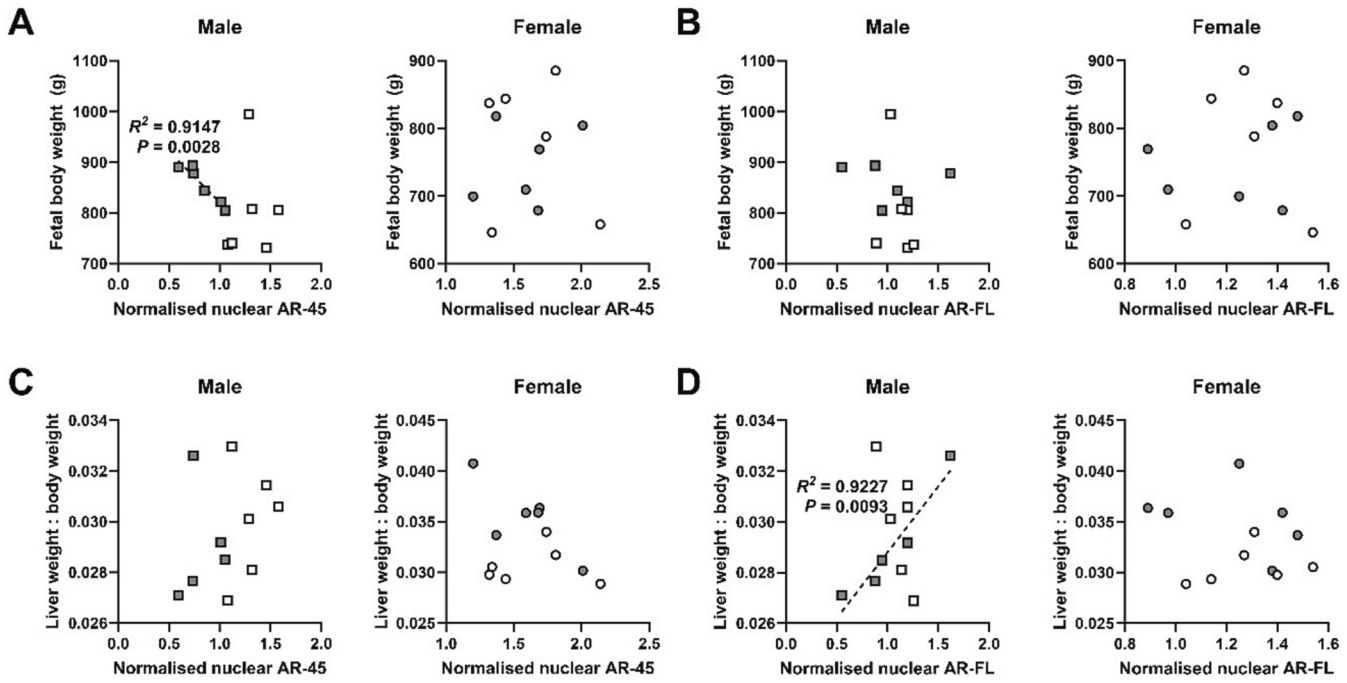

Fig. 5. Nuclear AR isoform expression is associated with fetal body and liver weight in a sex-specific manner. Linear regression analysis was performed between fetal body weight and nuclear localised (A) AR-45 and (B) AR-FL, and between relative liver weight and nuclear localised (C) AR-45 and (D) AR-FL. Open data points are samples from control pregnancies, closed data points are samples from maternal obesity (MO) pregnancies. Statistical analysis: simple linear regression; statistical significance <0.05.

from our model of MO pregnancies are exposed to increased testosterone concentrations. Despite this, when examining the AR isoform profile, there was an increase in nuclear AR-45 expression in female livers when compared with nuclear AR-FL; however, this was not altered by the complication of MO. These findings collectively indicate that the molecular regulation of androgen signalling in female livers is not impacted by MO. However, we also report that the nuclear expression of AR-45 was increased in MO females when compared with MO males. Indeed, prenatal androgenisation of female fetuses results in subclinical hepatic steatosis in offspring despite liver function remaining normal [8]. Therefore, the severity of programmed liver dysfunction and/or disease between the sexes may, in part, be the result of males favouring a pro-androgenic intrauterine environment by suppressing AR-45, whereas in females the opposite occurs. These reported sex differences in AR protein isoform expression and subcellular distribution may be regulated by the availability of bioactive androgens and may contribute to sex-specific neonatal risks associated with MO.

Indeed, a recognised neonatal risk factor associated with MO during pregnancy is macrosomia [41]. Interestingly, the rate of macrosomia is higher in males than females [42,43]. In our study we reported male fetuses were heavier than females, independent of maternal diet. This finding is consistent with a growing body of literature demonstrating sexually dimorphic fetal growth trajectories in normal and complicated pregnancies, such as those impacted by MO [36]. Of particular note, studies show that a high-fat diet results in minimal growth adaptations in females, whereas males prioritise growth and are subsequently less adaptable to the adverse intrauterine environment associated with poor nutrition [44,45]. The mechanisms by which this sexual dimorphic growth pattern is controlled may be, in part, via changes to fetoplacental androgen signalling pathways. Indeed, increased concentrations of insulin like growth factor 1 (IGF-1) in either the fetal or maternal circulation is a predictive marker of macrosomia [46]. *IGF1* is a known androgen-mediated downstream target [47] and fetal IGF-1 is primarily produced hepatically [48]; therefore, changes to hepatic-specific androgen signalling may influence IGF-1 production and thus fetal macrosomia in response to MO. While the current study did not measure IGF-1, we did report a significant, inverse relationship between nuclear AR-45 and fetal body weight in MO males only. Likewise, we found that nuclear AR-FL was positively associated with relative liver weight in MO males. In contrast, no associations between hepatic AR protein variants and neonatal morphometric measures were shown in females. Collectively, these findings indicate that the reduced expression of AR-45 in the nucleus likely enables enhanced AR-FL function, which may drive increased male fetal overgrowth and contribute to liver hypertrophy in response to MO. These responses may also contribute to an increased risk of insulin resistance and subsequent metabolic dysfunction in later life for males, but not females, in response to MO. Indeed, male offspring of MO pregnancies are more likely to be obese, develop hypertriglyceridemia and insulin resistance, when compared with female offspring [49,50]. Furthermore, excess androgen exposure to the developing fetus has been linked to insulin resistance in later life [51]. However, whether the observed male-specific risk for metabolic dysfunction in response to MO is due to the function of AR-FL function via hyperandrogenic-mediated suppression of AR-45 is not clear. It is therefore evident that future studies are required to characterise the significance of hepatic-specific androgen signalling on offspring metabolic health, particularly in males; however, this is beyond the scope of the current body of work.

5. Conclusion

Using a preclinical baboon model of MO, we have shown sex-specific differences in CYP mediated fetal liver testosterone metabolism and AR protein isoform subcellular expression and localisation. These distinct changes to CYP activity may contribute to a pro-androgenic environment for the male fetal liver that enables activated androgen signalling via AR-FL nuclear translocation. Our findings of sex differences in fetal liver androgen metabolism and signalling pathways may drive the observed male disadvantage phenotype within the neonatal period and contribute to programmed liver dysfunction and/or disease in later life (Fig. 6). Thus, modulation of in utero androgen signalling in MO pregnancies may ameliorate the male-specific risk for programmed liver

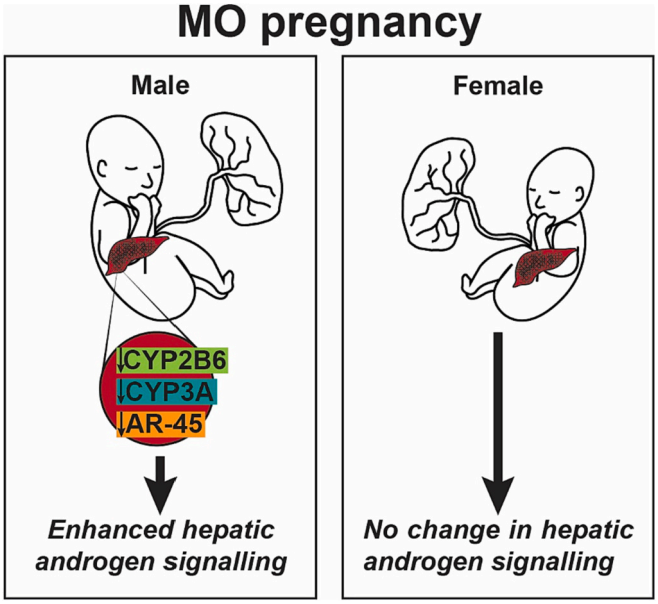

Fig. 6. Summary of sex differences in hepatic androgen signalling in response to maternal obesity (MO) during pregnancy. Unlike females, males prioritise a pro-androgenic environment that may contribute to 1) increased growth and thus a greater risk of macrosomia at birth and 2) programmed liver disease and/or dysfunction.

dysfunction/disease in later life.

Funding

The baboon cohort was funded by NIH R24 RR021367-01 and HD21350 to PWN. JLM and the molecular work were funded by an Australian Research Council Future Fellowship (Level 3; FT170100431).

CRediT authorship contribution statement

Ashley S. Meakin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Peter W. Nathanielsz:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Cun Li:** Data curation, Methodology, Project administration, Resources, Writing – review & editing. **Vicki L. Clifton:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Michael D. Wiese:** Methodology, Supervision, Writing – review & editing, Conceptualization. **Janna L. Morrison:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declared no conflict of interest.

Acknowledgements

We acknowledge the support of staff from Texas Centre for Pregnancy and Life Course Health and the Southwest National Primate Research Institute for their expert care of the primate colony and the Early Origins of Adult Health Research Group for their support with the molecular work.

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