Continued Postnatal Administration of Resveratrol Prevents Diet-Induced Metabolic Syndrome in Rat Offspring Born Growth Restricted

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OBJECTIVE—A prenatal hypoxic insult leading to intrauterine growth restriction (IUGR) increases the susceptibility to develop metabolic syndrome (MetS) later in life. Since resveratrol (Resv), the polyphenol produced by plants, exerts insulin-sensitizing effects, we tested whether Resv could prevent deleterious metabolic effects of being born IUGR.

RESEARCH DESIGN AND METHODS—Pregnant rats were exposed to either a normoxic (control; 21% O_2) or a hypoxic (IUGR; 11.5% O_2) environment during the last third of gestation. After weaning, male offspring were randomly assigned to receive either a high-fat (HF; 45% fat) diet or an HF diet with Resv (4 g/kg diet) for 9 weeks when various parameters of the MetS were measured.

RESULTS—Relative to normoxic controls, hypoxia-induced IUGR offspring developed a more severe MetS, including glucose intolerance and insulin resistance, increased intra-abdominal fat deposition and intra-abdominal adipocyte size, and increased plasma triacylglycerol (TG) and free fatty acids, as well as peripheral accumulation of TG, diacylglycerol, and ceramides. In only IUGR offspring, the administration of Resv reduced intra-abdominal fat deposition to levels comparable with controls, improved the plasma lipid profile, and reduced accumulation of TG and ceramides in the tissues. Moreover, Resv ameliorated insulin resistance and glucose intolerance as well as impaired Akt signaling in the liver and skeletal muscle of IUGR offspring and activated AMP-activated protein kinase, which likely contributed to improved metabolic parameters in Resv-treated IUGR rats.

CONCLUSIONS—Our results suggest that early, postnatal administration of Resv can improve the metabolic profile of HF-fed offspring born from pregnancies complicated by IUGR. ***Diabetes*** **60:2274–2284, 2011**

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The prevalence of obesity both in adults and children has reached pandemic proportions and constitutes a pressing public health problem that shows little sign of improving in the near future (1). Obesity is strongly associated with additional cardiovascular risk factors including high blood pressure, dyslipidemia, and insulin resistance, all of which have been grouped as the metabolic syndrome (MetS) (2). Disappointingly, the number and effectiveness of therapies for management of obesity is still very limited (3). Although obesity has a complex pathophysiology characterized by a strong influence of inherited factors (4) and environmental variables, it is clear that behaviors such as reduced physical activity and consuming hypercaloric and high-fat (HF) Western diets play a major role in its pathophysiology (5). Evidence from population health studies and animal models showed that the prenatal environment influenced the postnatal susceptibility for insulin resistance (6–8). We have recently shown that offspring born intrauterine growth restricted (IUGR) as a result of a prenatal hypoxic insult exhibit permanent metabolic changes that increase the susceptibility for several components of the MetS when consuming an HF diet (9).

Although these results suggest that individuals born IUGR should participate in regular exercise and consume diets low in fat, adherence to lifelong lifestyle interventions is poor. Remarkably, therapeutics for individuals diagnosed with IUGR are lacking (10) and the focus of current medical interventions for IUGR is to prevent the development of acute perinatal complications in the mother and the baby. Infancy is a potential window of opportunity to intervene and prevent the future development of metabolic diseases (11). However, several ethical issues exist with testing preventative pharmacological interventions in pediatric populations. Since the prenatal environment is not a modifiable risk factor for the individual later in life, our objective was to determine whether the programmed susceptibility to HF-induced MetS observed in our model of hypoxia-induced IUGR (9) could be treated pharmacologically with resveratrol (Resv).

Resv is a natural polyphenol produced by plants in response to environmental stress (12) and is present in low concentrations in plant-based foods common in our diets. Oral administration of Resv has already been shown to protect against the development of diet-induced insulin resistance in aged rodents fed a hypercaloric diet (13,14). Although the mechanism(s) of action of Resv has not been completely defined, there is evidence that some of the effects of Resv may be mediated via activation of AMP-activated

protein kinase (AMPK) (13,15–17). AMPK is a protein kinase pathway that is involved in the control of oxidative metabolism and lipid homeostasis in muscle (18) and liver (19) and exerts many of its effects on metabolism via the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) (20,21). In the liver, activation of the AMPK/ACC axis decreases fatty acid and triacylglycerol (TG) synthesis and lowers lipids (19), whereas activation of AMPK in skeletal muscle has been shown to improve glucose uptake independent of insulin (22). Together, these previous results suggest that Resv may contribute to an improved plasma lipid profile and increased glucose disposal in IUGR offspring fed an HF diet. However, the efficacy of Resv and its potential to prevent IUGR-induced susceptibility for MetS in young mammals has not been evaluated. Therefore, based on our preceding studies, we hypothesize that postnatal nutritional supplementation with Resv could prevent the early development of MetS associated with the interaction between being born IUGR and postnatal exposure to an HF diet.

RESEARCH DESIGN AND METHODS

Reagents. All antibodies were purchased from Cell Signaling Technologies. Human recombinant insulin (Novolin) was purchased from Novo Nordisk Canada Inc. ELISA kits for the determination of insulin were purchased from ALPCO Diagnostics, and leptin kits were purchased from Millipore. [γ^{32}P]-ATP was purchased from Perkin-Elmer, sn-1,2-dioleylglycerol was from Avanti, and most other reagents and chemicals were purchased from Sigma-Aldrich.

Experimental animals and diets. The University of Alberta Animal Welfare Committee approved all procedures in this study. The generation of the hypoxia-induced rat model of IUGR has been described in detail previously (23–25). At birth, litters were reduced to eight male pups and litters that contained fewer than eight viable male pups were reduced to eight offspring with as many males as possible. At 3 weeks of age, female offspring were killed and male pups were weaned and housed two per cage. Immediately after weaning, all offspring exposed to different prenatal interventions (control, n = 36 from seven litters; and IUGR, n = 36 from eight litters) started receiving an HF diet (45% fat; Research Diets D12451). In addition, half of the animals from each litter were randomly allocated to receive additional supplementation with Resv in the diet (4 g/kg of diet).

Therefore, four experimental groups were created: control offspring receiving HF diet (control HF-C; n = 18 from six litters), control offspring receiving HF diet plus Resv (control HF-R; n = 18 from six litters), IUGR offspring receiving HF diet (IUGR HF-C; n = 18 from six litters), and IUGR offspring receiving HF diet plus Resv (IUGR HF-R; n = 18 from six litters). After 9 weeks of nutritional intervention, experimental analyses were performed. The bioavailability of Resv administered in the diet is low (~0.1%), mainly because of intestinal breakdown, absorption, and liver metabolism (14). Therefore, the final plasma concentration of this molecule achieved with the dose administered in this study was within expected therapeutic values (10–20 μmol/L range) (26). To determine which measurements were performed on each animal, rats born from the same dam within each experimental group were randomly assigned to three possible subgroups (designated A, B, or C); see Supplementary Fig. 1 for details. Because all rats included in each subgroup belonged to a different litter, we used the offspring as the unit of analysis.

Determination of body composition and adiposity. Body weight and food intake was measured weekly from birth. After 9 weeks of nutritional intervention, body composition was determined using a whole body composition analyzer based on time-domain nuclear magnetic resonance technology (EchoMRI 4-in-1/1000; Echo Medical Systems). For the determination of abdominal fat in vivo, rats were anesthetized using inhaled isoflurane (~2% in compressed air) and placed into a micro single-photon emission computed tomography (SPECT) scanner FLEX Pre-Clinical Platform XO-XPET-XSPET instrument (γ-Medica Ideas). The γ-camera was programmed to scan 512 projections (sum of frames = 4, voltage 60 kDP and 390 uA). The observation window was set between the diaphragmatic membrane and the acetabulum for each animal (~91.78 mm), and the magnification factor on the γ camera was set to 1.29. The intra-abdominal fat volume was calculated using the software GMI-Amira 3.1.1. Threshold density was adjusted by internal volume so that internal organs and large vessels were not counted as intra-abdominal fat tissue and were excluded from calculations. For additional measurements of intra-abdominal fat content, mechanical extraction and weight of different abdominal fat depots (retroperitoneal, perirenal, mesenteric, epiploic, and subdiaphragmatic) was performed after dissection. The Pearson correlation between methods used to determine the intra-abdominal fat content (mechanical extraction and micro CT scan) was r = 0.65, P = 0.002. The Bland and Altman analysis demonstrated a bias between the techniques of −0.14 ± 14.7 g, which demonstrates that both techniques were highly correlated and that the average variability in the determinations made with these techniques was less than 1 g (<2% of the average value).

Analysis of tissues. Intra-abdominal organs including liver, spleen, pancreas, and kidneys were dissected and weighed before being frozen. In a subset of rats and after 2 h of fasting, rats were anesthetized using inhaled isoflurane, followed by cervical dislocation, collection of a sample of blood by cardiac puncture, and for the analysis of AMPK activity, tissues were dissected and immediately frozen in liquid nitrogen, as described (27). In a separate set of animals, rats were fasted 2 h before intraperitoneal injection with insulin (1 mU/kg) and killed as above 15 min after injection. Liver and gastrocnemius muscle were collected and snap frozen in liquid nitrogen. The length of the right tibia bone was measured in all animals. For immunoblotting, homogenates were prepared in ice-cold sucrose homogenization buffer as described (27). Intra-abdominal fat was extracted and weighed, and samples of omental adipose tissue were fixed in 10% formalin for 48 h and then in 10% methanol. Histological sections and hematoxylin/eosin staining were performed at the Alberta Diabetes Institute Histology Core (Edmonton, AB, Canada) following standardized protocols. Digital images of three representative fields were taken using a digital camera mounted on a light microscope at 40× magnification. All images were analyzed with ImageJ software (Ver 1.43u; National Institutes of Health).

Determination of liver, muscle, and plasma lipids. Plasma from rats following a 2-h fast was collected in the presence of EDTA. Lipids were extracted from 200 μL of plasma, and TG, cholesterol ester, and free fatty acids were separated by fast-protein liquid chromatography as described (28). Liver and skeletal muscle tissues were homogenized, and TG, cholesterol ester, and ceramide content was determined by fast-protein liquid chromatography (27). Diacylglycerol content was determined by a diacylglycerol kinase assay, according to a well-established procedure (29).

Indirect calorimetry and physical activity. Indirect calorimetry was performed using the Comprehensive Laboratory Animal Monitoring System (Oxymax/CLAMS; Columbus Instruments). After an initial 24-h acclimatization period, rats were monitored every 13 min for 24 h to complete a 12-h dark (active)/12-h light (inactive) cycle. The respiratory exchange ratio (RER), V_{O_2}, V_{CO_2}, heat production, and physical activity were measured.

Glucose and insulin tolerance tests. After a 5-h fast, rats were injected intraperitoneally with a 50% glucose solution (2 g/kg) for the glucose tolerance test (GTT). Blood glucose concentrations were determined using an ACCU-CHEK Advantage glucometer (Roche Diagnostics) using blood from the tail at baseline and after glucose injection (15, 30, 60, 90, and 120 min). For the insulin tolerance test (ITT), rats were injected intraperitoneally with insulin (1 mU/kg) after a 2-h fast and the blood glucose concentration was measured from the tail at baseline and after insulin injection (15, 30, 60, 90, and 120 min).

Statistical analyses. Data are presented as mean ± SEM. Differences in measurements performed among four groups were analyzed using two-way ANOVA and a Bonferroni post hoc test with both IUGR and administration of Resv as sources of variation. Measurements of body weight and food consumption over time, as well as GTT and ITT, were analyzed using a two-way ANOVA with both time and group as sources of variation. When interaction between sources of variation included in the two-way ANOVA was detected, interpretation of the overall ANOVA was dictated by the significance observed in the post hoc analyses. A P value < 0.05 was considered statistically significant.

RESULTS

Body weight gain, energy intake, and physical activity. Consistent with our previous report (9), exposure of rats to prenatal hypoxia had no effect on body weight gain (Fig. 1*A*) or body weight-to-tibia length ratio (Fig. 1*B*) of the offspring after 9 weeks of HF diet. Food intake was decreased in IUGR offspring compared with control rats independently of the administration of Resv, despite similar body weights (Fig. 1*C*). These data suggest that additional mechanisms must be involved in maintaining body weight in IUGR offspring to compensate for the decrease in food consumption. Consistent with this finding, physical activity was significantly reduced in IUGR offspring

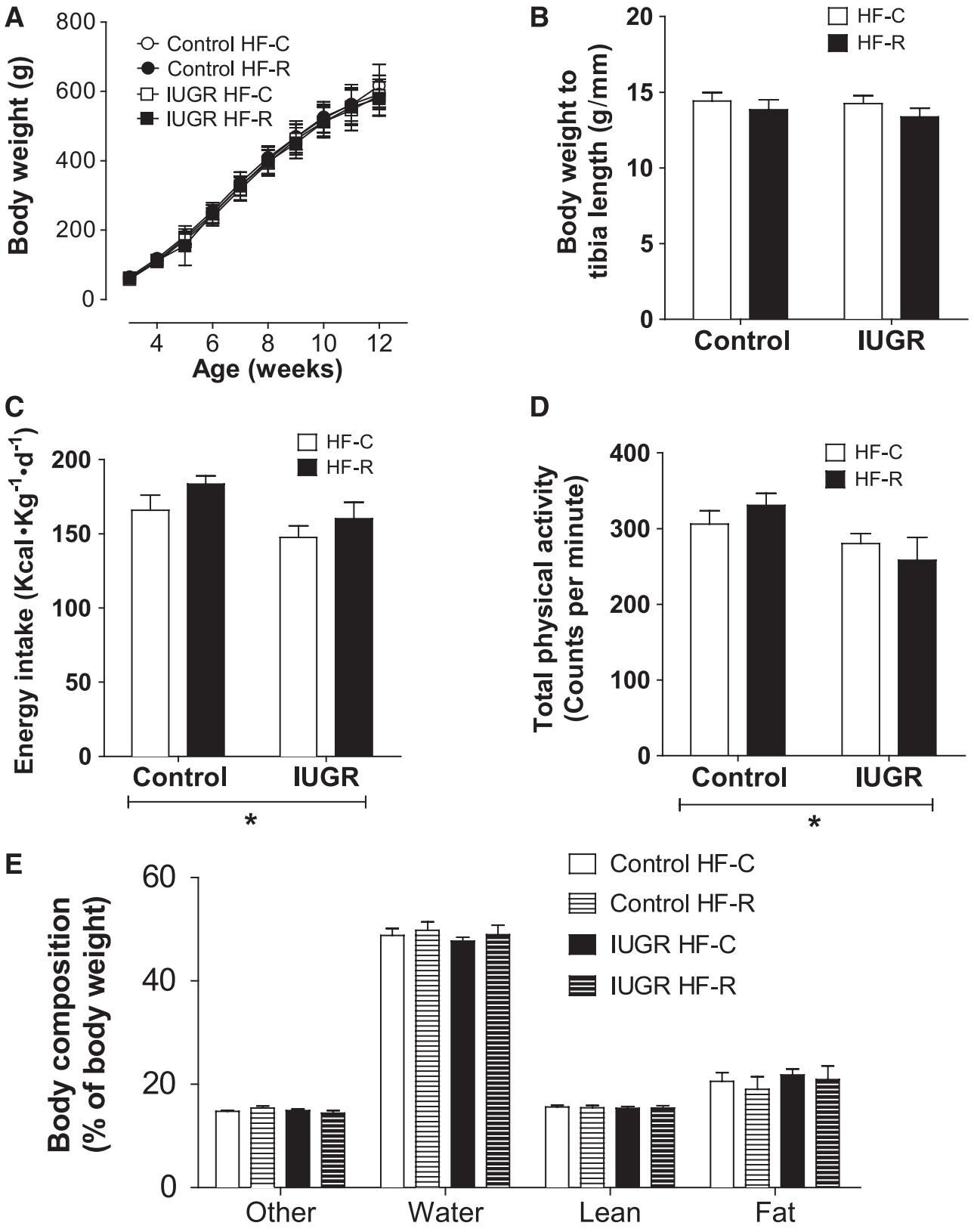

FIG. 1. Effect of IUGR and Resv on physical characteristics after HF diet feeding. After exposure to a normoxic (21% O_2) or a hypoxic (11.5% O_2) prenatal environment that caused IUGR, we examined the effect of postnatal feeding an HF (HF-C) diet or an HF diet supplemented with Resv (HF-R) to rat offspring for 9 weeks. *A*: Change in body weight over time. *B*: Body weight measured under anesthesia adjusted by tibia length. *C*: Absolute energy intake adjusted by body weight. *D*: Total physical activity in 24 h. *E*: Total body composition estimated by SPECT. HF is 45% fat, 4.73 kcal/g. Resv is 4 g/kg of diet. *$P < 0.05$ for the respective sources of variation (IUGR and Resv administration) using two-way ANOVA; †$P < 0.05$ vs. controls after a Bonferroni post hoc test comparing IUGR and control offspring receiving the same diet ($n = 6$ per group).

compared with control offspring (Fig. 1*D*). Although the mechanisms involved in maintaining body weight in control rats and IUGR rats may differ, neither IUGR nor Resv administration affected whole body lean and fat tissue composition (Fig. 1*E*).

As expected, rats fed an HF diet exhibited a low RER as a result of increased availability of fat as an energy source (Table 1). Changes in the RER were not observed in control offspring compared with offspring born IUGR. However, IUGR rats exhibited a reduction in V_{O_2}, V_{CO_2}, and heat production during both light and dark cycles relative to controls regardless of whether they were receiving Resv in their diets (Table 1).

Intra-abdominal fat distribution and adipocyte morphometry. Despite similar body weight and total body composition, offspring born IUGR and exposed to an HF diet exhibited increased total and relative (adjusted by total body fat) intra-abdominal fat distribution compared with control rats, whereas Resv reduced the abdominal fat content as determined by both abdominal tomographic imaging and surgical dissection of fat depots (Fig. 2*A*–*D*). The effect of IUGR and administration of Resv on fat distribution among the different intra-abdominal fat depots is reported in Supplementary Table 1. Rats receiving Resv displayed a reduction in the absolute abdominal fat content regardless of whether the rats were born IUGR (Fig. 2*C*). Although the administration of Resv to control offspring did not affect the content of abdominal fat as a percentage of body weight, Resv reduced this parameter in IUGR offspring to levels comparable with those observed in controls (Fig. 2*D*), suggesting that Resv causes a redistribution of fat to depots other than the abdomen in IUGR rats. Consistent with our previous results (9), IUGR offspring fed an HF diet had larger adipocyte diameters than control offspring receiving the same nutritional intervention (Fig. 2*F* and *G*), whereas Resv caused a comparable decrease in the relative adipocyte diameter in both IUGR and control offspring (Fig. 2*F* and *G*). In agreement with greater abdominal fat mass, circulating leptin levels were higher in HF-fed IUGR rats compared with control HF-fed offspring (Fig. 2*E*). More importantly, Resv decreased the plasma levels of leptin in offspring born IUGR but had little or no effect on controls (Fig. 2*E*).

Lipid profile, lipid accumulation, and glucose homeostasis. Interestingly, although administration of Resv had no effect on control offspring, Resv reduced circulating levels of TG and free fatty acids in offspring born IUGR (Table 2). In addition, liver and skeletal muscle TG, diacylglycerol, and ceramide levels were elevated in IUGR offspring compared with control offspring, and these values were significantly lower in Resv-treated rats (Table 2), demonstrating a plasma lipid–lowering effect of Resv as well as the prevention of accelerated peripheral organ steatosis during the consumption of an HF diet.

Because the major effect of high levels of circulating lipids and their accumulation in tissues is the development of insulin resistance and impaired glucose handling, we used GTT and ITT to investigate whole body glucose homeostasis. Although IUGR and Resv had no effect on fasting blood glucose levels after 9 weeks of HF diet (Fig. 3*A*), Resv prevented the development of hyperinsulinemia and elevated the homeostasis model assessment index in IUGR offspring (Fig. 3*B* and *C*). IUGR offspring also exhibited impaired glucose disposal (Fig. 3*D* and *F*) and insulin resistance (Fig. 3*G* and *I*) compared with control offspring, and Resv improved both glucose disposal (Fig. 3*E* and *F*) and insulin sensitivity in IUGR rats (Fig. 3*H* and *I*). Together, these results indicate that Resv administration prevents IUGR rats from developing HF-induced insulin resistance.

Insulin signaling. The insulin signaling cascade regulates both hepatic glucose output as well as glucose transport into skeletal muscle; therefore, we determined the phosphorylation status of protein kinases in the insulin signaling cascade in the tissues of rats isolated ~15 min after insulin injection. Feeding an HF diet to IUGR rats impaired insulin-stimulated Akt phosphorylation at Ser-473 (activating site) in both liver and gastrocnemius muscle (Fig. 4*A*). In agreement with the interpretation of data from GTT and ITT, the addition of Resv to the HF diet of IUGR rats prevented a decrease in insulin-stimulated Akt phosphorylation in the liver and skeletal muscle. Interestingly, insulin-stimulated Akt phosphorylation was higher in the muscles of both control and IUGR offspring fed an HF diet supplemented with Resv compared with those rats receiving an HF diet alone, demonstrating that the muscle insulin sensitizing effects of Resv were not confined to only IUGR offspring. To further characterize the source of the signaling defect, we measured the phosphorylation of upstream regulators of Akt. Phosphorylation of insulin receptor substrate (IRS)-1 at Ser-1101 (inhibitory site) was higher in liver and skeletal muscle of IUGR offspring compared

TABLE 1
Gas exchange ratio and heat production in control and IUGR rats fed HF diet with or without Resv

	HF diet		HF diet + Resv		Two-way ANOVA		
	Control	IUGR	Control	IUGR	IUGR	Resv	Int
Light cycle							
V_{O_2} (mL/kg/h)	1,124 ± 39	1,007 ± 34	1,098 ± 30	1,036 ± 27	*		
V_{CO_2} (mL/kg/h)	760 ± 23	691 ± 23	758 ± 28	699 ± 26	*		
RER	0.67 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.66 ± 0.02			
Heat (kcal/h)	3.2 ± 0.13	2.81 ± 0.11	3.04 ± 0.10	2.63 ± 0.09	*		
Dark cycle							
V_{O_2} (mL/kg/h)	1,233 ± 41	1,120 ± 35	1,221 ± 26	1,195 ± 34	*		
V_{CO_2} (mL/kg/h)	856 ± 27	784 ± 24	857 ± 28	817 ± 33	*		
RER	0.69 ± 0.01	0.68 ± 0.02	0.70 ± 0.01	0.68 ± 0.02			
Heat (kcal/h)	3.54 ± 0.1	3.18 ± 0.1	3.4 ± 0.1	3.0 ± 0.1	*		

Experiments were performed after 9 weeks of nutritional intervention (Int). *Values of $P < 0.05$ for the respective sources of variation (IUGR and Resv) using two-way ANOVA ($n = 6$ per group).

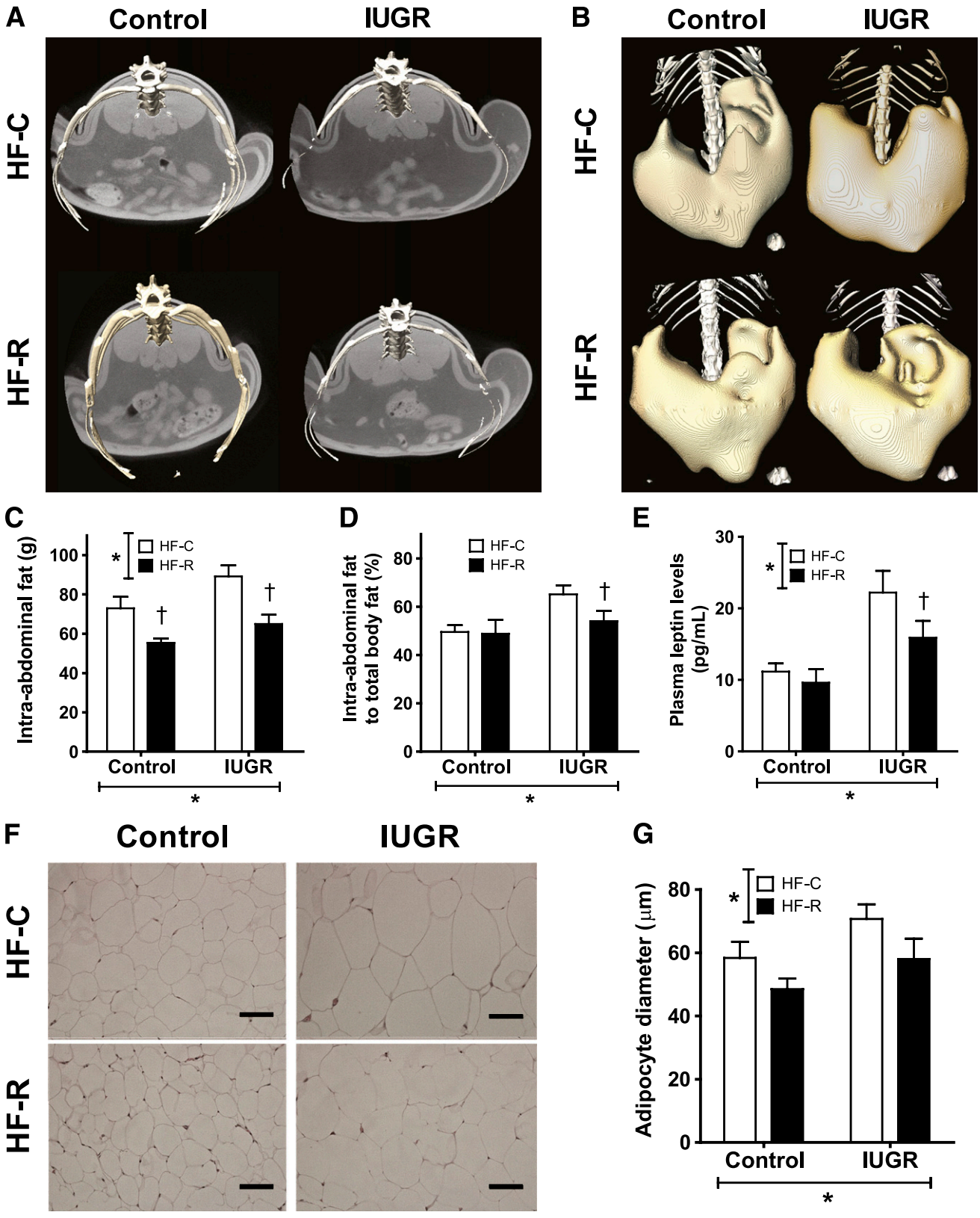

FIG. 2. Effect of IUGR and Resv on adiposity. Measurements were made after 9 weeks of an HF-C or an HF-R (Resv is 4 g/kg of diet). *A*: Representative axial views of the abdominal cavity obtained by X-ray computed tomography and (*B*) subsequent three-dimensional reconstructions of intra-abdominal fat deposits. Total (*C*) and relative (*D*) intra-abdominal fat adjusted by total body fat determined by SPECT is shown. *E*: Plasma leptin levels. *F*: Representative pictures of omental fat tissue histological preparations (hematoxylin-eosin; scale bar, 50 μm). *G*: Average intra-abdominal adipocyte diameter. **P* < 0.05 for the respective sources of variation (IUGR or Resv) using two-way ANOVA; †*P* < 0.05 vs. controls after a Bonferroni post hoc test comparing IUGR and control offspring receiving the same diet (*n* = 6 per group). (A high-quality digital representation of this figure is available in the online issue.)

TABLE 2
Circulating and tissue lipid concentrations of control and IUGR rats fed HF diet with or without Resv

	HF diet		HF diet + Resv		Two-way ANOVA		
	Control	IUGR	Control	IUGR	IUGR	Resv	Int
Plasma							
TG (mmol/L)	2.5 ± 0.5	6.2 ± 0.8†	2.4 ± 0.2	3.0 ± 0.5	*	*	*
Cholesterol ester (mmol/L)	3.6 ± 0.5	4.1 ± 0.5	3.0 ± 0.3	3.6 ± 0.2			
Cholesterol (mmol/L)	0.87 ± 0.07	1.05 ± 0.07	0.97 ± 0.17	1.00 ± 0.007			
FFA (mmol/L)	0.51 ± 0.07	0.86 ± 0.12†	0.40 ± 0.01	0.42 ± 0.03	*	*	*
Liver							
TG (μg/mg protein)	109.3 ± 32	196.4 ± 10.5†	64.1 ± 15.6	81.7 ± 20.3	*	*	
Cholesterol ester (μg/mg protein)	68.7 ± 3.6	82.1 ± 8.1	59.3 ± 3.0	67.7 ± 8.3			
Diacylglycerol (nmol/g)	342.7 ± 19	904.1 ± 57†	382.6 ± 26.6	513.7 ± 38.7	*	*	
Ceramides (pmol/g)	410.1 ± 29	486.2 ± 34.0†	230.4 ± 29.2	330.7 ± 41.3†	*	*	
Gastrocnemius muscle							
TG (μg/mg protein)	117.6 ± 5.5	155.4 ± 17.2†	101.8 ± 6.0	113.1 ± 7.2	*	*	
Cholesterol ester (μg/mg protein)	34.5 ± 1.1	40.2 ± 2.0†	36.8 ± 1.4	34.2 ± 1.1			
Diacylglycerol (nmol/g)	101.3 ± 14	327.4 ± 12†	62.2 ± 16	164.6 ± 12†	*	*	*
Ceramides (pmol/g)	68.4 ± 5.9	107.2 ± 16.0†	40.3 ± 8.9	60.7 ± 7.4	*	*	

Measurements were made after 9 weeks of HF diet with or without Resv 4 g/kg of diet. FFA, free fatty acids. *$P < 0.05$ for the respective source of variation such as prenatal hypoxia (IUGR), Resv, or Int using two-way ANOVA; †$P < 0.05$ vs. controls receiving the same diet after a Bonferroni post hoc test (n = 6 per group).

with controls (Fig. 4*B*). Moreover, the inhibitory phosphorylation of IRS-1 was reversed by Resv treatment in both organs (Fig. 4*B*). Because IRS-1 is directly phosphorylated by protein kinase C θ (PKCθ) at Ser-1101 and PKCθ is activated in tissues where lipids, especially diacylglycerols, accumulate, we measured the phosphorylation of PKCθ at Thr-538 (activating site). During HF-diet feeding, liver and skeletal muscle PKCθ activity was increased in IUGR rats compared with controls (Fig. 4*C*). More importantly, and consistent with the reduction of lipid accumulation in the tissues (Table 1) and prevention of glucose intolerance and insulin resistance (Fig. 3), PKCθ phosphorylation was reduced by Resv in the IUGR offspring (Fig. 4*C*). Together, these data indicate that Resv prevented molecular signaling defects observed in IUGR rats fed an HF diet, and these improvements contributed to the prevention of HF-induced insulin resistance in IUGR rats.

To further characterize the mechanisms for the metabolic benefits of supplementing the diets of IUGR rats with Resv, we investigated AMPK signaling. Although IUGR did not affect AMPK activity (Fig. 5*A*), Resv clearly activated AMPK as determined by its phosphorylation at its activating site (Thr-172). Consistent with the increased AMPK activity, Resv increased ACC phosphorylation at Ser-79 (inhibitory site) in rat liver and skeletal muscle (Fig. 5*B*). Given the ability of activated hepatic AMPK to decrease fatty acid and TG synthesis (19,20) and increased AMPK in skeletal muscle to promote glucose disposal (22), these data indicate that Resv-mediated activation of AMPK contributes to the prevention of HF-induced insulin resistance in IUGR rats.

DISCUSSION

Our previous work established that IUGR offspring subjected to an HF diet are more susceptible to developing several components of the MetS than control offspring fed the same diet (9). Because Resv has been demonstrated to be an insulin-sensitizing molecule (13,14,20,30), we tested the efficacy of Resv in alleviating the development of HF-mediated pathologic metabolic phenotypes in offspring born from pregnancies complicated with IUGR. Interestingly, even in the absence of increased body weight, IUGR offspring exposed to an HF diet exhibited increased abdominal fat, elevated abdominal adipocyte size, hyperlipidemia, insulin resistance, and impaired glucose disposal. Importantly, these significant pathophysiological alterations were alleviated when the HF diet was supplemented with Resv. Although Resv has been shown to improve insulin resistance and glucose disposal in other rodent models (13,14,20,30), to our knowledge this is the first study demonstrating that early postnatal administration of Resv in the diet of young rats improved the metabolic profile of HF-fed offspring born IUGR secondary to a prenatal hypoxic insult.

An interesting phenomenon observed with our IUGR model is that although prenatal hypoxia had no effect on total body composition after an HF diet, IUGR was associated with increased absolute and relative intra-abdominal fat content compared with control offspring. Although we have yet to determine the mechanism(s) responsible for this, we do show that Resv decreases the amount of fat located in the abdominal cavity and reduces adipocyte diameter. This observation is important given the relatively short length of the intervention (only 9 weeks) compared with other studies showing the beneficial effects of Resv on fat distribution when administered for up to 60 weeks (31–33). Because we also show that administration of Resv prevented the diet-induced increase in plasma lipids that is observed in IUGR offspring, it is likely that reduced circulating levels of lipids contribute to the reduced lipid storage by adipocytes. However, we cannot explain why Resv specifically reduces visceral fat without altering whole body fat content. With that said, previous work has shown that incubation of isolated adipocytes with Resv reduced lipogenesis and increased lipolytic rates (34). Based on this, we speculate that Resv preferentially targets abdominal adipocytes to increase lipolysis and reduce the mass of this fat depot. Because intra-abdominal fat is a major source of adipokines and cytokines associated with metabolic disorders (35,36), the reduced abdominal fat mass in IUGR offspring fed an HF diet supplemented with Resv

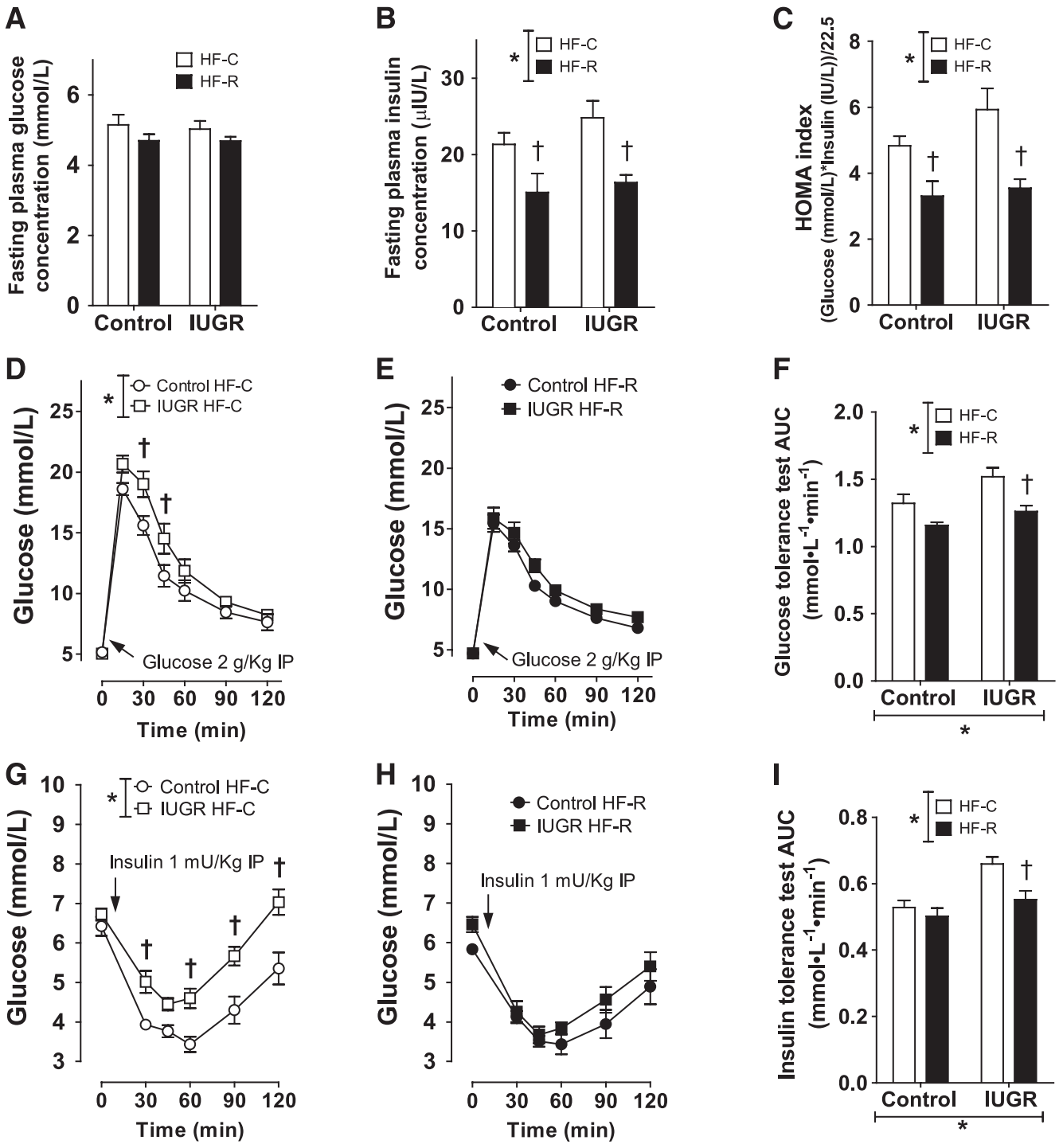

FIG. 3. Effect of IUGR and Resv on glucose homeostasis. Measurements were made after 9 weeks of HF-C or HF-R (Resv is 4 g/kg of diet) on: fasting blood glucose levels (*A*), fasting plasma levels of insulin (*B*), and homeostasis model assessment (HOMA) index (*C*). HF-C rats' GTT (*D*), HF-R–fed rats' GTT (*E*), and GTT summary information (*F*) are presented as area under the curve (AUC). HF-C rats' ITT (*G*), HF-R–fed rats' ITT (*H*), and ITT summary information (*I*) are presented as AUC. IP, intraperitoneal. **P* < 0.05 for the respective source of variation (IUGR or Resv) using two-way ANOVA (bar graphs) or a repeated-measures ANOVA (GTT and ITT); †*P* < 0.05 vs. controls receiving the same diet after a Bonferroni post hoc test (*n* = 6 per group).

corresponds with a lower level of leptin observed in these rats. These results are consistent with evidence showing that supplementation of HF diets with Resv can prevent diet-induced obesity in rodents (13,14,20), in part, by diminishing visceral adipose tissue (14,37), although this is the first evidence that Resv reduces adiposity in young rodents.

As mentioned, Resv reduced the HF diet-induced hyperlipidemia that is observed in IUGR offspring. Resv inhibited fatty acid and TG synthesis by isolated hepatocytes (37,38), suggesting that the lipid-lowering effects of Resv could be attributed to direct effects of this molecule on hepatic function. Consistent with this, we provide evidence that the lipid-lowering effects of Resv may be attributed to

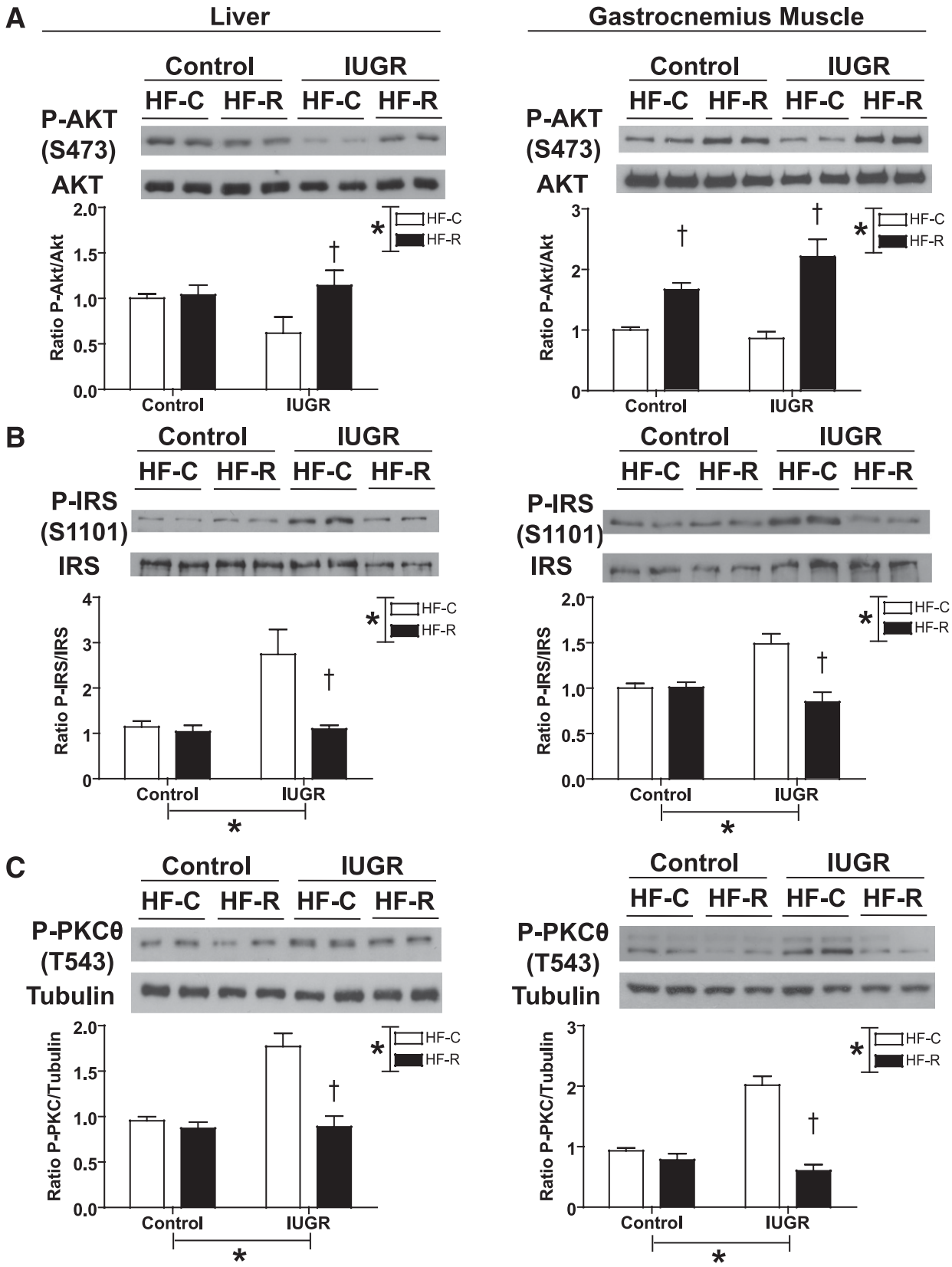

FIG. 4. Influence of IUGR and Resv on insulin signaling elements. Measurements were made in the liver and gastrocnemius tissues of rats after 9 weeks of HF-C or HF-R. Phosphorylation of Akt (P-Akt–to–Akt ratio; *A*), phosphorylation of the IRS-1 (P-IRS–to–IRS ratio; *B*), and phosphorylation of PKCθ (P-PKCθ/tubulin; *C*) are shown. **P* < 0.05 for the respective source of variation (IUGR or Resv) using two-way ANOVA (bar graphs); †*P* < 0.05 vs. controls receiving the same diet after a Bonferroni post hoc test (*n* = 6 per group).

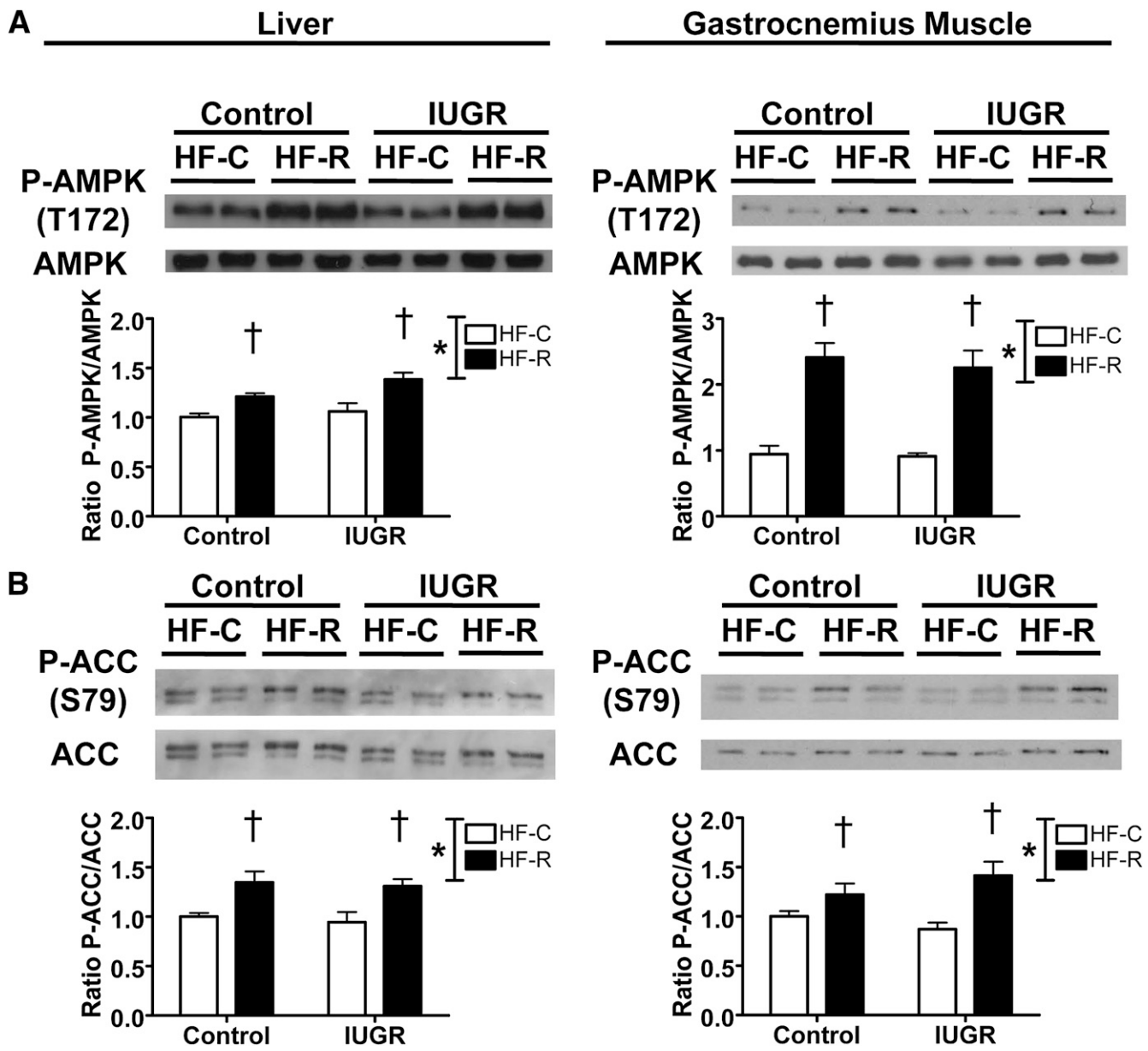

FIG. 5. Effect of IUGR and Resv on AMPK signaling. Measurements were made in the liver and gastrocnemius muscle tissues of rats after 9 weeks of HF-C or HF-R. *A*: Phosphorylation of AMPK (P-AMPK–to–AMPK ratio). *B*: Phosphorylation of ACC (P-ACC–to–ACC ratio). *$P < 0.05$ for the respective source of variation (IUGR or Resv) using two-way ANOVA (bar graphs); †$P < 0.05$ vs. controls receiving the same diet after a Bonferroni post hoc test ($n = 6$ per group).

activation of AMPK and reduced activity of ACC in the liver, thus resulting in the inhibition of fatty acid synthesis and decreased accumulation of TG in the liver. Because increased fatty acid utilization by skeletal muscle also contributes to lowering plasma lipid levels (39), we also show that activation of the AMPK/ACC axis occurs in the skeletal muscle as well. Because activation of the AMPK/ACC signaling axis in muscle increases fatty acid oxidation (18), this may also contribute to lowering plasma lipid levels in IUGR offspring when HF diets are supplemented with Resv. Therefore, the lipid-lowering effects of Resv in IUGR rats could be a result of AMPK-mediated reduction of hepatic lipid synthesis (17) and increased oxidation of fatty acids by the skeletal muscle (18).

Our finding that Resv activates AMPK in young rodents fed an HF diet is consistent with previous reports indicating that AMPK activation is the primary mechanism by which Resv mediates these beneficial effects (15–17). Recent findings using HF-fed AMPK-α1– and -α2–deficient mice demonstrated that the effects of Resv on insulin sensitivity required the presence of either AMPK isoform (16). Moreover, activation of AMPK in skeletal muscle increases glucose utilization in glucose-intolerant mice in an insulin-independent fashion (40). These findings are in agreement with our data showing that Resv activates AMPK.

Although our data implicate AMPK as being involved in the beneficial effects of Resv, we also investigated whether additional molecular signaling mechanisms may contribute. For example, the PKC/IRS/Akt signaling pathway is centrally involved in insulin sensitivity in the liver and skeletal muscle (41) and subsequently regulates glucose uptake in muscle (42). In agreement with this, previous

work suggested that impaired signaling through this pathway contributes to insulin resistance and decreased glucose disposal in association with the accumulation of lipotoxic lipid intermediates (43). Consistent with this, our data show that the PKC/IRS/Akt signaling pathway in the liver and skeletal muscle of IUGR offspring fed an HF diet is significantly impaired, whereas Resv restores this pathway and tissue lipids to levels observed in control offspring fed an HF diet. Thus we conclude that the ability of Resv to prevent insulin resistance and glucose intolerance in IUGR offspring exposed to an HF diet involves activation of AMPK and the prevention of impaired Akt signaling in liver and skeletal muscle.

In conclusion, our data suggest that the consumption of HF diets by IUGR offspring predisposes young rats for the development of glucose intolerance and peripheral insulin resistance without affecting insulin secretion. Most importantly, this is the first study demonstrating that postnatal administration of Resv in the diet of young rats can improve the metabolic profile of HF-fed offspring born from pregnancies complicated by IUGR. Specifically, we show that supplementation with Resv is well tolerated and can reduce the susceptibility to HF diet–induced metabolic alterations in fat distribution, adipocyte size, hyperlipidemia, glucose disposal, and insulin resistance. Moreover, we also provide molecular evidence that the improvement of glucose homeostasis by Resv may be attributed to insulin sensitization of the peripheral tissues by preventing impaired Akt signaling as well as by activation of AMPK and potentially improving glucose utilization via an insulin-independent mechanism. Based on these data, we suggest that Resv should be considered for further testing as a therapy for the pediatric population born IUGR. Indeed, postnatal therapies for the metabolic defects associated with being born IUGR are currently lacking, and Resv represents the first potential pharmacological treatment that, if administered to vulnerable pediatric patient populations consuming high-calorie diets, could reduce the susceptibility for an adverse metabolic profile.

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V.W.D. and C.F.R.-C. developed the model, performed the experiments, contributed to discussion, and wrote the manuscript. J.S.M. developed the model, contributed to discussion, and reviewed and edited the manuscript. S.T.D. and J.R.B.D. designed the experiments, contributed to discussion, and wrote the manuscript.

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