Journal of Developmental Origins of Health and Disease (2016), **7**(3), 237–243.

doi:10.1017/S2040174415007266
Themed Issue: Ibero-American DOHaD Society

ORIGINAL ARTICLE

Pre-gestational overweight in guinea pig sows induces fetal vascular dysfunction and increased rate of large and small fetuses

B. J. Krause[1]*, E. A. Herrera[3], F. A. Díaz-López[1,3], M. Farías[1], R. Uauy[2] and P. Casanello[1,2]

[1]*Division of Obstetrics & Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*
[2]*Division of Paediatrics, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*
[3]*Programa de Fisiopatología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile*

In humans, obesity before and during pregnancy is associated with both fetal macrosomia and growth restriction, and long-term cardiovascular risk in the offspring. We aimed to determine whether overweighted pregnant guinea pig sows results in an increased fetal weight at term and the effects on the vascular reactivity in fetal systemic and umbilical arteries. Pregnant guinea pigs were classified as control ($n = 4$) or high weight (HWS, $n = 5$) according to their pre-mating weight, and their fetuses extracted at 0.9 gestation (~60 days). Segments of fetal femoral and umbilical arteries were mounted in a wire myograph, where the contractile response to KCl (5–125 mM), and the relaxation to nitric oxide synthase-dependent agents (insulin, 10^{-10}–10^{-7} and acetylcholine, 10^{-10}–10^{-5}) and nitric oxide [sodium nitroprusside (SNP), 10^{-10}–10^{-5}] were determined. Fetuses from HWS (HWSF) were grouped according to their body weight as low (<76 g) or high (>85 g) fetal weight, based on the confidence interval (76.5–84.9 g) of the control group. No HWSF were observed in the normal range. Umbilical arteries from HWSF showed a lower response to KCl and insulin compared with controls, but a comparable response with SNP. Conversely, femoral arteries from HWSF showed an increased response to KCl and acetylcholine, along with a decreased sensitivity to SNP. These data show that overweight sows have altered fetal growth along gestation. Further, large and small fetuses from obese guinea pig sows showed altered vascular reactivity at umbilical and systemic vessels, which potentially associates with long-term cardiovascular risk.

Received 13 June 2015; Received 11 September 2015; Accepted 14 September 2015; First published online 22 October 2015

Key words: cardiovascular, fetus, maternal pregnancy, obesity

Introduction

In humans, female overweight and obesity at fertile age are becoming a worldwide burden, which in turn associate increased risk of altered fetal growth (i.e., restriction or macrosomia). Compelling data show that fetal macrosomia (>4000 g) or large for gestational age (LGA) newborns from overweight/obese mothers have increased risk of neonatal morbidity,[1] and the development of cardiovascular diseases at adulthood.[2–5] Recent reports show that chorionic plate arteries from placentae of obese women have a reduced relaxation in response to exogenous nitric oxide (NO)[6] and endothelial dysfunction[7] compared with placentae from women with normal body mass index (BMI). However, there is no data addressing whether these changes at the placental bed from overweight women would represent an altered systemic vascular reactivity in the fetus.

A comprehensive analysis reveals that in humans pre-pregnancy BMI is one of the main factors associated with fetal macrosomia and LGA newborns with cardiometabolic consequences at long term.[8] Many rodent models have been proposed to address the detrimental effects of an increased maternal weight previous and during gestation. In those models, maternal obesity is generated by altering the maternal metabolic status with long-term exposure to pro-obese diets (i.e., high fat, sucrose or fructose), genetic manipulation or induction of diabetes.[9–11] Interestingly, despite the strong metabolic intervention, maternal obesity in these models associates mainly with intrauterine growth restriction (IUGR) rather than macrosomia, in both mice and rats.[12–15] Notably, guinea pigs have a spontaneous capacity of becoming obese, which ultimately impacts on their reproductive outcomes.[16] In fact a pre-pregnancy weight over 700 g in guinea pigs sows associates with increased adiposity, reduced fertility and litter size, but apparently increased offspring birth weight.[16] In this study, we propose that increased pre-pregnancy weight in guinea pig sows resembles the human maternal obesity gestational outcomes with a higher fetal weight at term and associates with altered vascular reactivity in umbilical and systemic arteries.

Materials and methods

Groups

Pirbright White female guinea pigs (*Cavia porcellus*) of 3–4 month of age were feed with guinea pig diet (3.53 kcal/g; 26.4% protein, 13.3% fat and 60.3% carbohydrates)

*Address for correspondence: Dr. B. J. Krause, Division of Obstetrics and Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile.
(Email bjkrause@uc.cl)

(LabDiet, cat. no. 5025, US) in order to maintain the body weight between 500–700 g (25 g_{food}/day) or induce overweight (>800 g; 40 g_{food}/day). Sows were classified as control (CS, $n = 4$) or high weight (HWS, $n = 5$) based on pre-mating weight according to a previous report.[16] After confirmation of pregnancy by ultrasound at day 18 post-mating, diet was adjusted to 40 g_{food}/day until near to term for all pregnant sows. Sows from both groups have a similar amount of fetuses, with 3–4 fetuses per pregnancy. *In utero* fetal growth (biparietal diameter and abdominal circumference)[17] and umbilical resistance [pulsatility and resistance index in arbitrary units (a.u.)] were recorded during gestation by sonography and Doppler velocimetry, respectively (Sonoace R3 ultrasound system, Samsung Medison, Korea). At 0.9 gestation (60–63 days, term = 68) sows were anaesthetized (sodium thiopenthone 100 mg/kg), and fetuses were extracted by c-section, weight and dissected for further analysis.

Wire myography

Carotid, femoral and umbilical arteries were dissected from control fetuses (CSF) from control sows and fetuses from HWS (HWSF). Vessel segments of 2 mm were mounted in a wire myograph (model 620 M; Danish Myo Technology A/S, Aarhus, Denmark), maintained at 37ºC in Krebs buffer (in mmol/l: 118.5 NaCl, 25 $NaHCO_3$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 5.5 D-glucose) with constant bubbling (5% CO_2 in air). Isometric force was recorded using PowerLab data acquisition hardware (ADInstruments, Castle Hill, Australia) and LabChart (version 6; ADInstruments) software. After 30 min of equilibration, vessel internal circumferences were determined by measuring the maximal active force in response to KCl (65 mmol/l) as described.[18] This method allows the comparison between different vessels normalizing the vessel tone with similar *in vivo* levels.[19] In addition, the maximal wall tension was determined measuring the tension achieved to increasing concentration of KCl (5–125 mmol/l) and the vessel length as described by Delaey *et al.*[20] In order to determine the nitric oxide synthase (NOS)-dependent vasodilation, ring vessels were pre-constricted with half maximal KCl concentration (40.8 mmol/l) and the isometric force in response to cumulative concentrations of acetylcholine for carotid and femoral arteries (10^{-10}–10^{-5} mol/l) and insulin for umbilical arteries (10^{-10}–10^{-7} mol/l) was determined in the absence or presence of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 µmol/l). The NOS-independent response to NO was determined with sodium nitroprusside (SNP, 10^{-10}–10^{-5} mol/l) in pre-constricted vessels.

Statistical analysis

Values are expressed as mean ± S.E.M., where n indicates the number of animals studied. Comparisons between two groups were performed by *t*-student for determination of body and organ weight, as well as sonography and Doppler measurements. Comparisons among the three groups was performed by one-way ANOVA and Dunn's *post-hoc* analysis. Data from isolated vessels reactivity were adjusted to sigmoidal curves from which maximal responses and sensitivity (EC_{50} or pD_2) were obtained. Comparison of curves and maximal responses under different conditions were analysed by two-way ANOVA and Bonferroni's *post-hoc* analysis. All the analyses were carried out with GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA), where $P < 0.05$ was considered the cut-off for statistical significance.

Results

Pre-gestational weight and fetal growth

Four pregnant sows were included in the control group (CS) with a pre-gestational weight of 602 ± 11 g and five in HWS group with 879 ± 17 g ($P < 0.01$). Time course of weight gain showed that total gestational weight gain was comparable between the two groups (Fig. 1a and 1b). However, CS showed a relatively constant increase in weight along gestation. In contrast, HWS had a depressed weight gain during the first two out of three of gestation and a brisk increase in the last third of gestation, relative to CS (Fig. 1a and 1c).

Biparietal diameter (Fig. 2a) and abdominal circumference (Fig. 2b) during gestation in HWSF were comparable with CS fetuses at every point studied. Similarly, at near term (60 days) mean fetal weight in HWS group (82.7 ± 3.8 g) was comparable with CS fetuses (82.5 ± 1.8 g, $n = 14$). However, none of the HWSF showed a body weight in range of the confidence interval (78.6–86.4 g) of the control group (Fig. 3a). For further analysis HWS fetuses were classified according to their body weight as low (LWF, range 55.8–74.8 g) or high (HWF, range 88.7–107.7 g) regarding control group confidence interval. A retrospective analysis of fetal biometry during pregnancy considering this stratification in HWS showed a reduced abdominal circumference in LWF only at 40 days of gestation (Supplementary Fig. 1a) but no changes in the abdominal circumference growth rate (Supplementary Fig. 1b). LWF and HWF from HWS were present in a same litter and these characteristics were not associated to specific position in the uterus or horns. This was also observed in control sows as reported.[21]

Placental weight was not altered in HWF (6.56 ± 0.33 g) but reduced in LWF (4.60 ± 0.27 g) compared with controls (6.14 ± 0.23 g) (Fig. 3b), however, fetal to placental ratio was comparable among groups (Fig. 3c). Conversely, brain and heart weight were similar in the three fetal groups, whereas kidneys, liver and lungs weights were lower in LWF without significant changes in HWF compared with controls (Table 1). Furthermore the ratio between brain-to-liver weight, as well as brain-to-body weight, were increased in LWF (Fig. 3d–3e). In contrast, HWF have a decreased brain to body weight percentage relative to controls and LWF.

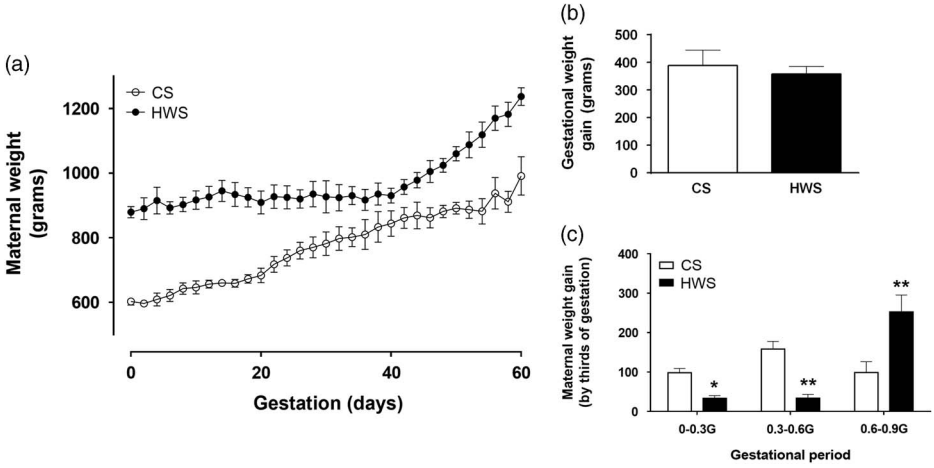

Fig. 1. Maternal weight during gestation in control and obese guinea pigs. Time course of maternal weight gain (*a*), total gestational weight gain (*b*) and weight gain for each third of gestation (*c*) in control (CS, open circles or bars) and high weight (HWS, solid circles or bars) sows. $^{*}P > 0.05$, $^{**}P > 0.01$ *v.* CS, ANOVA.

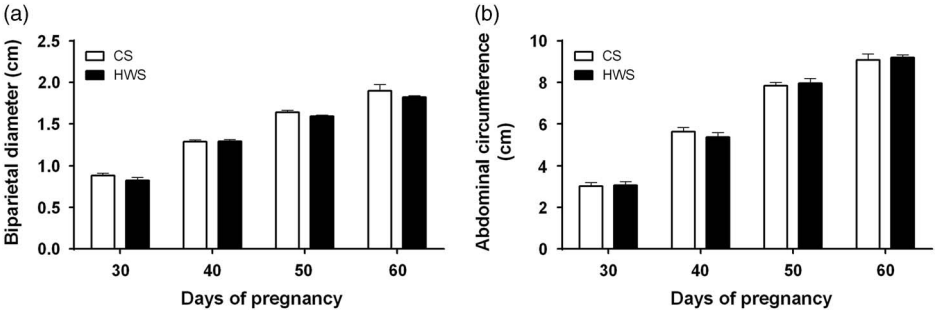

Fig. 2. Pre-gestational weight and fetal biometry during gestation. Biparietal diameter (*a*) and abdominal circumference (*b*) in fetuses from control (CS, open bars) and high weight (HWS, solid bars) sows.

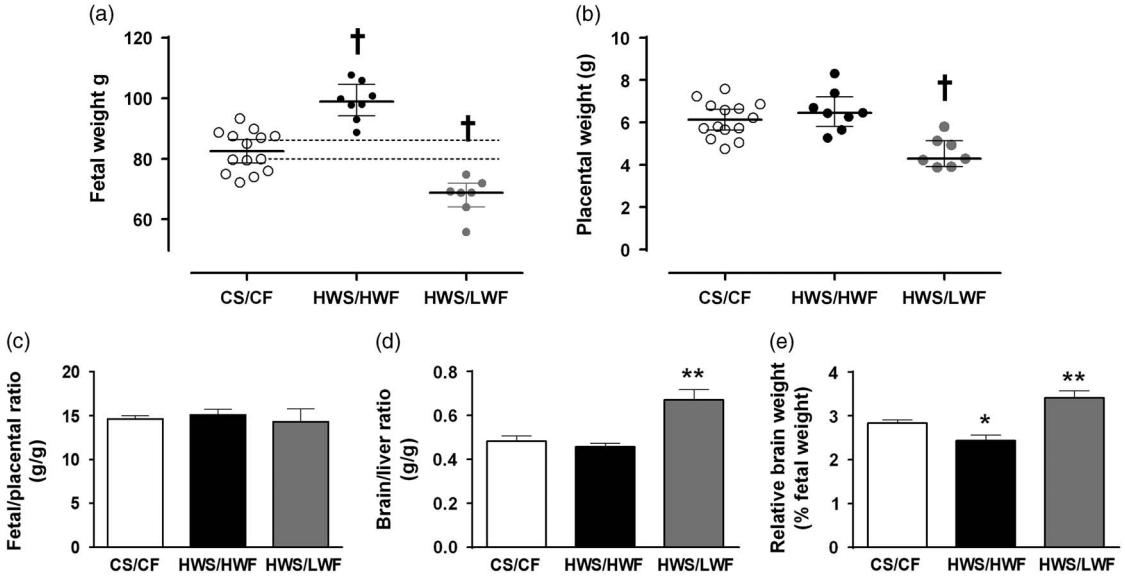

Fig. 3. Pre-gestational weight and fetal characteristic at term. Fetal body weight (*a*), placental weight (*b*), as well as fetal to placental (*c*), brain to liver (*d*) and brain to body (*e*) weight ratios, in fetuses from control sows (CS/CF, open circles or bars), fetuses with high weight from obese sows (HWS/HWF, solid circles or bars) and fetuses with low weight from obese sows (HWS/LWF, grey circles or bars). $^{*}P > 0.05$, $^{**}P > 0.01$, $^{\dagger}P < 0.01$ *v.* CS/CF, ANOVA.

https://doi.org/10.1017/S2040174415007266 Published online by Cambridge University Press

Umbilical artery blood flow and vascular reactivity

Umbilico–placental blood flow measured by Doppler velocimetry showed that pulsatility index (PI) was increased in HWSF at 30 (~1.3-fold increase), 40 (~1.2-fold increase) and 50 (~1.4-fold increase) days of gestation compared with controls (CSF) (Fig. 4a). At 60 days of gestation, there were no differences in the PI between control and HWS fetuses, however, LWF showed a substantial increase in umbilical artery PI (1.38 ± 0.12 a.u.) compared with their HWF siblings (0.90 ± 0.09 a.u.) and fetuses from control sows (0.70 ± 0.10 a.u.) (Supplementary Fig. 2). Resistance index throughout gestation was comparable between HWSF and CSF with a subtle increase at 50 days (data not shown).

Table 1. *Fetal organ weights at near term*

	CS/CF	HWS/HWF	HWS/LWF
Brain	2.33 (0.05)	2.43 (0.09)	2.31 (0.06)
Heart	0.56 (0.02)	0.58 (0.03)	0.49 (0.03)
Kidneys	0.41 (0.01)	0.46 (0.01)	0.32 (0.01)**
Liver	4.95 (0.20)	5.47 (0.25)	3.57 (0.20)**
Lungs	1.93 (0.06)	2.18 (0.07)	1.59 (0.07)*

CS/CF, control fetus from control sows; HWS/HWF, high weight fetuses from high weight sows; HWS/LWF, low weight fetus from high weight sows.
Values expressed as mean (S.E.M.).
*$P < 0.05$ *v.* CS/CF; **$P < 0.01$ *v.* CS/CF, one-way ANOVA.

In order to determine the effects of high pre-gestational weight in the mother on umbilical arteries reactivity, analysis of vasoactive responses was carried out on arteries isolated from HWF and LWF. *Ex vivo* vascular reactivity of umbilical arteries was similar in HWF and LWF, therefore we decided to group these responses. Umbilical arteries from HWSF have a decreased contractile response to increasing concentration of KCl (14.3 ± 1.0 N/m^2) relative to CSF (22.6 ± 2.2 N/m^2) (Fig. 4b) without changes in the EC_{50}. Conversely, umbilical arteries from guinea pig did not relaxed to acetylcholine (data not shown), but showed a NOS-dependent relaxing response to insulin as occurs in human umbilical arteries.[18] HWSF showed a reduced maximal relaxation (12.4 ± 3.2%Kmax) without changes in the pD_2 to insulin (7.72 ± 0.45) (Fig. 4c) compared with controls fetuses (26.4 ± 5.3%Kmax; pD_2 7.92 ± 0.33), and this response was completely inhibited by the NOS inhibitor L-NAME. In contrast, the response to exogenous NO (SNP) were comparable between the two groups (~85% Kmax) (Fig. 4d).

Femoral arteries vascular reactivity

Similar to umbilical arteries, isolated femoral arteries from low and high fetal weight showed comparable *ex vivo* vascular responses, therefore we averaged them. HWSF showed an augmented contractile response to KCl (11.1 ± 1.2 N/m^2) (Fig. 5a) relative to CSF (6.3 ± 0.7 N/m^2) without changes in the EC_{50}. However, the relaxation to acetylcholine in terms of maximal response (~50% Kmax) and pD_2 (~6.5) was

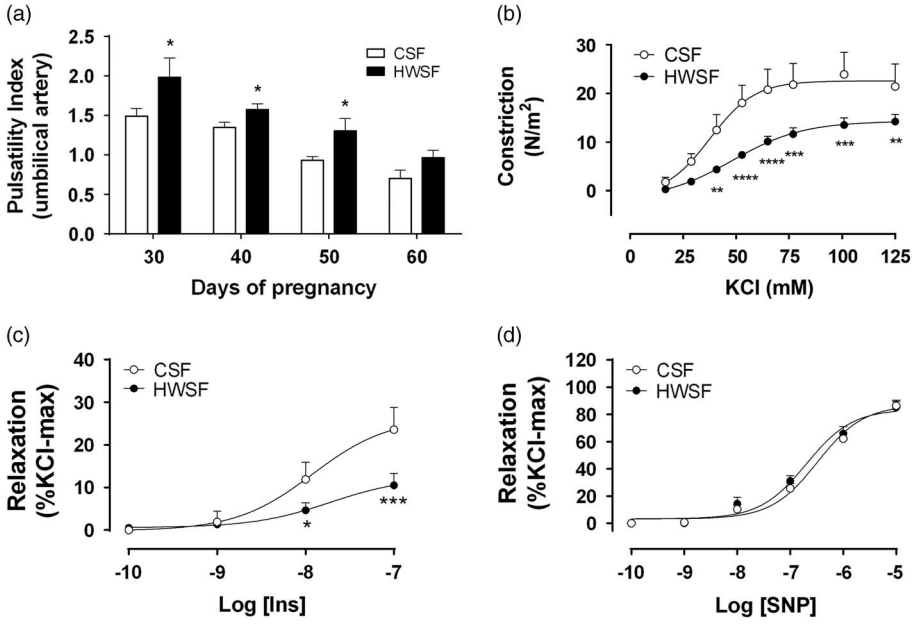

Fig. 4. Pre-gestational weight and umbilico–placental reactivity. (*a*) Umbilical artery pulsatility index during gestation in control sows (CSF, open bars) and high weight sows (HWSF, solid bars) fetuses. Concentration dependent constriction to KCl (*b*) and relaxation to cumulative concentration of insulin (*c*) and sodium nitroprusside (SNP) (*d*) in umbilical arteries from fetuses from control sows (CSF, open circles) and fetuses with high weight from obese sows (HWSF, solid circles). *$P > 0.05$, **$P > 0.01$ *v.* CSF, ANOVA.

https://doi.org/10.1017/S2040174415007266 Published online by Cambridge University Press

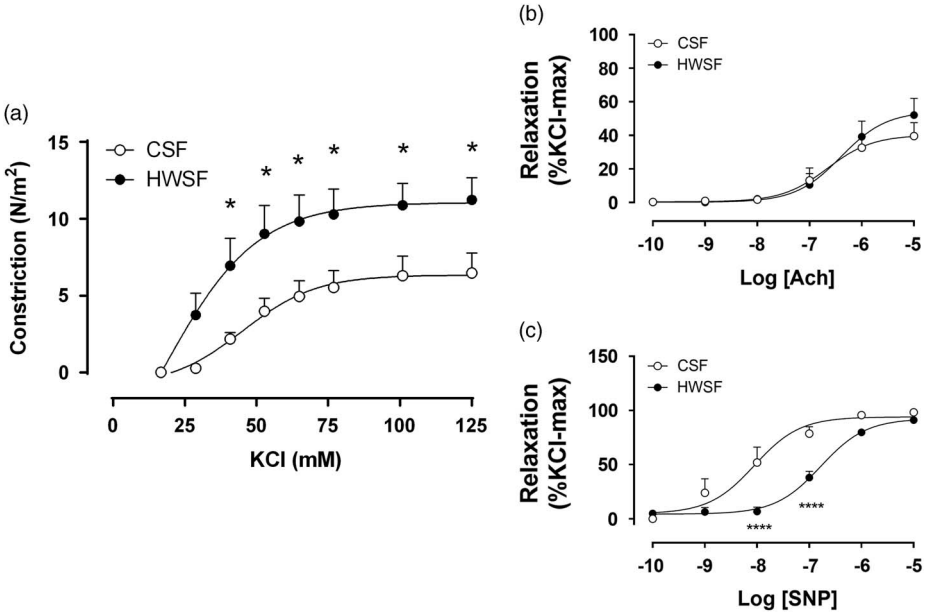

Fig. 5. Femoral artery vascular reactivity in large fetuses from obese sows. Concentration dependent constriction to KCl (*a*) and relaxation to cumulative concentration of acetylcholine (*b*) and sodium nitroprusside (SNP) (*c*) in femoral arteries from fetuses from control sows (CSF, open circles) and fetuses with high weight from obese sows (HWSF, solid circles). *$P > 0.05$, **$P > 0.01$ *v.* CS/CF, ANOVA.

comparable between the two groups (Fig. 5b). Conversely, femoral arteries from HWSF showed a comparable maximal response (~90% Kmax), but a decreased pD_2 to SNP (Fig. 5c) compared with CSF (CSF $pD_2 = 8.05 \pm 0.17$, HWSF $pD_2 = 6.78 \pm 0.09$, $P < 0.01$ *t*-test).

Discussion

This study showed that overweight at conception and during gestation in guinea pigs is associated with altered fetal weight, and umbilical and systemic vascular impairment at term. In guinea pig, pre-gestational overweight induced increased risk of both small and large fetuses, with an asymmetric growth in small fetuses suggesting an IUGR. The HWS fetuses, independent of their growth, showed an increased umbilical artery PI during gestation, which normalized near term only in the large fetuses. These changes were accompanied by reduced *ex vivo* contractility and relaxing capacity in all the fetuses for HWS. In contrast, femoral arteries from HWSF, as examples of periphery arteries, showed increased contractile force with heterogeneous changes in the endothelial-dependent relaxation as well as the response of smooth muscle layer to NO. Altogether, this data show that maternal overweight in guinea pigs associates with altered placental blood flow that may associate with the vascular changes in systemic arteries.

In this study, increased pre-gestational weight was induced in female guinea pigs by a ~33% increase in total daily caloric intake without changes in nutrients composition, differing with other rodent models in which substantial increase in lipid or carbohydrates are applied.[9–11] During pregnancy, total weight gain was comparable between control and HWS, however, the timing of weight gain differs among these groups. Thus, for the present model any alteration in fetal physiology would be related mainly to the pre-gestational maternal metabolic condition as occurs in human population in which controlling maternal weight gain during gestation in overweight/obese women has little effects on neonatal outcomes.[8,22,23]

Following the fetal growth by echography did not show differences between groups, but stratification of fetuses at term according to their weight showed that offspring from HWS had an altered fetal weight, either decreased or increased, relative to offspring from sows with normal weight before pregnancy. In the case of HWF, the differences in weight were not reflected at levels of specific organs suggesting an increased weight of the carcass, however, a further analysis is required to clarify a potential obesogenic body composition. On the other hand, in LWF from HWS, the increased brain to liver ratio and the altered umbilical artery Doppler suggest the presence of a fetal growth restriction. This effect could have been unnoticed in the fetal biometry follow-up owing to the inherent limitations of this technique,[21] but it could represent a potential early onset of IUGR as occurs in other models of maternal obesity[15] in which fetal growth could be compromised at embryonic stages.[14] Altogether, this data suggest that pre-gestational overweight in guinea pigs increases the risk of IUGR and macrosomia as occurs in human pregnancies.[23]

There is no clarity about how increased pre-gestational BMI impacts on placental blood flow during gestation in human pregnancies. The most accepted view is that there are little effects on utero–placental blood flow as maternal BMI increases,

https://doi.org/10.1017/S2040174415007266 Published online by Cambridge University Press

particularly when associated with gestational diabetes.[24,25] However, recent reports suggest that pre-pregnancy maternal obesity in humans associates with altered fetal cardiac function,[26] even at early stages of fetal development,[27] which could be driven by impaired umbilico–placental perfusion. In fact, recently, it has been shown that there is a positive correlation between umbilical artery PI and maternal BMI in pregnancies.[28] The latter suggests that increased placental vascular resistance may be associated to maternal overweight and obesity. Similarly, in this study we found that increased maternal weight is associated with higher umbilical artery PI throughout gestation, which ultimately was manifested as a decreased *ex vivo* maximal relaxation to insulin. The altered umbilical PI was specially maintained in LWF up to term, clear evidence of umbilio–placental dysfunction. Furthermore, umbilical artery from HWS had a reduced contractile capacity but a normal response to exogenous NO, which could represent a compensatory mechanism to deal with an increased placental resistance resulting from endothelial dysfunction in chorionic vessels.[7]

Changes in the PI during gestation is a predictor of blood pressure at early infancy in a control population.[29] Therefore, considering the changes in PI and vascular reactivity at umbilical level, we aimed to associate these effects in fetal femoral arteries. Femoral arteries from HWS fetuses showed an increased contractile capacity, but a lower sensitivity to NO without changes in the response to acetylcholine. Several studies have shown that maternal overweight/obesity and excessive gestational weight gain is associated with increased risk for later cardiovascular diseases in the offspring.[22,30] This effect has been mainly attributed to the increased adiposity and metabolic complications that subjects develop during their early infancy. In fact, non-human primates born from mothers exposed to high-fat diets have an increased intima-media thickness and impaired endothelial function in the aorta at 1 year of age. Interestingly, these aortic impairments may be substantially reversed by modifications in the post-natal diet.[31] In contrast, a rat model of increased maternal adiposity during pregnancy shows impaired endothelial function in mesenteric arteries in the offspring, independent of the body weight at adult age,[32] suggesting that the vascular dysfunction in the offspring from obese mothers has an independent origin. In this context, it has been shown that in human neonates the aortic intima-media thickness positively correlates with the maternal BMI,[33] a fact that could be related with the increased vascular risk in the offspring. At the best of our knowledge, in this study we showed for the first time that increased maternal weight before gestation induces changes in the vascular reactivity of fetal systemic vessels at term, with a heterogeneous effect on endothelial NOS and NO-dependent responses but having with a consistent increased contractile force as a hallmark. Altogether, these data support the concept that the cardiovascular risk in the offspring born from mothers with pre-gestational obesity has its origins *in utero*.

In conclusion, this study shows that altered fetal growth in pregnancies affected by increased pre-gestational weight take place under a reduced fetal–placental blood flow and associates with an altered endothelial and vascular function in the fetal umbilical and systemic circulation. We proposed that the increased placental vascular resistance along with the augmented nutrients supply generate the fetal hypoxic-like characteristic of these pregnancies,[34,35] which is an important mechanism involved in the programming of vascular function.[36,37]

Acknowledgment

None.

Financial Support

This work was supported by Fondecyt Chile (P.C., grant number 1120928), (M.F., grant number 1121145), (B.J.K., grant number 1130801), (E.A.H., grant number 1151119).

Conflicts of Interest

None.

Ethical Standards

The procedures and animal handling were approved by the Ethics Committee of the Faculty of Medicine of the University of Chile (CBA 694 FMUCH) and the Faculty of Medicine of Pontificia Universidad Catolica de Chile (CEBA-MedUC 1130801). The studies on animals were performed according with the ARRIVE guidelines, The Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and adheres to APS's Guiding Principles in the Care and Use of Animals.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S2040174415007266

References

1. Higgins L, Greenwood SL, Wareing M, Sibley CP, Mills TA. Obesity and the placenta: a consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. *Placenta*. 2011; 32, 1–7.
2. Reynolds RM, Allan KM, Raja EA, *et al*. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. *BMJ*. 2013; 347, f4539.
3. Edvardsson VO, Steinthorsdottir SD, Eliasdottir SB, Indridason OS, Palsson R. Birth weight and childhood blood pressure. *Curr Hypertens Rep*. 2012; 14, 596–602.
4. McGuire W, Dyson L, Renfrew M. Maternal obesity: consequences for children, challenges for clinicians and carers. *Semin Fetal Neonatal Med*. 2010; 15, 108–112.
5. Ornellas F, Souza-Mello V, Mandarim-de-Lacerda CA, Aguila MB. Programming of obesity and comorbidities in the progeny: lessons from a model of diet-induced obese parents. *PLoS One*. 2015; 10, e0124737.

6. Hayward CE, Higgins L, Cowley EJ, *et al*. Chorionic plate arterial function is altered in maternal obesity. *Placenta*. 2013; 34, 281–287.
7. Schneider D, Hernandez C, Farias M, Uauy R, Krause BJ, Casanello P. Oxidative stress as common trait of endothelial dysfunction in chorionic arteries from fetuses with IUGR and LGA. *Placenta*. 2015; 36, 552–558.
8. Yu Z, Han S, Zhu J, *et al*. Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis. *PLoS One*. 2013; 8, e61627.
9. Li M, Reynolds CM, Sloboda DM, Gray C, Vickers MH. Effects of taurine supplementation on hepatic markers of inflammation and lipid metabolism in mothers and offspring in the setting of maternal obesity. *PLoS One*. 2013; 8, e76961.
10. Gurecka R, Koborova I, Jansakova K, *et al*. Prenatal dietary load of Maillard reaction products combined with postnatal Coca-Cola drinking affects metabolic status of female Wistar rats. *Croat Med J*. 2015; 56, 94–103.
11. Toop CR, Muhlhausler BS, O'Dea K, Gentili S. Consumption of sucrose, but not high fructose corn syrup, leads to increased adiposity and dyslipidaemia in the pregnant and lactating rat. *J Dev Orig Health Dis*. 2015; 6, 38–46.
12. Sloboda DM, Howie GJ, Pleasants A, Gluckman PD, Vickers MH. Pre- and postnatal nutritional histories influence reproductive maturation and ovarian function in the rat. *PLoS One*. 2009; 4, e6744.
13. Connor KL, Vickers MH, Beltrand J, Meaney MJ, Sloboda DM. Nature, nurture or nutrition? Impact of maternal nutrition on maternal care, offspring development and reproductive function. *J Physiol*. 2012; 590(Pt 9), 2167–2180.
14. Luzzo KM, Wang Q, Purcell SH, *et al*. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One*. 2012; 7, e49217.
15. Busso D, Mascareno L, Salas F, *et al*. Early onset intrauterine growth restriction in a mouse model of gestational hypercholesterolemia and atherosclerosis. *Biomed Res Int*. 2014; 2014, 280497.
16. Michel CL, Bonnet X. Influence of body condition on reproductive output in the guinea pig. *J Exp Zool Part A Ecol Genet Physiol*. 2012; 317, 24–31.
17. Turner AJ, Trudinger BJ. A modification of the uterine artery restriction technique in the guinea pig fetus produces asymmetrical ultrasound growth. *Placenta*. 2009; 30, 236–240.
18. Krause BJ, Prieto CP, Munoz-Urrutia E, *et al*. Role of arginase-2 and eNOS in the differential vascular reactivity and hypoxia-induced endothelial response in umbilical arteries and veins. *Placenta*. 2012; 33, 360–366.
19. Mulvany MJ, Aalkjaer C. Structure and function of small arteries. *Physiol Rev*. 1990; 70, 921–961.
20. Delaey C, Boussery K, Van de Voorde J. Contractility studies on isolated bovine choroidal small arteries: determination of the active and passive wall tension-internal circumference relation. *Exp Eye Res*. 2002; 75, 243–248.
21. Turner AJ, Trudinger BJ. Ultrasound measurement of biparietal diameter and umbilical artery blood flow in the normal fetal guinea pig. *Comp Med*. 2000; 50, 379–384.
22. Santangeli L, Sattar N, Huda SS. Impact of maternal obesity on perinatal and childhood outcomes. *Best Pract Res Clin Obstet Gynaecol*. 2015; 29, 438–448.
23. King JC. Maternal obesity, metabolism, and pregnancy outcomes. *Annu Rev Nutr*. 2006; 26, 271–291.
24. Santolaya J, Kahn D, Nobles G, Ramakrishnan V, Warsof SL. Ultrasonographic growth and Doppler hemodynamic evaluation of fetuses of obese women. *J Reprod Med*. 1994; 39, 690–694.
25. Quintero-Prado R, Bugatto F, Sanchez-Martin P, *et al*. The influence of placental perfusion on birthweight in women with gestational diabetes. *J Matern Fetal Neonatal Med*. 2014; 27, 1–4.
26. Ece I, Uner A, Balli S, *et al*. The effects of pre-pregnancy obesity on fetal cardiac functions. *Pediatr Cardiol*. 2014; 35, 838–843.
27. Ingul CB, Loras L, Tegnander E, Eik-Nes SH, Brantberg A. Maternal obesity affects foetal myocardial function already in first trimester. Ultrasound Obstet Gynecol. 2015; doi: 10.1002/uog.14841.
28. Sarno L, Maruotti GM, Saccone G, *et al*. Maternal body mass index influences umbilical artery Doppler velocimetry in physiologic pregnancies. *Prenat diagn*. 2015; 35, 125–128.
29. Khoury J, Knutsen M, Stray-Pedersen B, Thaulow E, Tonstad S. A lower reduction in umbilical artery pulsatility in mid-pregnancy predicts higher infant blood pressure six months after birth. *Acta Paediatr*. 2015; doi: 10.1111/apa.13020.
30. Fraser A, Tilling K, Macdonald-Wallis C, *et al*. Association of maternal weight gain in pregnancy with offspring obesity and metabolic and vascular traits in childhood. *Circulation*. 2010; 121, 2557–2564.
31. Fan L, Lindsley SR, Comstock SM, *et al*. Maternal high-fat diet impacts endothelial function in nonhuman primate offspring. *Int J Obes (Lond)*. 2013; 37, 254–262.
32. Gray C, Vickers MH, Segovia SA, Zhang XD, Reynolds CM. A maternal high fat diet programmes endothelial function and cardiovascular status in adult male offspring independent of body weight, which is reversed by maternal conjugated linoleic acid (CLA) supplementation. *PLoS One*. 2015; 10, e0115994.
33. Begg LM, Palma-Dias R, Wang J, Chin-Dusting JP, Skilton MR. Maternal adiposity and newborn vascular health. *Arch Dis Child Fetal Neonatal Ed*. 2013; 98, F279–F280.
34. Dollberg S, Marom R, Mimouni FB, Yeruchimovich M. Normoblasts in large for gestational age infants. *Arch Dis Child Fetal Neonatal Ed*. 2000; 83, F148–F149.
35. Sheffer-Mimouni G, Mimouni FB, Dollberg S, *et al*. Neonatal nucleated red blood cells in infants of overweight and obese mothers. *J Am Coll Nutr*. 2007; 26, 259–263.
36. Camm EJ, Hansell JA, Kane AD, *et al*. Partial contributions of developmental hypoxia and undernutrition to prenatal alterations in somatic growth and cardiovascular structure and function. *Am J Obstet Gynecol*. 2010; 203(495), e424–e434.
37. Giussani DA, Davidge ST. Developmental programming of cardiovascular disease by prenatal hypoxia. *J Dev Orig Health Dis*. 2013; 4, 328–337.