REVIEW Role of Fetal Programming and Epigenetic Regulation on the Development of Endocrine and Metabolic Alterations

Developmental origins of nonalcoholic fatty liver disease as a risk factor for exaggerated metabolic and cardiovascular-renal disease

Frank T. Spradley, 1,2,3 Jillian A. Smith, 1 Barbara T. Alexander, 2,3 and Christopher D. Anderson 1,2

1 Department of Surgery, Division of Transplant and Hepatobiliary Surgery, School of Medicine, The University of Mississippi Medical Center, Jackson, Mississippi; 2 Cardiovascular-Renal Research Center, The University of Mississippi Medical Center, Jackson, Mississippi; and 3 Department of Physiology and Biophysics, The University of Mississippi Medical Center, Jackson, Mississippi

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INTRODUCTION

Environmental perturbations in early life predispose offspring to chronic disease. This is the basis for the “developmental origins of health and disease” theory set forth by Dr. David Barker and colleagues. The importance of understanding the developmental programming of chronic disease is becoming increasingly relevant due to the rising exposure rates of offspring to poor intrauterine environments, such as maternal smoking and drug use, maternal under- or overnutrition, obesity, maternal diabetes, environmental toxins, or hypertensive disorders of pregnancy (25, 57, 107, 216). Hypertensive disorders of pregnancy, including preeclampsia (PE), affect ~9% of pregnancies in the United States (retrieved February 12, 2018: https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pregnancy-complications-data.htm). Hypertensive disorders of pregnancy are associated with IUGR (216). IUGR programs offspring for obesity, proinflammatory status (157), insulin resistance, and type 2 diabetes mellitus (T2DM) (51).

In addition to metabolic abnormalities, intrauterine growth restriction (IUGR) is associated with developmental programming of cardiovascular-renal disease (CVRD). CVRD is the leading cause of death worldwide, with the main modifiable
risk factor being hypertension (232). Blood pressure in IUGR adolescents is already increased by 6 yr of age (186) implicating a higher risk of hypertension in IUGR offspring as they age. Obesity and resulting metabolic diseases, like T2DM, are thought to contribute to 70% of essential hypertensive cases (85). While evidence demonstrates that T2DM exacerbates hypertensive renal disease in experimental animals (213), it is unknown whether developmental programming of metabolic disease risk factors contributes to exaggerated risk for CVRD in IUGR offspring.

It is well established that nonalcoholic fatty liver disease (NAFLD) and T2DM coexist (38). Yet, how these two morbidities are linked is not fully understood. Several studies implicate a role for insulin resistance in the etiology of steatosis (fatty liver) (97, 176), whereas there is no consensus that NAFLD initiates T2DM (16, 76, 106). Complexity in the timing of presentation of these comorbidities is evidenced in adolescents and young adults, whereby some individuals with T2DM have no evidence of NAFLD at the time of diagnosis of T2DM while others have both T2DM and NAFLD (154). Abnormal birth weight is a suggested contributor to the development of advanced NAFLD in children (153), but it is unclear if a history of complications from IUGR predicts or exaggerates the risk for metabolic disease. Although not all patients with NAFLD and T2DM were IUGR, experimental studies suggest that IUGR programs earlier insulin resistance with additional studies indicating an increased risk for NAFLD in IUGR offspring. It has not yet been examined whether early insulin resistance drives accelerated development of NAFLD in IUGR. It is established that accumulation of hepatic lipids fosters hepatic insulin resistance (165, 218). Furthermore, progression of NAFLD and liver injury may worsen insulin resistance making diabetes more difficult to treat. Liver-specific knockout of the insulin receptor (IR) results in insulin resistance, glucose intolerance, and reduced insulin-mediated suppression of hepatic glucose production suggesting the importance of protecting against hepatic insulin resistance (142). Combined hepatic insulin resistance, NAFLD, and diabetes may contribute to the greater risk for cardiovascular disease (81). Currently, it has not been substantiated whether IUGR hastens the progression of hepatic insulin resistance to NAFLD and liver injury and whether the latter worsens T2DM and diabetes-induced CVRD in these at-risk offspring. Therefore, the purpose of this review is to propose that developmental programming for increased susceptibility to metabolic diseases, like insulin resistance, contributes to the incidence of IUGR-induced chronic disease.

DEVELOPMENTAL PROGRAMMING OF CHRONIC DISEASE: IUGR AND EPIDEMIOLOGY

Barker et al. (23, 24) proposed that developmental programming of chronic disease is dependent on reduced fetal growth independent of gestational age. The definition of impaired fetal growth varies. Low birth weight (LBW) is defined as birth weight <10th percentile (2,500 g) regardless of gestational week. LBW is often used as a surrogate marker for IUGR. IUGR, when associated with an increased brain-to-liver weight ratio, is indicative of asymmetric IUGR and predicts an enhanced risk for future pathologies in the offspring. However, being born LBW is not always associated with inappropriate gestational age at birth. Small-for-gestational age (SGA) is defined as birth weight <10th percentile for gestational week; those associated with IUGR are termed “SGA associated with IUGR.” Alarming, the prevalence of IUGR is increasing in the United States (132).

Although the origins of IUGR are multifactorial, a main cause within the Western world is due to placental insufficiency and ischemia (84). Placental ischemia is an initiating event in PE (75). PE is defined as new-onset hypertension in the presence of neural, vascular, uterine, and/or renal abnormalities in the mother occurring after the 20th week of gestation. PE is a contributor to both maternal and fetal morbidity immediately during pregnancy and long term. PE is one of the major risk factors for IUGR in conjunction with unemployment, smoking, drug abuse, and maternal age >34 yr (109). PE, which includes preterm PE with delivery <34 wk, contributes to a respective 2.7 and 4.3 times increase in the odds of having an IUGR baby at <10 and <5% of fetal growth, as calculated using gestational age at delivery and absolute birth weight (192). There is no current treatment for IUGR beyond close monitoring of the pregnancy and early delivery (22), which highlights the necessity for preclinical research to identify novel prophylactic strategies. Preclinical research using experimental animal models is required to develop cost-effective therapeutic strategies to treat pregnancy-related disorders, such as PE, to attenuate the incidence of IUGR. Furthermore, it is critical to study IUGR offspring to identify mechanistic targets to prevent long-lasting adversities on chronic health, which include the developmental programming of NAFLD and T2DM.

In this review, we will highlight the epidemiology of NAFLD and the evidence suggesting that it magnifies metabolic dysfunction. Next, we will provide an overview of evidence from experimental models that suggest the development of accelerated insulin resistance in IUGR offspring, which is likely mediated via programming of pancreatic β-cell loss and dysfunction of insulin-signaling pathways in adipose tissue and liver leading to steatosis. Earlier onset of insulin resistance may promote more progressive steatosis, NAFLD, and liver injury in patients with a history of IUGR. We will detail mechanisms of liver injury, as discovery of approaches to reverse liver injury may also allow for improvement in clinically uncontrolled metabolic diseases, such as T2DM (52). Lastly, we will explore the hypothesis that metabolic disease mediates the known exaggerated risk for hypertensive CVRD in IUGR offspring with a focus on a role for proinflammatory pathways.

DIAGNOSING NAFLD

NAFLD is the most common form of chronic liver disease (184) and is divided into nonalcoholic fatty liver (NAFL) and its advanced form of nonalcoholic steatohepatitis (NASH). Upon biopsy and histological inspection, NAFL is defined as isolated hepatic steatosis with or without mild lobular inflammation, while advancement to NASH includes features of inflammation and hepatocyte ballooning (39). The appearance of fibrosis is indicative of ongoing NASH (2). Although patients are diagnosed with NAFLD by noninvasive factors, presently, and despite its invasive nature, the gold standard for diagnosing NAFLD is by histological staging of liver biopsies.
(11). This diagnosis is defined by the NAFLD activity score (NAS) and includes the presence of steatosis (0–3), inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–4) (12). NAS scores ≥5 are diagnosed as NASH (110). In a follow-up study of a median 6.6 yr, 37% of NAFL patients and 43% of NASH patients had progressive fibrosis (139). The fibrotic process of NAFLD may be worsened by T2DM, and NAFLD is proposed to render control of diabetes more challenging (38). Therefore, it is important understand the role that NAFLD plays in the etiology of T2DM and whether approaches to target and attenuate this liver disease allow for better control of metabolic disease.

NAFLD IS A MAJOR RISK FACTOR FOR UNCONTROLLED METABOLIC DISEASE

Health care burden of NAFLD. It is observed that 10–20% of patients with NAFL progress to NASH (139, 205), and ~20% of patients with NASH advance to cirrhosis (173). Between 1999 and 2013, the death rate from chronic liver disease and cirrhosis rose by 22% in America (45). In 2013, chronic liver disease and cirrhosis accounted for 101,000 discharges as the first-listed diagnosis and contributed to 36,427 deaths in America (79). According to the National Institute of Diabetes and Digestive and Kidney Diseases, NASH is the third leading cause of cirrhosis after hepatitis C and alcoholic liver disease in patients awaiting liver transplantation ( Cirrhosis; retrieved Sept. 1, 2017: https://www.niddk.nih.gov/health-information/liver-disease/cirrhosis). Forty-six percent of patients with NASH develop decompensated cirrhosis within 10 yr and require transplantation (72, 179). Currently, the only steadfast treatment for liver failure is transplantation (135, 136). NASH has increased as a cause for liver transplantation from 1.2 to 9.7% (128) and is projected to become the primary reason for liver transplantation (3, 220, 236).

NAFLD exaggerates metabolic dysfunction in T2DM. Studies suggest that diagnosing NAFLD may identify those individuals at greater risk for metabolic dysfunction in T2DM (143, 228). Epidemiological studies suggest that NAFLD and T2DM coexist (56, 66). In a Chinese cohort, NAFLD was implicated in advancing T2DM (123). The prominent histological feature in patients with NAFLD that is associated with long-term outcomes, including advancement of T2DM, is liver fibrosis (15). NAFLD-induced liver fibrosis and injury may exaggerate insulin resistance. Indeed, in a Korean population, ultrasonography, fibrotic scoring (49, 196), and the fatty liver index (calculated using body mass index, waist circumference, triglycerides, and GGT levels) revealed that diabetes is greater with increasing NAFLD and liver fibrosis scores (227). The World Health Organization reports that T2DM or high blood glucose is associated with 3.8 million deaths in 2012 (WHO Diabetes Fact Sheet, retrieved October 7, 2017: http://www.who.int/en/news-room/fact-sheets/detail/diabetes). T2DM is projected to be the seventh leading cause of worldwide death by 2030 (137) underscoring the importance of understanding the mechanisms that encourage its pathology, which includes determining the impact of developmental programming of metabolic disease risk factors, like NAFLD.

Do IUGR and developmental programming contribute to the increasing incidence of NAFLD? In America, between 80 and 90% of obese adults have some level of NAFLD, resulting in ~95 million Americans with some stage of the disease. The incidence of NAFLD is increasing in all races in the United States and throughout the world, including in adolescents (215). The incidence has risen in obese children to 40–70% (58). The rising prevalence of NAFLD may be partly explained by fetal programming of NAFL and NASH by the in utero environment. Children with LBW and high birth weights are at increased odds for this disease compared with their normal birth weight counterparts (153). Moreover, experimental studies highlight that early adversities predisposed offspring for chronic metabolic disease are suggested in male rats born from high-fat diet (HFD)-fed dams that develop exaggerated HFD-induced obesity, increased liver weight and circulating leptin levels, significantly reduced adiponectin levels, an insulin-sensitizing adipokine, and insulin resistance (185, 202). Similar diet protocols in male mice offspring from dams on a HFD have exaggerated HFD- or methionine choline-deficient diet-induced NASH, as detected by profibrotic gene expression (150, 214). To aid in understanding the mechanisms mediating fetal programming of chronic disease in adult offspring, including NAFLD, researchers have utilized the knowledge that placental ischemia and insufficiency are potent stimuli for IUGR. Experimental animal models of placental ischemia and insufficiency have been developed to study the impact of IUGR on programming of chronic disease.

EXPERIMENTAL MODELS OF IUGR

Models of placental ischemia and insufficiency used to identify placental factors mediating IUGR. Induction of placental ischemia and insufficiency contributes to the pathogenesis of hypertension in PE and the development of IUGR. The model of surgical-induced reduced uterine perfusion pressure (RUPP) promotes hypertension in the dam (Fig. 1A) and reduces placental mass (Fig. 1B) and fetal weight (Fig. 1C) by gestational day 19 in normotensive pregnant rats. To produce the RUPP model, a silver clip is placed on the abdominal aorta, directly above the iliac bifurcation and on both branches of the ovarian arteries at day 14 of gestation. At delivery, the RUPP dams give birth to male and female pups with reduced body weight indicative of IUGR (Fig. 1D). Along with the RUPP rat, additional experimental models are proving useful to support the causal role of placental insufficiency on inducing IUGR and that these IUGR offspring are programmed for increased odds of chronic disease as they age.

Antiangiogenic placental factors induce IUGR. It is reported that factors released from the ischemic placenta induce IUGR. Circulating and placental levels of the angiogenic factor soluble Flt-1 like tyrosine kinase (sFlt-1), which binds and quenches bioavailable vascular endothelial growth factor and placental growth factor that are important for maintenance of vascular health, are increased in RUPP dams (191). In pregnant Sprague-Dawley rats, chronic excess levels of sFlt-1 elicit hypertension and IUGR (37). Furthermore, adenosine-driven chronic increases in sFlt-1 during pregnancy in mice result in smaller offspring at weaning and a significant increase in blood pressure and vascular dysfunction in adult male but not female IUGR offspring (41) (130). When chronic sFlt-1 excess is
combined with maternal HFD, these offspring have a further increase in mean arterial pressure and circulating inflammatory markers relative to IUGR counterparts retained on regular fat chow (42). Furthermore, the chronic sFlt-1 excess model is associated with mechanisms that support the pathogenesis of NAFLD. Offspring of chronic sFlt-1 dams have increased fetal hepatic peroxisome proliferator-activated receptor (PPAR)-α levels (194). In contrast, hepatic mRNA PPAR-α levels are reduced in male and female IUGR rats at gestational day 20 in the nutrient-restricted rodent model of IUGR (238). It has not been examined whether these changes in expression remain as IUGR offspring age. Yet, it is important to define the timing of altered PPAR-α expression and signaling during development. PPAR-α is implicated to mediate early lipogenesis in the development of steatosis (229) whereas it is also reported to be protective against inflammation and fibrosis in progression to NASH (161). The role of hepatic PPAR-α in controlling the progression of NAFLD and liver injury has not been examined in adults from IUGR models of developmental insult. Moreover, it is unclear if antiangiogenic factors are involved in fetal programming of liver disease directly by signaling in the fetal liver or indirectly by encouraging placental insufficiency and IUGR.

**Proinflammatory placental factors induce IUGR.** Inflammatory factors contribute to the etiology of IUGR. In the RUPP dams, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and agonistic autoantibodies to the angiotensin II type 1 receptor (AT1-AA) are elevated and additional studies indicate each of these contribute to the development of maternal hypertension by late gestation in this model of PE (190). Adoptive transfer of CD4+ T cells or B cells from RUPP dams induces hypertension and increase sFlt-1 and TNF-α levels in once normotensive pregnant rats. Although sFlt-1 or TNF-α antagonism reduces blood pressure in RUPP dams, long-term fetal metabolic or CVRD outcomes of such treatments have only just started to be examined. Pharmacological-induced proliferation of endogenous anti-inflammatory regulatory T cells using a rat CD28 superagonist improves blood pressure by 50% in the RUPP hypertensive model reducing circulating levels of agonistic autoantibodies to the angiotensin II type 1 receptor and a slight improvement in fetal weights (90). This improvement in maternal blood pressure and fetal weights is also found following antibody-mediated depletion of natural killer cells in RUPP dams (60), although this did not increase fetal weights to those values observed in normal pregnant dams. The inability of these treatment strategies to fully restore fetal weight in RUPP dams is most likely due to the chronic constriction produced by the clipping procedure. Thus a shortcoming of the RUPP model is the inability to fully realize the potential protective impact of targeted in utero therapies against IUGR in the face of mechanical uterine vascular constriction. Genetic models are also available and support the targeting of proinflammatory mechanisms during pregnancy to attenuate IUGR. In pregnant rats that are transgenic for the human renin-angiotensin system, the CD28 superagonist significantly improves fetal weight and brain-to-liver weight ratio without reducing maternal blood pressure (169). In summary, these data collectively support that the placental ischemia-derived milieu of antiangiogenic and proinflammatory factors induce IUGR. As mentioned above, investigators have used IUGR offspring from models of placental ischemia and insufficiency to highlight the impact that IUGR has on developmental programming of CVRD and metabolic diseases. We propose that this predisposition for metabolic disease synergizes with the programming of cardiac and renal abnormalities in IUGR offspring to accelerate the progression of CVRD-related morbidity and mortality.

**Developmental programming of cardiac and renal abnormalities in IUGR offspring.** Various preclinical models of IUGR are utilized to investigate the developmental programming of CVRD. These include models of surgical-induced placental ischemia and insufficiency, including induction of the RUPP procedure in rats (191) and mice (96), dams with bilateral uterine ligation (189), or maternal hypoxia (178);
infusion of antiangiogenic or proinflammatory factors into dams (41, 68); and models of dietary-induced placental insufficiency, such as maternal protein restriction (74, 208), maternal HFD or high-sucrose diets (67, 182), or maternal administration of betamethasone (34). Importantly, it is demonstrated by utilizing each of these models that there are overarching similarities by which IUGR offspring are predisposed to CVRD, which supports the developmental programming of chronic disease. IUGR offspring from many of these models have altered cardiomyocyte biology, including early reductions in cardiomyocyte numbers reported in offspring from dams with bilateral uterine ligation (30), decreased cardiomyocyte proliferation by postnatal day 1 in offspring of dams with maternal hypoxia (171), decreased cardiac expression of genes involved in heart’s ability to produce energy from fatty acid oxidation in mice offspring exposed to maternal HFD by 27 wk of age (112), and increased contractile function of isolated left ventricles by 12 wk of age in offspring from nutrient-restricted dams (80). Moreover, data from these various models of developmental programming of CVRD in IUGR offspring show reductions in nephron endowment. For example, a reduced nephron complement is observed in IUGR offspring following maternal uterine ligation (71), maternal hypoxia (223), maternal low-protein diet, and maternal betamethasone administration (34). The reduced nephron number is also detected in human IUGR offspring (102). This nephron deficit is accompanied by persistent glomerular hyperfiltration to maintain renal excretory function at the cost of accelerated development of renal injury, loss of renal function, and hypertension (131). Indeed, glomerular sclerosis and increased blood pressure are present by 4 mo of age in offspring of rabbits exposed to maternal HFD and high-sucrose diets (221) and in IUGR rats from dams with altered nutritional status (35). Hypertension and vascular dysfunction, noted by reduced endothelium-dependent vasorelaxation, are evident by adulthood in offspring from RUPP dams (5, 162), bilateral uterine ligation (30), maternal excess of sFlt-1 (41, 130), and HFD (221). Coronary artery endothelial dysfunction is present in IUGR in offspring from ewes with placental disease produced by placental embolization following injection of microspheres into the fetal circulation (40). In summary, the utilization of different experimental models of IUGR provide credence to the developmental programming of CVRD in offspring (14).

Developmental programming of accelerated insulin resistance in IUGR offspring. Experiments utilizing a number of IUGR models have implicated reduced maternal health in the developmental programming of metabolic dysfunction, including mechanisms related to insulin resistance, hepatic steatosis, and gluconeogenesis (122). Fetuses of obese mothers have insulin resistance when examined in utero (47). Moreover, in a series of elegant studies from the laboratory of Simmons et al. (28, 54, 175, 189, 193), they demonstrated that IUGR is associated with abnormal insulin biology. These studies indicated that bilateral ligation of the uterine arteries in the pregnant rat produces metabolic abnormalities in offspring including increased basal insulin secretion, hepatic glucose production, hyperinsulinemia, and hyperglycemia and attenuated glucose-stimulated insulin secretion. β-Cell dysfunction is detected in IUGR from models induced via maternal nutrient restriction (28, 54, 175, 189, 193) and bilateral uterine ligation (189, 193). Also, reduced insulin-mediated suppression of hepatocyte glucose production is detected in IUGR offspring from bilateral uterine artery-ligated dams (99, 170). These hepatic changes occur by 6 days of life and before the onset of hyperglycemia at 3–6 mo old (211). Prevention of reductions in β-cell vascularity and islet number and increased inflammation by treatment in early life with IL-4-neutralizing antibodies reduced T-helper 2 lymphocytes and macrophages and abolished fasting hyperglycemia, but assessments of hepatic insulin signaling were not reported. Fasting hyperglycemia is also detected in male and female IUGR offspring from RUPP rats (93, 94). Under standard chow conditions, by 12 mo of age, male IUGR offspring from RUPP dams have an increased area under the curve to glucose tolerance testing without a significant change in insulin secretion in response to a fasting blood glucose challenge (94). Interestingly, these male IUGR offspring had increased fasting glucose (Fig. 2), which is indicative of hepatic insulin resistance (167), and a tendency for increased liver fat content without dramatic changes in body fat composition compared with their normal birthweight controls. In females, although steatosis was not examined, IUGR offspring from RUPP have increased body fat, reduced oral glucose tolerance, and increased fasting blood glucose. In these IUGR females, there was unaltered insulin tolerance or circulating insulin levels but glucose simulation of insulin release is impaired in response to a glucose challenge in the fasted state (93). Molecular mechanisms whereby female IUGR offspring from RUPP have impaired glucose homeostasis may involve lower pancreatic expression of glucose transporter (GLUT)2. There is no difference in muscle GLUT4 or IRβ expression, but dorsal white adipose tissue has reduced GLUT4 and increased IRβ expression implicating dysfunctional insulin signaling in adipose tissue as a contributor to developmental programming of hyperglycemia. Thus several models of IUGR provide evidence for developmental programming of insulin resistance in males and females. Whether the mechanisms leading to this point are similar between both sexes or involve sex-specific mechanisms including a role for the sex hormones is understudied. Overall, these data implicate IUGR in the acceleration of insulin resistance in these at-risk offspring. However, it is not yet known if IUGR-mediated programming of insulin resistance fosters a heightened risk for development of NAFLD (177).

Fig. 2. By 12 mo of age, male intrauterine growth restriction (IUGR) offspring from reduced uterine perfusion pressure (RUPP) pregnancies have elevated fasting glucose levels on normal chow diet. *P < 0.05 vs. control offspring from normal pregnant rats. [Adapted from Intapad et al. © 2017 (94), licensed under Creative Commons Attribution CC-BY 4.0.]
PROGRAMMING OF INSULIN RESISTANCE IN IUGR OFFSPRING AND ITS IMPACT ON PROMOTING NAFLD

In humans, IUGR is associated with the development of insulin resistance and T2DM (217). As stated above, studies indicate that the predisposition for T2DM in IUGR offspring is dependent on reduced pancreatic β-cell size (32). However, experimental evidence suggests that additional mechanisms outside of the pancreas contribute to developmental programming of insulin resistance in IUGR offspring. In a study by Um et al. (207), mouse dams with global S6 kinase (S6K1) deficiency resulted in reduced placental weight and IUGR offspring that exhibit reduced β-cell number and size and lower whole body insulin content but increased insulin sensitivity. This latter finding is due to the ability of this enzyme to reduce insulin signaling. To determine if the reduced placental weight potentially mediates β-cell dysfunction in these knockout offspring, placental growth was restored in the knockout offspring using a technique to generate blastocyst-stage embryos, which were produced by aggregation of tetraploid wild-type embryos with diploid S6K1 knockout embryonic stem cells. These were implanted into uteri of pseudopregnant CD1 outbred female recipient mice. At embryonic day 16.5, the fetuses and placentas were examined. These fetuses were genotyped and determined to be S6K1 knockouts, but they had increased placental weight and rescued fetal growth. However, there was still lower fetal insulin content in the S6K1 knockout mice even though there was restoration of placental weight. This implicates S6K1 as being critical for proper insulin production regardless of placental disease and IUGR. Conversely, repression of S6K1 specifically in β-cells of S6K1 global knockout embryos restored circulating insulin levels but did not protect against IUGR in these offspring. This pancreatic repression of S6K1 in the S6K1 knockout offspring did not completely restore the insulin tolerance test to levels seen in control wild-type normal birthweight offspring. Thus dysfunction of pancreatic S6K1 during fetal development is capable of reducing β-cell growth and inducing the long-term development of insulin resistance regardless of IUGR. However, another important take-home message from the studies by Um et al. is that manipulation of pancreatic S6K1 did not completely restore insulin tolerance in IUGR offspring, but the contribution to changes in extra-pancreatic tissue S6K1 expression was not studied. Others have shown that skeletal muscle and liver are sites for increased S6K1 signaling in the development of insulin resistance in HFD-induced obesity (188), but it is unknown whether changes in S6K1 contribute to the insulin resistance found in adult IUGR offspring from dams exposed to hypoxia (177) or other models of IUGR. Interestingly, S6K1 expression is reduced in myotubules isolated from skeletal muscle of neonatal pigs with reduced birth weight (50) suggestive of early compensatory mechanisms to preserve insulin sensitivity in the various IUGR models mentioned above with reduced insulin production. However, the temporal role of S6K1 as a regulator of insulin signaling, including its role in adipose tissue or liver, in IUGR offspring requires further investigation. In humans, expression of S6K1 in visceral adipose tissue is upregulated in obesity and associated with insulin resistance and inflammation (46), but the impact of IUGR of these signaling pathways is unknown. Collectively, these studies advocate for further study of mechanisms whereby IUGR provokes insulin resistance to identify potential therapeutic targets to intervene in developmental programming of metabolic disease.

Insulin resistance in adipose tissue. Several studies suggest that proper insulin signaling in adipose tissue promotes glucose homeostasis. While overstimulation of insulin signaling in adipose tissue promotes obesity and hyperglycemia (31), insulin resistance in adipocytes attenuates glucose uptake and contributes to hyperglycemia (198). Adipose tissue inactivation of GLUT4 results in blunted insulin-stimulated glucose uptake, glucose intolerance, and hyperinsulinemia along with attenuated insulin-mediated prevention of β2-adrenergic receptor-induced lipolysis in small and large adipocytes (1). As mentioned above, female IUGR offspring from RUPP dams have adipose tissue inactivation of GLUT4 resulting in reduced insulin-stimulated glucose uptake in muscle, although GLUT4 expression remains normal in this tissue, and reduced insulin-mediated suppression of hepatic glucose production. Intriguingly, ewes with spontaneous IUGR have increased fetal hepatic gluconeogenic capacity marked by increased expression of proliferator-activated receptor-γ coactivator-1α and phos-cAMP-response element-binding protein/total cAMP-response element-binding protein ratio in their livers (203) (Fig. 3). With regards to NAFLD, studies suggest that compensatory mechanisms allow buffering of insulin-mediated lipolysis and subsequent ectopic accumulation of hepatic lipids, but it is unknown if these systems fail leading to increased risk for NAFLD and metabolic dysfunction in the face of IUGR. For example, adipocytes from obese humans and mice have increased expression of Snail1, which is a Snail transcription factor family member shown to inhibit adipocyte differentiation and expression of lipogenic and gluconeogenic genes. Insulin stimulates production of Snail1. Mice having adipocyte deletion of Snail1 have increased adipose tissue lipolysis and liver fat under normal or HFD conditions (195), but this is not yet studied in developmental programming of insulin resistance in IUGR offspring.

Pathological insulin signaling in immune cells might also lead to downstream adipose tissue dysfunction and allow for the progression of NAFLD. Increased insulin signaling in inflammatory cells is implicated in development of adipose

![Fig. 3. Hepatic protein expression of gluconeogenic factors in intrauterine growth restriction (IUGR) ewes at approximately gestation day (GD) 134, which were generated by exposing pregnant ewes to elevated ambient room temperature from approximately GD 38–120. Control fetuses were from pregnant ewes exposed to normal ambient temperatures. *P < 0.05 vs. control. Adapted from Thorn et al. (203) with permission from Oxford University Press. Copyright © 2009 The Endocrine Society.]
tissue dysfunction. In myeloid cells, IR deficiency attenuates HFD-induced macrophage invasion into white adipose tissue and circulating TNF-α (138). In these mice with IR deficiency in their myeloid cells, the euglycemic- hyperinsulinemic clamp studies reveal that they are protected against obesity-induced insulin resistance by decreased basal hepatic glucose production and increased insulin-stimulated glucose disposal in skeletal muscle. These studies suggest that increased insulin signaling in inflammatory cells during obesity leads to hepatic insulin resistance. This may act in concert with the finding that, in mice, hyperinsulinemia leads to reduced hepatic insulin receptor substrate (IRS)-2 expression while continuing to increase production of sterol regulatory element-binding protein-1c (SREBP-1c), a transcription factor that elicits fatty acid synthesis (187). In IUGR female rats from food-restricted dams, there are slight increases in SREBP-1c mRNA levels by gestational day 20 that persist as they age (238). These data allude to increases in insulin signaling in immune cells and adipose tissue, but insulin resistance in liver, as pathogenic in the development of NAFLD. It remains to be determined whether inflammatory mechanisms mediate these NAFLD and metabolic responses in IUGR in both males and females, especially in the face of diet-induced NAFLD.

Increased inflammation in adipose tissue is thought to maintain ectopic lipid accumulation in NAFLD and propagation of hepatic insulin resistance and T2DM (101, 180). This is based on evidence that hepatic lipid overload is mediated by increased hepatic de novo lipogenesis from glucose diverted from lipid-overloaded, insulin-resistant skeletal muscle. This excess glucose reduces hepatic insulin sensitivity and glycogen storage and allows for lipogenesis and increased release of VLDL cholesterol. The resulting hyperglycemia and hyperlipidemia subsequently leads to adipose tissue inflammation, which directs additional fat to the liver culminating in hepatic gluconeogenesis and maintained hyperglycemia (209). In mesenteric white adipose tissue, a visceral adipose tissue depot, inflammation and IL-6 signaling promote lipolysis and contribute to obesity-associated hepatic steatosis and insulin resistance (222). In humans, omental fat IL-6 mRNA levels are positively correlated with hepatic steatosis and insulin resistance and the greater visceral adipose-to-subcutaneous adipose tissue ratio in these individuals. Several studies suggest that visceral adipose tissue produces greater proinflammatory cytokines, like IL-6, than subcutaneous fat (144). Infiltration of macrophages in visceral adipose tissue in obese adolescents is associated with reduced ability of subcutaneous adipose tissue to expand and store lipids along with hypertriglyceridemia, hypoalphaproteinemia, and systemic insulin resistance (113). Collectively, these studies suggest that shunting of excess lipids to visceral adipose depots or ectopically to organs, like the liver, sets off a cascade of events that promote inflammation and the onset of metabolic dysfunction. A complete understanding of how alterations in adipose tissue expansion and inflammation encourage the progression of NAFLD during the developmental programming of hyperglycemia and diabetes is warranted.

**Adipocyte dysfunction and inflammation.** Adipocyte expansion allows for proper lipid storage in times of energy surplus (147). However, a chronic increase in adipocyte size is an independent risk factor for NAFLD (166). In obesity, adipocyte hypertrophy is associated with adipocyte apoptosis (9) and the onset of inflammation (36) and steatosis (10). In humans, increased adipose tissue expression of proinflammatory cytokines is associated with the severity of NAFLD (103). In mice, inhibition of adipocyte apoptosis by knocking out Bid, a proapoptotic member of the Bcl-2 family, protects against macrophage infiltration into adipose tissue and the development of hepatic steatosis (8). Studies support that this subsequently leads to the development of hepatic insulin resistance. One potential mechanism mediating this response is increased adipose tissue SREBP-1α function. SREBP-1α, sterol regulatory element-binding protein-1α, is a transcription factor that increases expression of enzymes in adipose and liver involved in fatty acid and cholesterol synthesis (13). SREBP-1α overexpression in adipose tissue in mice results in adipocyte hypertrophy, increased fatty acid secretion, increased circulating free fatty acids, and steatosis (86). This is associated with increased white adipose tissue levels of fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase, and acetyl-coA carboxylase, which are enzymes linked to biosynthesis of fatty acid and triacylglycerol. Increases in these enzymes are detectable in adipose tissue isolated from models of IUGR. In an IUGR rat model produced by maternal food restriction, male IUGR offspring at 6 mo of age have greater fatty acid de novo synthesis in both subcutaneous and visceral adipose tissues with increases in acetyl-coA carboxylase-α and fatty acid synthase in these tissues (231) (Fig. 4). In pigs with IUGR, which are defined as newborns with a body weight of 2 SD below the average birth weight of all piglets from the same litter, HFD feeding from postnatal days 28–178 results in greater body fat mass, circulating triglycerides, and insulin along with total hepatic and semitendinosus muscle fat (230). Hepatic IL-6 mRNA levels are greater in IUGR pigs when fed HFD (124). In a guinea pig model of IUGR induced surgically by uterine artery ablation, there are increased lipid synthesis-related genes in visceral epididymal white adipose tissue (181). Thus there is valid rationale to examine the pathways of adipose tissue expansion, hypertrophy, and inflammation in the pathogenesis of metabolic dysfunction and NAFLD in IUGR offspring.

**Adipokines in the pathogenesis of NAFLD.** Adipose tissue also produces adipokines that have a role in the pathogenesis of NAFLD. Preventing adipose tissue hypertrophy in global knockout of caspase-2 mice prevents HFD-induced abdominal adiposity, T2DM, dyslipidemia, and steatosis (133). Furthermore, the epididymal white adipose tissue in the caspase-2 knockout mice is more proliferative with smaller adipocytes; they have increased mitochondrial function suggestive of ability to burn energy surplus; and they are resistant to adipocyte hypertrophy and death. These changes correspond with alterations in circulating adipokine levels noted by lower leptin and greater adiponectin levels mirrored by their expression in the fat tissue. In humans, in both lean and overweight individuals, the levels of leptin increase with the severity of NAFLD (87), and there are increased circulating leptin receptor levels in the morbidly obese with insulin resistance and NAFLD (140), suggestive of reduced leptin signaling. It is established that obesity is a state of leptin resistance, and there is hepatic leptin resistance in NAFLD (226). This promotes early fat accumulation, as suggested in mice with selective deletion of leptin receptors in hepatocytes where they have a slight but significant increase in liver triglyceride and cholesterol content (89).
This occurs despite no change in body weight and while being maintained on a standard chow diet. Reversal studies in humans with lipodystrophy found that leptin treatment for ~6 mo results in increased serum leptin from 1.4 ± 0.3 to 25.7 ± 9.0 ng/ml and reduced steatosis (100). However, in the setting of a proinflammatory environment, as mimicked by low-dose endotoxin, leptin exaggerates the progression to NASH during HFD (91). Therefore, the role of leptin in the pathogenesis of progressive NASH is complex with even less understood about its effects in liver pathology in IUGR offspring.

With regards to adiponectin, its levels are reduced in association with increasing adipocyte size (83). Adiponectin is important in maintaining hepatic insulin sensitivity (21). Adiponectin levels are lower in mothers with IUGR (114). With regards to fetal levels, the available data are variable ranging from no change to reduced levels (210), as measured in blood from doubly clamped umbilical cords. By 7 yr of age, adiponectin levels are increased in lean SGA offspring and lower from no change to reduced levels (210), as measured in blood from food-restricted dams vs. control dams present with growth restriction (IUGR) male offspring produced by maternal protein restriction. *P < 0.05 vs. control offspring. [Adapted from Yee et al. (231) with permission from John Wiley and Sons. Copyright © American Oil Chemists’ Society 2016 (AOCS).]

PROINFLAMMATORY MECHANISMS MEDIATING NAFLD-INDUCED LIVER INJURY: ARE THESE MECHANISMS MORE PRONOUNCED IN IUGR TO ACCELERATE ONSET OF CHRONIC LIVER DISEASE?

Immune cells and cytokines. A study by Campanati et al. (43) demonstrated that 24 wk of treatment with etanercept, a soluble TNF-α receptor antagonist, reduces the AST/ALT ratio, circulating levels of the inflammatory marker C-reactive protein, and fasting insulin levels in patients with established NAFLD implicating a role for proinflammatory mediators in the etiology of NAFLD-induced liver injury. The role of TNF-α as a mediator of NAFLD risk is supported by experimental studies, which demonstrate that knockout of the TNF-receptor 1 in mice protects against diet-induced steatosis and liver injury (63). In vitro studies indicate that hepatocyte TNF receptor-associated factor 3 stimulates HFD-induced liver steatosis and insulin resistance (212). In vivo studies in HFD-fed mice demonstrate that TNF-α promotes lipid accumulation in the liver (159). Furthermore, an increase in free fatty acids stimulates TNF-α production in hepatocytes (63), collectively implicating a vicious cycle in the etiology of NAFLD. However, there is a paucity of data linking these proinflammatory mechanisms to NAFLD and liver injury in IUGR.

Although observational studies in humans demonstrate an association between IUGR and NAFLD (7, 61), these reports have not examined whether proinflammatory pathways mediate this increased risk for NAFLD in IUGR offspring. The benefit of using experimental animals is the ability to test the hypothesis that proinflammatory mechanisms mediate an accelerated risk for NAFLD in IUGR offspring. This has not yet been tested directly, but studies provide rationale to the validity of this hypothesis. Adult male and female IUGR offspring from food-restricted dams vs. control dams present with greater NAFLD under HFD as adults (238). Regarding exam-
ination of inflammatory factors, surgical-induced utero-placent-
tal insufficiency programs an increase in serum and adipose
tissue levels of TNF-α by postnatal day 21 in male IUGR
(172). Others show that poor maternal nutrition programs
greater liver inflammation, oxidative stress, and fibrosis (200).
However, it has not been determined if intervening in proin-
flammatory mechanisms blocks the increased risk for NAFLD
and liver injury in IUGR offspring.

Perry et al. (163) showed that blocking actions of the
proinflammatory cytokine IL-6 by utilizing infusion of neutral-
izing antibodies attenuated HFD-induced hepatic insulin resis-
tance and T2DM in rats. These investigators propose that one
of the primary sites for action of this mechanism is mac-
rophage-mediated inflammation driving lipolysis and release of
acetyl CoA from white adipose tissue. The acetyl CoA targets
the liver where it attenuates insulin-induced suppression of
hepatic glucose production. In another study, use of oral
clecloxib, an inhibitor of the proinflammatory enzyme cyclo-
oxigenase (COX)-2, lessened HFD high-sucrose diet-induced
NAFLD along with being beneficial for insulin tolerance,
HOMA-IR, liver expression of the proinflammatory transcription
factor, NF-κB, and ALT and AST levels (204). COX-2 may be
localized to infiltrating immune cells in the progression of
this disease. In vivo studies suggest that homing of immune
cells to the liver in the progression of NASH whereby bone
marrow-derived macrophages in hyperlipidemic LDLr/−/−
mouse are important in the progression of NASH (29). A study
using a model of chronic liver injury generated by oral carbon
tetrachloride (CCl4) demonstrated that local hepatic Kupffer
cells, a resident macrophage cell type in the liver, and infiltr-
ating macrophages express COX-2 during the development of
liver injury (219). In contrast, COX-2 expression in hepatocytes
protects against models of NASH and liver injury, including
CCl4 treatment (149); this protective effect may be depen-
dent on COX-2 production of prostaglandin E2. In summary,
the relative role of hepatocyte vs. immune cell COX-2 (88)
should be examined in the development of NAFLD.
Furthermore, these mechanisms and interventions should be
utilized to probe the hypothesis that proinflammatory cytokines
and immune cells mediate the accelerated risk for obesity-
induced NAFLD in IUGR offspring.

Kupffer cells, an innate immune cell to the liver, mediate
hepatic monocyte recruitment via production TNF-α and
monocyte chemoattractant protein-1 in the pathogenesis of
NASH (206). Kupffer cells also have the ability to produce the
cXC chemokine receptor 3 (148), which studies suggest is a
major regulator in promoting lipid accumulation, immune cell
infiltration, and tissue levels of TNF-α and NF-κB (239). The
cXC chemokine receptor 3 signaling mechanism also contrib-
utes to NASH via impaired cell stress sensing pathways,
including endoplasmic reticulum (ER) stress and autophagy
(239). Overall, ER stress-induced autophagy is thought to
provide protection against tissue injury (48, 237). Collectively,
remains unexplored whether ER stress or autophagy directly
contributes at any step to the accelerated progression of obe-
sity-induced inflammation, insulin resistance, or NAFLD and
liver injury in IUGR.

ER stress and inflammation. Folding of transmembrane and
secretory proteins occurs in the ER before mediating their
biological functions (234). In instances of heavy protein work-
load, accumulation of unfolded proteins in the lumen of the ER
can trigger ER stress. If proteins are not folded in their allotted
time, they are targeted for ER-associated degradation by sev-
eral chaperone proteins including the immunoglobulin heavy
chain binding protein/glucose-regulated protein 78 (Bip/
Grp78). Restoration of cellular homeostasis and adaptation is
supported by the unfolded protein response with Grp78-in-
duced activation of ER transmembrane receptors PKR-like ER
kinase (PERK), activating transcription factor (ATF)6, and
inositol-requiring enzyme 1 and downstream signaling inter-
mediates eukaryotic initiation factors-2α/ATF4/stanniocalcin
2, cleaved ATF6, and X-box binding protein-1 (XBP-1), re-
spectively (225). Under irremediable ER stress, PERK acti-
vates ATF4 that increases expression of the CCAAT-enhancer-
binding homologous protein (CHOP) transcription factor and
CHOP-mediated inflammation and apoptosis (120, 155). Pro-
longed ER stress promotes apoptosis and profibrotic mecha-
nisms (44).

It is hypothesized that ER stress-mediated apoptosis is an
important upstream mechanism mediating immune cell infil-
tration in the liver (4). A main initiator of this process is lipotoxicity (174). Preventing hepatic triglyceride and choles-
terol accumulation in a preclinical model of NASH is associ-
ated with lower levels of the ER stress marker CHOP, an ER
stress protein that induces apoptosis and cytokine production in
macrophages (155), and cytokine levels of IL-1β in liver tissue
along with the development of NASH (26). However, this
study did not examine immune cell infiltration or inflam-
masome activation. The inflammasome is a multiprotein sys-
tem expressed in myeloid cells that promotes inflammation and
apoptosis in target tissues (77). Interestingly, ER stress induces
IL-1β expression that activates the inflammasome and resultant
hepatocyte death (116). Inflammasome activation is thought to
also involve mitochondria to propagate the cell-death response
(116). Chronic enrichment of ER-mitochondria contacts leads
to hepatic mitochondrial dysfunction in obesity (19). Using a
controlled-release mitochondrial protonophore to pharmaco-
logically induced mitochondrial uncoupling, which is hypoth-
esisized to protect cells from conditions that favor reactive
oxygen species production (146), is beneficial in preventing the
development of HFD-induced NAFLD in rats along with
reducing hypertriglyceridemia, insulin resistance, hepatic ste-
atosis, diabetes, and NASH in methionine/choline-deficient
rats (164). Increased physical and molecular interactions be-
tween the mitochondria and ER elicit apoptosis. Collectively,
these studies suggest that signaling between the ER and mito-
chondria play a major role in the core mitochondrial apoptosis
pathway (82). This background information is necessary for
the discussion below detailing mechanisms of NAFLD-medi-
atated progression of liver injury.

ER stress in NAFLD and liver injury. Studies in human liver
biopsies implicate altered ER stress signaling in the develop-
ment of NAFLD. In patients with NASH, there are signifi-
cantly greater levels of the ER stress markers of spliced
XBP-1s and STC2 with a trend for increases in c-Jun NH2-
terminal kinase (JNK), ATF4, and CHOP (115). In a separate
study, intervening with a chemical chaperone, tauroursodeoxy-
cholic acid (TUDCA), to inhibit aberrant ER stress increases
liver insulin sensitivity in obese men and women (105). In an
experimental model of obesity, insulin resistance, and NASH,
foz/foz mice on 6-wk HFD have activation of JNK, ATF4, and
CHOP, whereas XBP-1 levels are not altered (119). Inhibition

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of ER stress reduces steatosis but does not improve glucose intolerance or hepatic inflammation and apoptosis in these mice. This lack of an effect on these injurious mechanisms may be due to the fact that other ER stress mediators, like XBP-1, are not altered in this specific model. To probe the specific ER stress factors that may promote the development of steatosis and liver injury, ER stress factor-deficient models have been developed. Although XBP-1-deficient mice have exaggerated fructose diet-induced ER stress noted by increased factors, like JNK, they have increased hepatic insulin sensitivity and reduced hepatic lipids (104). Furthermore, it was found that XBP-1 deficiency specifically in hepatocytes attenuates HFD and high-sucrose diet-induced steatosis, whereas these mice have exaggerated diet-induced mRNA levels for markers of inflammation, apoptosis, and injury associated with increased CHOP and phosphorylated-JNK and increased hepatic injury and fibrosis (125). Moreover, in mice with Xbp1 deficiency specifically in the liver, it was shown that this ER stress mediator is important for resolution of chronic ER stress-induced liver injury (156). These data suggest that XBP-1 is an important factor promoting steatosis but is protective against the progression of NAFLD-mediated liver injury. Not as much mechanistic information is known about the ER stress pathway in mediating the predisposition NAFLD or liver injury in IUGR offspring.

Information is beginning to surface regarding IUGR and its impact on ER stress proteins during the pathogenesis of NAFLD. In a mouse model of maternal caloric restriction, expression of the hepatic ER stress markers, like spliced XBP-1s, is increased in IUGR offspring on normal diet. These IUGR offspring have greater HFD-induced steatosis accompanied by lower hepatic SREBP1 levels and increased fatty acid synthase levels and liver macrophages (152). However, following the HFD regimen, XBP-1 expression was no longer different to normal birthweight counterparts. TUDCA reduced steatosis and circulating small dense LDL cholesterol more dramatically in the IUGR offspring and reduced XBP-1s only in this offspring group along with other ER stress mediators that were exaggerated in the HFD IUGR offspring. Furthermore, TUDCA reduced the greater ALT and AST levels in these IUGR offspring on HFD indicative of attenuated liver injury (Fig. 5). Similar trends were found for reduced nonfasting glucose, but this did not reach statistical significance. However, this study did not examine these responses in comparison to age-matched IUGR or control offspring maintained on normal diet, and it is unclear if the HFD-induced ER stress, metabolic disease, and liver injury were exaggerated in IUGR in comparison with control IUGR offspring on a control diet and over the HFD responses in normal birth weight counterparts. Furthermore, it has not yet been teased apart if different ER stress factors mediate the development of steatosis vs. liver injury in HFD IUGR offspring. Collectively, these data advocate for further study of the impact that ER stress has on metabolic disease and NAFLD.

ER stress and autophagy in liver injury. When discussing ER stress control of cell survival, autophagy is important to mention. Autophagy is an adaptive response activated during cell stress that delivers proapoptotic cytoplasmic components, like damaged cellular organelles or unused proteins, to the lysosome for degradation to encourage cell survival (121). Increases in splicing of the ER stress-associated protein XBP-1 and induction of ER stress is an early adaptive mechanism to protect against excessive cell stress by activation of autophagy (108). An inability to proceed through autophagy is thought to drive apoptosis in NAFLD (183) and marks the transition to cirrhosis (53). Studies using mice with hepatocyte-specific deletion of MAP kinase kinase transforming growth factor-β-activated kinase 1 (TAK1) revealed that this factor suppresses autophagy in response to HFD allowing for exaggeration of HFD-induced steatosis, inflammation, and fibrotic injury in the liver (92). In that same study, pharmacologic-mediated increases in autophagy by rapamycin-induced increases in mam-

![Fig. 5. Steatotic grade (A) and plasma small dense LDL (B) and circulating markers of liver injury ALT (C) and AST (D) in male intrauterine growth restriction (IUGR) offspring from calorically-restricted (CR) pregnant C57Bl/6 mice. All offspring were on a high-fat diet (60% lipids) from 9 to 22 wk treated with vehicle or the endoplasmic reticulum stress inhibitor tauroursodeoxycholic acid (TUDCA; 0.5 g·kg⁻¹·day⁻¹). *P < 0.05 vs. vehicle-treated control rats from ad libitum dams; **P < 0.05 vs. TUDCA-treated control rats. #P < 0.05 vs. vehicle-treated controls. [Adapted from Ramatsu-Kato et al. (152), licensed under Creative Commons Attribution CC-BY 4.0.]

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malian target of rapamycin complex 1 (mTORC1) significantly prevented hepatic apoptosis, injury, and hepatocellular carcinoma (HCC) in the TAK1-deficient mice. Intriguingly, and with regards to IUGR, it was found that newborn IUGR piglets have reduced protein expression of factors linked to activation of autophagy, including mTOR, protein phosphatase 2A, and microtubule-associated protein 1A/1B-light chain 3 along with reduced autophagic vacuoles in their livers (126) (Fig. 6). At this juncture, it is unknown if this early indication of dysfunctional autophagy persists as IUGR offspring age to mediate programming of accelerated progression of NAFLD and liver injury.

NAFLD AS A NOVEL RISK FACTOR FOR CVRD

Does NAFLD exaggerate risk for CVRD? NAFLD is linked to increased risk for coronary heart disease (69, 117, 141) independent of other metabolic factors, including obesity (20, 160). The incidence of atrial fibrillation is also higher in hospitalized patients with NAFLD and T2DM (199). It is likely that greater CVRD in these patients presenting with NAFLD and T2DM could be due to uncontrolled diabetes. Portillo-Sanchez et al. (168) report that patients with NAFLD and T2DM exhibit greater uncontrolled diabetes over those with T2DM alone. According to the American Heart Association (Cardiovascular Disease & Diabetes; retrieved September 2, 2017: http://www.heart.org/HEARTORG/Conditions/More/Diabetes/WhyDiabetesMatters/Cardiovascular-Disease-Diabetes_UCM_313865_Article.jsp#.WiWSK7aZPXS), adults with diabetes have quadrupled the risk of death from heart disease. In a 33-yr follow-up study, individuals with NASH had reduced survival mainly due to cardiovascular disease (59, 145). Longitudinal studies suggest that NAFLD and hepatic insulin resistance are associated with the onset of hypertension (33). Moreover, in a sample of patients from the UK, 80% of those individuals with biopsy-proven steatosis and progressive fibrosis were diabetic at follow-up compared with 25% that did not have progressive fibrotic disease (139). With the use of both ultrasound and histology, NAFLD was present in >50% of patients with T2DM and was higher in NASH (66). In genetically obese foz/foz mice, HFD-induced NASH and liver fibrosis were greater and corresponded with hyperinsulinemia, hyperglycemia, HOMA-IR, hypoadiponectinemia, and hepatic levels of TNF-α (62). It is not yet clear the relative role that NAFLD-related fibrosis has on advancement of metabolic disease and CVRD.

T2DM as a mediator of exaggerated CVRD risk in NAFLD. Rodent studies indicate a synergistic action between high glucose levels and hypertension in promoting renal injury in male nonobese Goto-Kakizaki rats, a model of nonobese T2DM and hepatic insulin resistance (213). A positive relationship was detected between hypertension and the onset of NAFLD (64). Patients with baseline hypertension, hypertriglyceridemia, impaired fasting glucose, or T2DM have increased odds for steatosis (33). In spite of this finding, the relationship of hypertension, NAFLD, and liver injury is unknown. Although hydralazine-mediated lowering of blood pressure reduces immune cell counts in hypertensive rodents (98), the importance of proinflammatory mediators has not been examined in livers from hypertensive models of obesity and progressive NAFLD. In a series of studies performed by investigators at Kagoshima University, hepatic lipid levels (17) increased ALT and AST were increased in male spontaneously hypertensive rats compared with their normotensive Wistar-Kyoto counterparts on a normal diet of 10% fat (118). A hypertensive insult of chronic high-salt diet exaggerated their hypertension accompanied by increased liver weight/body weight and liver fibrosis (17) along with increased insulin, fasting blood glucose, and insulin resistance (111). Antihypertensive therapy in already hypertensive rats reduced these parameters accompanied by a reduction in IL-6 and increased anti-inflammatory and vasoprotective IL-10 levels in serum. It is unknown whether the liver is a source of the circulating

Fig. 6. Expression of proteins associated with activation of autophagy in liver tissue from newborn piglets with intrapartum growth restriction (IUGR). A: mammalian target of rapamycin. B: catalytic subunit of protein phosphatase 2A (PP2Ac). C: microtubule-associated protein 1A/1B-light chain 3 (LC3). D: autophagic vacuoles detected by electron microscopy. *P < 0.05 vs. control, normal-birthweight piglets. [Adapted from Long et al. (126) with permission from American Chemical Society. Copyright © 2016, American Chemical Society.]
cytokines or whether they contribute to alterations in hypertension-induced CVRD and damage. Future studies are required to fully examine the contributions of hypertension and inflammation on the development of NAFLD and ensuing metabolic dysfunction and CVRD (56, 127).

Inflammatory markers implicated in the pathogenesis of NAFLD and liver injury may also be responsible for the exaggerated CVRD in IUGR offspring. The above highlights the complexity of the pathogenesis of programming for cardiometabolic disease and the need for research to explore the contribution of inflammatory pathways. Inflammatory markers that are implicated in the pathogenesis of NAFL and NASH may also be responsible for CVRD associated with IUGR. A redundancy in these inflammatory pathways may explain how a history of IUGR is related to the association between T2DM and NAFLD and exaggerated CVRD risk. The aforementioned anti-inflammatory treatment studies in IUGR pregnancies did not examine hepatic or CVRD outcomes in the offspring, including their known programming for decreased cardiomyocyte proliferation (171), increased cardiac fibrosis (14), or decreased renal nephron endowment (233). Moreover, hypertension is observed in the IUGR offspring of maternal nutrient restriction, hypoxia, and RUPP dams (5). In humans, there is an association between higher fetal-placental vascular resistance and systolic blood pressure in IUGR offspring (70), but the role of inflammation in mediating the increased CVRD risk in IUGR offspring has not been examined in humans or animal models.

**PERSPECTIVES AND SIGNIFICANCE**

NAFLD is an increasing public health concern (27). Evidence in humans implicates that a risk factor for accelerated progression of NAFLD is IUGR. The various experimental models of IUGR discussed in this review will allow for research to assist in a better understanding of the mechanisms mediating increased risk for NAFLD in IUGR offspring. Studies already being conducted in these models of IUGR suggest that developmental programming of insulin resistance is a determinative factor in predisposing for NAFLD in these at-risk offspring. Obesity-induced inflammation is an initial signal to drive the development of hepatic insulin resistance and steatosis, which ultimately results in progressive NAFLD and liver injury. This injury response is mediated by hepatic lipotoxicity-induced inflammation and alterations in ER stress and autophagy signaling. Further research is needed to determine if intervening in these pathways, which are illustrated in Fig. 7, will halt the developmental programming of NAFLD and liver injury in IUGR offspring. It is important to fully understand these mechanisms and to identify those populations at increased risk for NAFLD, such as those with a history of IUGR, because NAFLD is projected to become the major cause of chronic liver disease. We are not proposing that every infant with IUGR will develop NAFLD, but it is suggested that IUGR offspring are at greater risk for progression of this chronic liver disease when exposed to environmental insults that foster its pathogenesis, such as adverse diets and obesity.

Regarding treatment strategies, Bril and Cusi (38) suggest that finding approaches to manage NAFLD and liver injury may assist in the treatment of uncontrolled metabolic diseases, like T2DM. T2DM is a leading cause of CVRD. In this review, it was discussed that IUGR offspring are at greater risk for cardiovascular-renal abnormalities that predispose to CVRD morbidities, like hypertension. Although IUGR encourages multiple risk factors for CVRD, including metabolic diseases and hypertension, it is unknown if these chronic diseases have synergistic effects to mediate the exaggerated risk for CVRD in IUGR offspring. Understanding the etiology of the developmental origins of chronic disease will allow investigators to uncover treatment strategies to intervene in the mother and her offspring to halt CVRD. For instance, it is established that antidiabetic agents like metformin or inhibitors dipeptidyl peptidase-4 are beneficial in reducing advancing NAFLD and T2DM (18). Metformin during pregnancy may also provide benefit by reducing the incidence of preterm delivery and maternal hypertension (235), but the long-term effects of metformin treatment in pregnancies with IUGR on metabolic and CVRD outcomes in offspring are only now starting to be examined. Indicative of its protective effect in developmental programming of metabolic dysfunction is that metformin reduces central adiposity and insulin resistance in prepubertal children that were born SGA at full-term (55). Regarding the
potential for other advances in the development of targeted strategies; downregulation of the transcription factor PPAR-γ contributes to hepatic lipid dysregulation and inflammation in IUGR (134). Furthermore, the Toll-like receptor-9 seems to be a potent factor in promoting immune cell infiltration in NASH (151) and compounds targeting this system are being developed for inflammatory diseases (73). Studies are needed in experimental models to determine if IUGR impacts the adverse pathways detailed in this review to allow for NAFLD-mediated exaggeration of metabolic dysfunction and subsequent hypertension-induced CVRD and damage.

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AUTHOR CONTRIBUTIONS
F.T.S., J.A.S., B.T.A., and C.A. conceived and designed research; F.T.S., J.A.S., B.T.A., and C.A. performed experiments; F.T.S., J.A.S., B.T.A., and C.A. analyzed data; F.T.S., J.A.S., B.T.A., and C.A. interpreted results of experiments; F.T.S., J.A.S., B.T.A., and C.A. prepared figures; F.T.S., J.A.S., B.T.A., and C.A. drafted manuscript; F.T.S., J.A.S., B.T.A., and C.A. edited and revised manuscript; F.T.S., J.A.S., B.T.A., and C.A. approved final version of manuscript.

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