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Intrauterine growth-restricted Yucatan miniature pigs experience early catch-up growth leading to greater adiposity and impaired lipid metabolism as young adults

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ABSTRACT

Early nutrition has critical influences on cardiovascular disease risk in adulthood. The study objectives were to evaluate the impact of low birth weight on fasting and postprandial lipid metabolism and endothelium function in Yucatan miniature pigs. Intrauterine growth-restricted (IUGR) piglets ($n = 6$, 3 d old, 0.73 ± 0.04 kg) were paired with normal weight (NW) same-sex littermates ($n = 6$, 1.11 ± 0.05 kg) and fed milk replacer *ad libitum* for 4 wk. Thereafter, all pigs were fed a standard diet *ad libitum* for 5 h/d with growth, intakes and blood samples collected for 8 mo. At 9 mo old, pigs were surgically fitted with venous catheters and an oral fat tolerance test was performed. At 10 mo old, pigs were killed and endothelium-dependent and -independent vasodilations of isolated coronary arteries were measured using wire-myographs. IUGR pigs demonstrated catch-up growth ($P < 0.05$) in body weight and abdominal circumference prior to sexual maturity (<7 mo old) and had more ($P < 0.05$) subcutaneous fat at 10 mo old compared to NW pigs. IUGR pigs had consistently higher fasting plasma triglyceride concentrations from 5 to 10 mo old and higher liver triglyceride and total cholesterol concentrations at 10 mo old ($P < 0.05$). The fat tolerance test revealed delayed postprandial triglyceride clearance in IUGR pigs, but no differences in plaque formation or vascular reactivity. To conclude, IUGR and early postnatal catch-up growth are associated with increased overall body fat deposition and altered triglyceride metabolism in adult Yucatan miniature swine.

Keywords: growth, lipid metabolism, dyslipidemia, obesity, animal model.

Introduction

The fetal origins of adult disease hypothesis proposes that adverse *in utero* conditions could result in altered programming of metabolic and physiological functions, which can have lifelong effects and increase the risk of chronic disease later in life, including cardiovascular diseases (McMillen and Robinson 2005). Numerous epidemiological and animal studies have established the association between low birth weight and later development of high blood pressure as a marker of cardiovascular disease (Barker et al. 2007; Luyckx et al. 2013). However, the association of early insults with many other cardiovascular risk factors are not as well established; for example, epidemiological studies on the relationships between birth weight and various blood lipids are equivocal (Laurén et al. 2003; Huxley et al. 2004; Sohi et al. 2011). Indeed, although there are several studies showing that low birth weight is associated with abnormal lipid profile in adulthood (Barker et al. 1993; Huxley et al. 2004; Kajantie et al. 2008; Skilton et al. 2011), associations between various fasting plasma lipid concentrations and birth weight are notably inconsistent.

Studies have emerged suggesting that the postprandial responses of blood lipids to dietary macronutrients are more predictive of cardiovascular disease than corresponding fasting levels (Bansal et al. 2007; Nordestgaard et al. 2007; Perälä et al. 2011). Moreover, it has been suggested that postprandial lipid responses might explain the associations between early growth and adult cardiovascular diseases more than fasting lipid levels (Perälä and Eriksson 2012). In particular, postprandial TG-rich lipoproteins are pro-atherogenic leading to inflammation and accumulation in endothelial cells (Goldberg et al. 2011; Kolovou et al. 2011), potentially promoting endothelial dysfunction (Klop et al.

2012). Vascular endothelial dysfunction has also been shown to be associated with birth weight in young adults (Goodfellow et al. 1998; Salonen et al. 2010; Skilton et al. 2011). In spite of these intriguing clinical studies, an appropriate animal model is needed to elucidate the interactions among fetal growth, postprandial lipidemia and endothelial function.

The *in utero* environment is affected by placental infusion, maternal nutrition and other factors (McMillen and Robinson 2005). However, in Western societies, reduction in uteroplacental perfusion is a greater contributor to intrauterine growth restriction (IUGR) than maternal malnutrition (Henriksen and Clausen 2002). As such, we recently developed a naturally occurring spontaneous IUGR Yucatan miniature pig model to assess the relationship between early development and cardiovascular disease risks in early adulthood (Myrie et al. 2011; Myrie et al. 2012a). Similar to other studies in IUGR models (Poore et al. 2002; Woods and Weeks 2004), previous data from our mini-pig model showed that IUGR was associated with higher blood pressure and salt sensitivity in early adulthood (Myrie et al. 2011; Myrie et al. 2012a). Because the pig is one of the most relevant species for modeling lipoprotein metabolism in humans (Larsen et al. 2002; Sébert et al. 2005; Torres-Rovira et al. 2012), the objective of this study was to evaluate the effects of birth weight, as a proxy for IUGR, on lipid metabolism and endothelial function as indicators of cardiovascular disease risk in a Yucatan miniature pig model.

Materials and methods

Animals and study protocol: The pigs used in this study were part of a larger experiment testing a variety of disease outcomes and some of the growth and feeding data have been

previously published (Myrie et al. 2011; McKnight et al. 2012). All animal procedures were approved by Memorial University of Newfoundland Animal Care Committee in accordance with the Canadian Council on Animal Care Guidelines. Yucatan miniature pigs from six sows from the University's swine herd were studied (average litter size was 7 ± 1). Within each litter, the runt (i.e. IUGR) piglet (0.73 ± 0.04), defined as ~65% of the birth weight of the largest littermate (Hegarty and Allen 1978), was sex-matched to a larger (i.e. normal weight (NW)) littermate (1.11 ± 0.05). To avoid normal competition among suckling piglets, after allowing for colostrum consumption, each IUGR-NW piglet pair was removed from the sow at 3 d old and housed together, but individually provided with rehydrated sow milk replacer (Grober Nutrition Inc., Cambridge, ON, Canada) 8-10 times daily *ad libitum* with all intakes recorded. All pigs were weaned onto standard pelleted grower pig diet (based on wheat, barley and canola; Eastern Farmers Co-op, St. John's, NL, Canada), providing 67% of energy as carbohydrate, 12% as fat and 21% as protein. Pigs were weaned at 4 wk old and siblings (IUGR and NW) remained group-housed throughout the study; however, to quantify individual pig daily feed intake, each pig was fed separately for 5 h/d (1200 – 1700 h) during which time the diet was freely available and intakes recorded. All animals had 24-h *ad libitum* water access and were maintained on a 12-h day-night cycle (lights on 0700 – 1900 h).

Body measurements and monthly blood samples: Serial growth measurements were taken throughout the study. During the milk-feeding phase (birth to 1 mo old), body weight, crown to rump length and abdominal circumference were measured at least twice weekly; thereafter until the end of the study, these measurements were made twice monthly. Using jugular venipuncture, monthly blood samples were collected for all

animals from 1 to 9 mo old. Overnight fasted blood samples were collected in EDTA tubes (BD; Franklin Lakes, NJ, USA) and immediately centrifuged at 4000 x g for 15 min at 4°C for separation and collection of plasma. Lipid assays, as described below, were performed on fresh samples stored at 4°C and the remainder of the plasma was stored at -80°C.

Surgical catheter implantation: At ~9 mo old, the pigs underwent surgery for implantation of blood sampling catheters; because this investigation was part of a larger series of studies, a blood pressure telemeter was also implanted in the femoral artery, as previously reported (Myrie et al. 2011). For implantation of the venous catheters, briefly, anesthesia was induced with ketamine (22 mg/kg *i.m.*) and xylazine (2 mg/kg *i.m.*) and maintained with 1.0-1.5% halothane and 3:2 oxygen:nitrous oxide mixture. Two blood sampling catheters (I.D. 1.0 mm, O.D. 1.8 mm; Tygon® Medical Tubing, Saint Gobain Performance Plastics Corp, Akron, OH, USA) were inserted in the left femoral vein, tunneled under the skin and exteriorized between the shoulder blades. For recovery from surgery, the pigs were given 0.07 mL/kg *i.v.* trimethoprim (40 mg/mL) and sulfadoxine (20 mg/mL) (Borgal, Intervet Ltd., Whitby, ON, Canada) and 300 µg buprenorphine hydrochloride (Temgesic, Schering-Plough Ltd., Hertfordshire, UK), as previously reported (Myrie et al. 2011). After surgery, each animal was housed individually, with 8-10 d post-surgery recovery.

Oral fat tolerance test: Animals were fasted overnight for 12-14 h before the oral fat tolerance test (OFTT). On the day of the test, a baseline blood sample (10 mL in EDTA) was collected; immediately thereafter the pigs were given the high fat meal bolus (1.5 g fat/kg body weight), which consisted of a mix of saturated and unsaturated fatty acids

provided via margarine (25% of total fat in the bolus; Central Dairies, St. John's, NL, Canada) and lard (75% of the total fat in the bolus; Loblaw Inc., Toronto, ON, Canada), combined with some ground pig grower diet (~5% of the total bolus) and sucrose (~0.4% of the total bolus; Lantic, Montreal, QC, Canada), since absorption of fatty acids depends on the presence of some carbohydrate and protein and a mixed meal is recommended for such tests (Kolovou et al. 2011). The pigs were allowed 2 h to consume the entire bolus; on average, the bolus was completely consumed within 1 h. Water was available during the test.

In addition to the baseline sample, blood samples (10 mL) were collected hourly until 10 h after the meal was initially given. Each blood sample was immediately centrifuged at $4\,000 \times g$ for 15 min at 4°C ; plasma fractions were frozen immediately with an aliquot stored at 4°C until the next day when further centrifuged at $15\,500 \times g$ for 20 min at 12°C for separation into chylomicron (CM) and chylomicron-free (CMF) fractions (McAteer et al. 2003). Plasma, CM and CMF fractions were analyzed for TG concentrations using an enzymatic assay kit (Diagnostics Chemicals, Saint John, NB, Canada). The TG responses during the OFTT were quantified as the total area under the curve (AUC) (calculated using the trapezoid method from baseline to final TG measurements for each fraction), peak TG concentration, peak TG concentration adjusted for baseline TG, and time to peak TG.

Necropsy: At ~10 mo of age, pigs were anesthetized with 105 mg/kg sodium pentobarbital (Euthanyl, Biomeda-MTC Cambridge, ON, Canada), ventilated and maintained with 0.5 – 1% halothane gas mixed with oxygen. Blood samples were collected in EDTA tubes and assayed for lipids as described below. Organs were

removed, weighed and samples frozen with liquid nitrogen and stored at -80 °C. Back fat thickness, a measure of subcutaneous fat, was measured on the carcass at the midline of the back, immediately caudal to the last rib. Total visceral organs (i.e. intra-peritoneal tissues) from each pig were removed, frozen at -20 °C, homogenized (Hobart 4346 Mixer/Grinder, Troy, OH, USA) and analyzed for fat content using a chloroform:methanol (2:1, v/v) extraction technique (Folch et al. 1957). The lipid-containing phase was evaporated to yield the dry weight of lipids expressed as a percentage of the original sample mass.

Plasma lipid analyses: Monthly plasma samples were analyzed for concentrations of total cholesterol, high-density lipoprotein (HDL)-cholesterol, and TG using enzymatic reagent kits (Diagnostics Chemicals, Saint John, NB, Canada; Stanbio Laboratories, Boerne, TX, USA) on fresh plasma. HDL was precipitated using HDL reagent # 200-26A (Diagnostics Chemicals, Saint John, NB, Canada). The concentration for plasma low-density lipoprotein (LDL)-cholesterol was calculated from the concentrations of total cholesterol, HDL-cholesterol and TG (Friedewald et al. 1972). Fresh necropsy blood samples were separated into lipoprotein fractions; plasma was centrifuged at 15 500 x g for 20 min at 12°C to separate any chylomicrons (McAteer et al. 2003), followed by separation of the infranatant by sequential density ultracentrifugation (Salter et al. 1998) into lipoprotein fractions: very-low-density lipoprotein (VLDL), LDL and HDL. Because pigs were in a postprandial state at necropsy, chylomicron fractions were discarded and all other fractions were analyzed for TG and total cholesterol.

Liver lipid analyses: Lipids were extracted from liver samples (Folch et al. 1957); for the determination of the concentration of lipids in the samples, aliquots of the lipid extracts

were dried and the lipids were re-dissolved using isopropanol, and then analyzed for concentrations of TG and total cholesterol by enzymatic assays as described above.

Coronary artery isometric tension measurements: At necropsy, whole hearts were removed from pigs and placed in ice-cooled Krebs physiological salt solution (KPSS) (114 mM NaCl, 4.7 mM KCl, 0.8 mM KH_2PO_4 , 1.2 mM MgCl_2 , 2.5 mM CaCl_2 , 25 mM NaHCO_3 , 11 mM D-glucose (pH 7.4), bubbled with a mixture of 95 % O_2 / 5 % CO_2) (Myrie et al. 2012b). Left anterior descending coronary arteries were isolated from whole hearts and cut into 4 to 8 rings of 1 to 2 mm lengths. Coronary artery rings were mounted on 200 μm diameter hooks in myograph chambers (DMT 610M, 620M, Aarhus, Denmark) containing KPSS (pH 7.4) at 37°C. Under isometric conditions, the initial resting tensions of artery rings were set to 90% of the internal circumference estimated to produce wall stress equivalent to 13.3 kPa (100 mmHg) (Mulvany and Halpern 1977). After 1 h of equilibration, tissue viability was tested by addition of high K^+ , osmotic balanced, KPSS (Na^+ substituted by equimolar amounts of K^+); arteries with contractions >2 mN/mm length passed (Chia et al. 2011). Vasomotor reactivity of individual arterial rings was determined by measuring separately vascular smooth muscle contraction (U46619), endothelium-dependent relaxations (bradykinin and PAR2-activating peptide 2fLIGRLO) and endothelium-independent relaxation (sodium nitroprusside; SNP). Bradykinin has been reported to elicit a combination of NO- and prostanoid-dependent vasodilation (Muller et al. 1994). PAR2-activating peptide agonists elicit NO-dependent vasodilation of pig coronary arteries and under conditions of inhibited NO synthase, may cause vasoconstriction (El-Daly et al. 2014). Relaxations were measured in arteries contracted submaximally by thromboxane receptor agonist U46619. Drugs were added

directly to the bath chambers and isometric tensions recordings were acquired continuously using Mydaq/Myodata (J.P. Trading, Copenhagen, Denmark) and Chart (ADI PowerLab, Colorado Springs, CO, USA) software. Contractility data for U46619 are normalized relative to contractions elicited by KPSS. Relaxation data are reported as the reversal of the contractions by U46619 (i.e. 100 % relaxation equals complete return of the elicited vasomotor tone to resting tension).

Aorta plaque assay: Fatty streak formation was assessed using gross anatomic staining with Sudan IV (Holman et al. 1958). At necropsy, the entire intact aorta (i.e. from the ascending aorta to the abdominal aorta) was removed, rinsed with saline, trimmed of arteries, opened longitudinally along the ventral surface, pinned out flat, and fixed with neutral-buffered formalin (10% v/v). After fixation, the intact aorta was gross stained with Herxheimer's solution (5 mg/mL Sudan IV Red, 70% (v/v) ethyl alcohol and acetone (1:1, v/v)) and then rinsed with 70% (v/v) ethyl alcohol to determine if there were atherosclerotic lesions.

Statistical analyses: Data are reported as means $\pm$ SEM. Growth data were analyzed using a general linear model (GLM) (Minitab v.15.1, Minitab Inc., State College, PA, USA). Plasma lipid parameters were analyzed using two-way ANOVA, accounting for treatment and time effects, with Bonferroni post-hoc test (GraphPad Software Inc., San Diego, CA, USA). For blood vessel data, concentration-response relationships for each pig (n = number of animals) were determined in replicate (two to eight rings). The maximal response ($E_{\max}$) for each agonist was determined directly from the data. pD_2 values, which are estimates of drug sensitivity (negative logarithm base 10 of the EC_{50} value (M)), were determined from the best-fit curves calculated using a sigmoidal dose

response equations with fixed parameters (minimum value = 0; maximum value = $E_{\max}$; Hill slope = 1; or variable Hill slope for SNP data only) fitted to the averaged data in each group (Kagota et al. 2011). An F test was used to determine whether the variables (pD_2 , hill slope for SNP, $E_{\max}$) describing the best-fit curves ($r^2 > 0.90$) were the same between the two groups. Pearson correlations were performed to assess relationships between some variables. Statistical significance was set at $P \leq 0.05$ for all analyses.

Results

Growth: Because sexual maturity in Yucatan pigs ranges from 4 to 7 mo old (Swindle 2007), we partitioned the growth data by developmental periods (Table 1). Birth weights of the IUGR piglets averaged ~30% lower than the NW piglets, and the difference remained significant until 4 mo old; however, by 7 mo old, there were no significant differences in body weight between groups. The catch-up growth in IUGR pigs was due to higher fractional growth rates in body length and abdominal circumference parameters from 0 to 1 mo old, with greater fractional growth rate in body weight from 0 to 4 mo old. IUGR pigs had significantly greater abdominal circumference per body weight from birth until 4 mo old; abdominal circumference measurements were correlated with percent visceral fat at 10 mo old ($r = 0.76$, $P = 0.004$) (Fig. 1). Pre-sexual maturity (i.e. 1-4 mo old), IUGR pigs had greater average daily feed intake and feed efficiency. By the end of the study at 10 mo old, there were no significant differences in body weights between groups, and IUGR pigs had more subcutaneous fat compared to large littermates (0.90 ± 0.18 vs. 0.82 ± 0.17 mm back fat/kg body weight, $P = 0.04$).

Fasting lipid concentrations: Because pigs, like humans, spend a large percentage of their waking hours in the postprandial state, elevated fasting plasma TG may be indicative of delayed TG clearance. From 5 mo old until the end of the study at 10 mo old, plasma fasting TG levels were higher in IUGR pigs compared to NW pigs (Fig. 2A) ($P = 0.01$). Conversely, although fasting total cholesterol decreased as the animals aged, there were no differences between groups (Fig. 2B). At 10 mo old after an overnight fast, only plasma chylomicron TG was higher in IUGR pigs; TG or total cholesterol in other plasma fractions were not different between groups (Table 2). Liver TG and total cholesterol concentrations were also significantly greater in IUGR pigs (Table 2).

Oral fat tolerance test: After the bolus fat challenge, the AUC of chylomicron plasma TG concentrations was greater in the IUGR compared to NW littermates suggesting delayed postprandial TG clearance (Fig. 3). Further analysis of the response curves indicated significantly greater peak TG area, higher peak TG concentration and adjusted peak TG in the IUGR pigs (Table 3); time to reach TG peak tended to be longer in IUGR ($P = 0.09$). By 9 h after the meal, the TG concentrations of all the NW pigs returned to baseline; in contrast, TG concentrations in all of the IUGR pigs failed to return to baseline values by 10 h.

Vascular reactivity: Contractions of arterial rings by thromboxane receptor agonist U46619 did not differ between NW and IUGR groups (Fig. 4A), indicating there were no differences in vascular smooth muscle contractility e.g. over-responsiveness. In arteries from IUGR pigs, the endothelium-dependent relaxations by bradykinin (Fig. 4B) and PAR2-activating peptide were not different than in NW pigs (Fig. 4C) ($P > 0.05$), suggesting that the endothelium's vasodilator function was normal in IUGR pigs.

Similarly, the direct acting vascular smooth muscle relaxant SNP (Fig. 4D) was equally effective in both groups, supporting evidence that smooth muscle responsiveness to exogenous nitric oxide was normal in the coronary circulation. Using the Sudan IV staining technique, we observed no visible evidence of fatty streak formation in the aortae of any of the pigs (data not shown).

Discussion

One of the most interesting findings in the present study was that early adaptation in IUGR Yucatan miniature swine had a deleterious impact on lipid metabolism during adolescence and early adulthood. IUGR pigs had persistent elevation of fasting plasma TG concentrations as well as significantly delayed postprandial clearance of TG after an oral fat challenge. Small birth weight with early catch-up growth also led to greater adiposity, which may have played a role in the development of dyslipidemia, possibly via altered hepatic lipid metabolism.

From 5 to 10 mo old, IUGR pigs had consistently higher fasting plasma TG levels relative to their siblings (Fig. 2A). Persistent elevation of fasting plasma TG levels may be indicative of delayed postprandial TG clearance, suggesting defects in lipid metabolism; the OFTT was used to assess postprandial TG responses. The higher total postprandial TG concentration (i.e. total peak area) during the OFTT suggested that IUGR pigs had a reduced ability to metabolize dietary lipids due to impairment in lipid metabolic processes. First, the tendency for a longer time to reach peak chylomicron-TG concentrations for IUGR pigs may suggest problems with digestive processes, such as pancreatic lipase secretion and/or activity, or packaging of chylomicrons. Moreover, the

higher peak TG concentration indicated that IUGR pigs may have a lower ability to clear circulating chylomicron-TG. Chylomicrons are TG-rich lipoproteins that are synthesized and secreted by the enterocyte to transport dietary fat to peripheral tissues. In the postprandial state, the clearance of circulating chylomicrons is largely dependent on adipocyte and muscle lipoprotein lipase (LPL) activities (Watts et al. 1998; Paglialunga and Cianflone 2007). Because LPL activity has been shown to be lower in piglets with IUGR (Liu et al. 2013; He et al. 2015), the reduced ability of IUGR pigs to clear circulating chylomicron-TG in our study could possibly be due to a lower LPL activity relative to their larger littermates. We conclude that IUGR pigs have problems clearing postprandial TG resulting in higher fasting and postprandial plasma TG concentrations; impairments of chylomicron metabolism and LPL activity should be the focus of future investigations into potential mechanisms for these outcomes. Regardless, this delayed postprandial clearance of TG-rich chylomicrons with fetal growth restriction is considered pro-atherogenic and could lead to vascular dysfunction (Goldberg et al. 2011; Kolovou et al. 2011; Klop et al. 2012).

The liver is a key regulator of overall plasma lipid levels (Nguyen et al. 2008), with some research suggesting that delayed clearance of TG after a high fat meal might be related to the presence of a large pool of endogenous TG in the liver and plasma (Watts et al. 1998; Paglialunga and Cianflone 2007). Consistent with this hypothesis, in this study, the prolonged increases in postprandial plasma TG concentrations from the OFTT in IUGR pigs corresponded to chronically elevated fasting plasma TG concentrations as well as higher hepatic concentrations of total cholesterol and TG at necropsy. Others have similarly demonstrated higher total cholesterol and TG

concentrations in plasma and liver of IUGR piglets (He et al. 2015), likely because IUGR piglets have lower hepatic LPL activity (Liu et al. 2013). The combination of hepatic lipid dysregulation and accumulation is a major mechanism of non-alcoholic fatty liver disease (Wang et al. 2014). Indeed, the risk for fatty liver disease is 4 times higher in children born with IUGR versus normal weight (Nobili et al. 2008). Overall, it appears that altered hepatic lipid metabolism is programmed early in life with IUGR and persists into adulthood. This is consistent with other research suggesting that an adult's lipid profile may depend in part on the conditions under which the liver was developed before birth (Souza-Mello et al. 2007; Wang et al. 2009; Heltemes et al. 2010).

Results of this study showed that small birth weight with early catch-up growth also leads to increased adiposity, which may play a role in the development of dyslipidemia. The *ad libitum* feeding regimen allowed us to demonstrate that the IUGR pigs had greater feed intake compared to their siblings, which consequently led to early catch-up growth prior to sexual maturity (i.e., ~4 to 7 mo old in Yucatan miniature pigs; Swindle 2007). Although mechanisms are unclear, this early compensatory growth involved accelerated length and weight growth in the first few weeks, which is a common phenomenon in animals born small (McMillen and Robinson 2005). However, beyond this early 'window' of compensatory growth, higher weight gain is likely associated with greater adipose tissue gain rather than lean tissue gain. Indeed, we found that this early catch-up growth was associated with increased adiposity in adult IUGR pigs, as measured by back fat thickness. In pigs, back fat thickness measurement can be used as an indicator of body fatness as it is positively correlated with whole body or carcass fatness (Doornenbal 1967; Fortin and Elliot 1985). Indeed, in this study, back fat thickness was

significantly correlated with chemically determined carcass fatness ($r = 0.40$, $P = 0.045$). Consistent with our current data, other epidemiological and animal studies have shown that rapid catch-up growth following IUGR leads to increased adiposity, which was associated with an elevation in fasting plasma lipid concentrations (Remacle et al. 2004; Boden 2008). This accumulation of fat in adipose tissue could be the consequence of higher hepatic TG concentrations and impaired chylomicron clearance in IUGR pigs.

However, central body measurements, such as abdominal circumference, reflect visceral fat and are therefore considered better markers of cardiovascular disease risk outcomes (Barker et al. 1993; Laurén et al. 2003). In this study, we found that the IUGR pigs experienced early abdominal circumference ‘growth’ rate (Table 1), and throughout the study, abdominal circumference measurements were highly correlated with percent visceral fat content of the body at necropsy ($r = 0.76$, $P = 0.004$). Because average daily feed intake did not differ between groups, it is possible that IUGR pigs had lower basal metabolic rate (BMR) or activity levels. In the blood pressure telemetry measurements in these pigs (Myrie et al. 2011), average ‘locomotor activity’ during the 48 hours of continuous measurements was not different (IUGR: 1.85 ± 0.36 arbitrary units vs NW: 1.48 ± 0.16 ; $P = 0.46$) (unpublished data); however, it should be noted that these pigs were not particularly active (i.e., ~70% inactive during light hours). A lower BMR in IUGR pigs is possible, although we do not have any direct measurements. Regardless of the mechanism, in terms of cardiovascular disease risk factors, intra-abdominal fat is a more important risk factor than overall weight (Montague and O’Rahilly 2000). Moreover, intra-abdominal fat increases the clusters of risk factors associated with the metabolic syndrome (i.e. glucose intolerance, dyslipidemia, hypertension and obesity)

(Despres 1993). Although none of the pigs in this project presented with glucose intolerance (McKnight et al. 2012), we found dyslipidemia, increased adiposity and higher blood pressure (Myrie et al. 2011) in IUGR pigs. Overall, the data suggest that the early increase in abdominal circumference in the IUGR pig is partly due to increased deposition of intra-abdominal fat, which could lead to hepatic TG accumulation and the observed dyslipidemia.

Impaired postprandial dyslipidemia combined with higher blood pressure in IUGR pigs (Myrie et al. 2011) suggests endothelial dysfunction could be involved. We hypothesized that IUGR pigs may show evidence of altered vasoreactivity in the coronary circulation, which could be evidence of either smooth muscle or endothelial cell dysfunction. There were no differences in vascular smooth muscle contractility or endothelium-mediated vasodilation responses between groups. Vasodilations of pig epicardial coronary arteries by both bradykinin and a PAR2 agonist were normal in the IUGR group, which suggests that synthesis of endothelium-derived prostanoids and nitric oxide were unaffected. Increasing evidence suggests a role for PAR2 promoting inflammation and metabolic dysfunction in obesity, diabetes and metabolic syndrome (Kagota et al. 2016). In a rat model of fetal programming, adult offspring of fat-fed and chow-fed rat dams also did not show evidence of endothelial dysfunction despite raised blood pressure (Khan et al. 2004). They suggested that the endothelial 'protective' trends in their rat model might be related to improved insulin sensitivity due to reduced plasma glucose and TG concentrations (Khan et al. 2004). However, we previously found no significant differences in plasma glucose concentrations, glucose tolerance or insulin sensitivity between IUGR and NW pigs (McKnight et al. 2012). Thus, factors other than

insulin sensitivity may contribute to endothelial ‘protection’ in the IUGR pigs in our model. Furthermore, despite chronically higher fasting and postprandial TG levels in the IUGR pigs, gross staining indicated no visible evidence of fatty streak formation in the aortae of any of the pigs in the study. Overall, the lack of arterial fatty streaks or endothelial dysfunction in these pigs is probably related to their still fairly young age (i.e. early adulthood) and the fact that the increase in blood pressure was rather modest at 4-7 mmHg (Myrie et al. 2011); moreover, the lifelong diet was not designed to be atherogenic.

In summary, the results of the present study indicate that IUGR followed by early postnatal catch-up growth led to increased adiposity and altered lipid metabolism in Yucatan miniature swine during early adulthood. In this model, both postprandial and fasting levels of TG were negatively associated with birthweight, consistent with epidemiological studies (Laurén et al. 2003). Such altered metabolism occurred even on a healthy lifelong diet and may contribute to early development of metabolic syndrome.

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Table 1: Growth rates and feeding characteristics of intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs.

Developmental period	Age	IUGR	NW
Body Weight (kg)			
Birth	1 d	0.73 ± 0.04*	1.11 ± 0.05
Neonate (Weaning)	1 mo	4.60 ± 0.22*	6.04 ± 0.42
Pre-sexual maturity	4 mo	29.54 ± 1.10*	34.19 ± 1.02
Sexual maturity	7 mo	55.70 ± 2.19	60.93 ± 1.46
Post-sexual maturity	10 mo	69.42 ± 3.10	74.48 ± 2.53
Fractional Body Weight Growth Rate (g·kg body weight⁻¹·d⁻¹)			
Neonate	0 - 1 mo	48.4 ± 1.6*	45.0 ± 1.2
Pre-sexual maturity	1 - 4 mo	19.0 ± 0.4*	16.6 ± 0.6
Sexual maturity	4 - 7 mo	7.1 ± 0.4	6.0 ± 0.8
Post-sexual maturity	7 - 10 mo	2.8 ± 0.2	2.6 ± 0.3
Crown-Rump Length (cm)			
Birth	1 d	24.2 ± 0.7*	27.4 ± 0.5
Neonate (Weaning)	1 mo	44.8 ± 1.0*	48.1 ± 0.7
Pre-sexual maturity	4 mo	84.7 ± 1.4*	91.0 ± 1.3
Sexual maturity	7 mo	104.8 ± 1.2*	110.2 ± 1.9
Post-sexual maturity	10 mo	107.3 ± 1.6	111.5 ± 1.4
Fractional Body Length Growth Rate (cm·m body length⁻¹·d⁻¹)			
Neonate	0 - 1 mo	28.1 ± 1.7*	25.0 ± 1.3

Pre-sexual maturity	1 - 4 mo	9.9 ± 0.5	9.8 ± 0.4
Sexual maturity	4 - 7 mo	2.7 ± 0.2	2.3 ± 0.1
Post-sexual maturity	7 - 10 mo	2.3 ± 1.3	1.8 ± 1.3
Abdominal Circumference (cm)			
Birth	1 d	$23.4 \pm 0.6^*$	28.0 ± 0.6
Neonate (Weaning)	1 mo	$42.1 \pm 1.1^*$	46.6 ± 0.8
Pre-sexual maturity	4 mo	$77.7 \pm 1.8^*$	81.9 ± 1.9
Sexual maturity	7 mo	96.3 ± 2.5	100.2 ± 2.6
Post-sexual maturity	10 mo	99.2 ± 2.6	102.8 ± 2.0
Abdominal Circumference per Body Weight ($\text{cm} \cdot \text{kg}^{-1}$)			
Birth	1 d	$32.7 \pm 2.2^*$	25.5 ± 1.5
Neonate (Weaning)	1 mo	$8.9 \pm 0.4^*$	7.5 ± 0.3
Pre-sexual maturity	4 mo	$2.6 \pm 0.1^*$	2.4 ± 0.1
Sexual maturity	7 mo	1.7 ± 0.1	1.6 ± 0.0
Post-sexual maturity	10 mo	1.6 ± 0.1	1.6 ± 0.1
Fractional Abdominal Circumference Growth Rate ($\text{cm} \cdot \text{m abdominal circumference}^{-1} \cdot \text{d}^{-1}$)			
Neonate	0 - 1 mo	$27.1 \pm 2.2^*$	21.7 ± 1.1
Pre-sexual maturity	1 - 4 mo	9.1 ± 0.4	8.4 ± 0.4
Sexual maturity	4 - 7 mo	2.8 ± 0.2	2.6 ± 0.4
Post-sexual maturity	7 - 10 mo	1.2 ± 0.9	1.8 ± 1.1
Average Daily Feed Intake ($\text{g} \cdot \text{kg body weight}^{-1} \cdot \text{d}^{-1}$)			

Neonate [†]	0 - 1 mo	370.2 ± 16.6	364.1 ± 15.2
Pre-sexual maturity	1 - 4 mo	50.9 ± 0.8*	49.1 ± 1.2
Sexual maturity	4 - 7 mo	30.0 ± 0.5	28.4 ± 1.4
Post-sexual maturity	7 - 10 mo	21.2 ± 1.1	21.4 ± 0.4
Feed Efficiency (body weight gain / feed intake) (g·kg⁻¹)			
Neonate [†]	0 - 1 mo	146.3 ± 8.9	140.0 ± 6.7
Pre-sexual maturity	1 - 4 mo	401.7 ± 16.6*	389.8 ± 16.7
Sexual maturity	4 - 7 mo	271.1 ± 22.4	266.4 ± 14.0
Post-sexual maturity	7 - 10 mo	165.2 ± 6.6	150.6 ± 21.5

Note: Data are means ± SEM, *n* = 6 pigs.

*Data are different than NW pigs, *P* ≤ 0.05 as assessed by paired t-test.

[†]Feed intake before 1 month old was reconstituted milk replacer (mL).

Table 2. Liver and plasma triglyceride and cholesterol profile in intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs at 10 months old.

	IUGR	NW
Cholesterol		
Liver, Total ($\text{mmol} \cdot \text{g protein}^{-1}$)	$480 \pm 90^*$	380 ± 31
Plasma, Total ($\text{mmol} \cdot \text{L}^{-1}$)	1.311 ± 0.177	1.236 ± 0.097
Chylomicron ($\text{mmol} \cdot \text{L}^{-1}$)	1.371 ± 0.166	1.299 ± 0.091
VLDL ($\text{mmol} \cdot \text{L}^{-1}$)	0.022 ± 0.013	0.017 ± 0.011
LDL ($\text{mmol} \cdot \text{L}^{-1}$)	0.432 ± 0.119	0.346 ± 0.093
HDL ($\text{mmol} \cdot \text{L}^{-1}$)	0.482 ± 0.121	0.480 ± 0.070
Triglycerides		
Liver, Total ($\text{mmol} \cdot \text{g protein}^{-1}$)	$170 \pm 43^*$	86 ± 18
Plasma, Total ($\text{mmol} \cdot \text{L}^{-1}$)	0.195 ± 0.037	0.145 ± 0.036
Chylomicron ($\text{mmol} \cdot \text{L}^{-1}$)	$0.388 \pm 0.050^*$	0.290 ± 0.052
VLDL ($\text{mmol} \cdot \text{L}^{-1}$)	0.108 ± 0.032	0.129 ± 0.026
LDL ($\text{mmol} \cdot \text{L}^{-1}$)	0.054 ± 0.027	0.063 ± 0.030

Note: Data are means $\pm$ SEM, $n = 6$ pigs.

*Data are different than NW pigs, $P \leq 0.05$ as assessed by paired t-test.

Table 3. Summary of oral fat tolerance test performed in intrauterine growth-restricted (IUGR) and normal weight (NW) Yucatan miniature pigs at 10 mo old.

	IUGR	NW
Baseline TG (mmol·L ⁻¹)	0.42 ± 0.12	0.40 ± 0.06
Total peak area (mmol·L ⁻¹ ·h ⁻¹)	8.95* ± 1.22	6.62 ± 0.84
Peak TG (mmol·L ⁻¹)	2.94* ± 0.40	2.01 ± 0.28
Adjusted [†] peak TG (mmol·L ⁻¹)	2.59* ± 0.40	1.67 ± 0.23
Time to peak TG (h)	6.2 ± 0.4	5.2 ± 0.4

Note: Data are means ± SEM, *n* = 6 pigs. TG, triglycerides.

*Data are different than NW pigs, *P* ≤ 0.05 as assessed by paired t-test.

[†]Adjusted for baseline TG within pig.

Figure Captions

Fig. 1: Visceral fat (%) correlated positively with abdominal circumference (cm) at 10 mo old ($r = 0.76$, $P = 0.004$) in intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs. Each symbol represents an individual pig ($n = 12$): IUGR (■), NW (○).

Fig. 2: Monthly fasting plasma triglyceride (TG) (A) and cholesterol (B) concentrations in intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs. Each symbol represents the mean + SEM in $n = 6$ IUGR pigs and mean – SEM for $n = 6$ NW pigs. There was a main effect of group for TG concentrations ($P \leq 0.05$), but not time, as assessed by two-way ANOVA; Bonferroni post-hoc differences at each time are indicated by * ($P \leq 0.05$).

Fig. 3: Plasma chylomicron triglyceride (TG) concentrations in intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs before and after administration of a high fat meal. Each symbol represents the mean $\pm$ SEM in $n = 6$ pigs for each group. Differences between groups over time were assessed by two-way ANOVA with Bonferroni post-hoc test indicating significance (*, $P \leq 0.01$).

Fig. 4. Vascular reactivity of epicardial arterial rings treated with (A) U46619, (B) bradykinin, (C) PAR2-activating peptide 2fLIGRLO, and (D) sodium nitroprusside. Tissues from intrauterine growth-restricted (IUGR) and normal weight (NW) littermate Yucatan miniature pigs at 10 mo old were mounted under isometric conditions and exposed to increasing concentrations of agents. Symbols are the mean $\pm$ SEM for $n = 6$ pigs per group. The best-fit sigmoidal dose response curves are shown for IUGR (solid

line) and NW (dashed line). For each drug tested, the concentration-response relationships were not different between groups ($P > 0.05$).

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