Original Paper

Childhood-Adolescent Obesity in the Cardiorenal Syndrome: Lessons from Animal Models

Melvin R. Hayden^{a, c} James R. Sowers^{a–d}

^aDivision of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, and ^bDepartment of Medical Pharmacology and Physiology, University of Missouri-Columbia School of Medicine, ^cDiabetes and Cardiovascular Center, and ^dHarry S. Truman VA Medical Center, Columbia, Mo., USA

Key Words

Adolescence • Childhood • Diabetes • Obesity • Rodent models • Ultrastructural remodeling

Abstract

Background/Aims: Childhood-adolescent overweight and obesity have grown to pandemic proportions during the past decade. The onset of obesity in younger adults will likely be manifested as earlier onset of myocardial and renal end-organ disease in younger adults. For the first time, it is estimated that the current generation may not live to be as old as their parents. Thus, it is important to develop animal models of childhood obesity to understand fundamental pathological organ changes. **Methods:** In this regard, we utilize transmission electron microscopy evaluation to evaluate early remodeling changes of two adolescent rodent obesity models: the Zucker obese (fa/fa) rat and the db/db mouse models of obesity. We have concentrated on the initial ultrastructural remodeling (obese adipose tissue, skeletal muscle, and islet remodeling) and the associated changes in target end organs (including the myocardium and kidney) in young rodent models of obesity and insulin resistance, collectively manifesting as the cardiorenal metabolic syndrome (CRS). **Results:** Briefly, tissues revealed the following ultrastructural remodeling abnormalities: inflammation, hypertrophy, and early fibrosis in adipose tissue; loss of mitochondria in skeletal muscles, hyperplasia, fibrosis, and depletion of insulin-secreting granules in pancreatic islets; increased intramyocardial lipid accumulation, fibrosis, and mitochondrial deposition in the myocardium, and obesity-related glomerulopathy and tubulopathy in the kidney. **Conclusion:** Based on the current knowledge and ultrastructural observations of organ pathology, we propose mechanisms whereby obesity appears to be the driving force behind the development of the CRS.

Copyright © 2011 S. Karger AG, Basel

Introduction

Childhood-adolescent overweight and obesity are emerging major global public health concerns [1, 2]. Childhood-adolescent overweight is defined as a body mass index (BMI) greater than the 85th percentile but less than the 95th percentile, whereas childhood-adolescent obesity is defined as a BMI greater than the 95th percentile [3]. The International Obesity Task Force has estimated that 22 million children younger than 5 years of age are overweight or obese. They also estimated that 1 in 10 children (approximately 155 million) are overweight. This emerging pandemic is largely thought to be triggered by the same sociologic/environmental factors that result in adulthood obesity. Important factors include: increasingly sedentary lifestyles and excess compact caloric consumption. Childhood obesity is associated with an increased prevalence (up to 50%) of the metabolic syndrome, and this relationship is increased on a graded scale based on increasing half units of BMI [4]. Overweight and obesity in childhood are the predominant drivers of early insulin resistance, dyslipidemia, impaired glucose tolerance, overt type-2 diabetes mellitus (T2DM) and long-term complications, including hypertension-associated cardiovascular disease (CVD) and chronic kidney disease (CKD) [1, 2, 5–21]. In this regard, childhood obesity is associated with increased inflammation with elevation of the biomarkers of C-reactive protein, interleukin-6, tumor necrosis factor- α (TNF- α) and decreased levels of adiponectin, a marker of insulin sensitivity, which is synthesized and secreted exclusively by adipocytes [2, 6, 12, 22]. Indeed, there is increasing evidence that inflammation and oxidative stress associated with overweight and obesity states represent a nexus between components of the cardiorenal metabolic syndrome (CRS), namely CVD and CKD [19–21].

The CRS is a constellation of numerous risk factors (obesity and insulin resistance), metabolic dyslipidemia, myocardial diastolic dysfunction, and diminished renal function with or without proteinuria. Developing CRS at an early age predisposes individuals to CKD and CVD, which are associated with marked morbidity and mortality causing both a social and an economic burden on our global health care system [15–21, 23, 24]. Multiple metabolic abnormalities are associated with overnutrition, an inflammatory/procoagulant state, and the excess production of reactive oxygen species (ROS). In turn, increased systemic inflammation and elevated tissue levels of ROS result not only in systemic depletion of antioxidant enzymes but are also directly damaging to proteins, lipids, and nucleic acids. In the kidney, these abnormalities lead to micro- and macroalbuminuria in the early stages of the CRS. Microalbuminuria reflects systemic endothelial dysfunction associated with CVD, as well as glomerular endothelial and glomerular filtration barrier dysfunction ultimately leading to CKD [16, 20]. Central obesity is a pivotal component of the CRS and predisposes to the early renal abnormalities that affect the kidney and other organs in the CRS. Therefore, we chose to focus more heavily on the central/omental adipose tissue (instigator/driver of the CRS) and the subsequent end-organ involvement of skeletal muscle, which predisposes to systemic insulin resistance [25]. Concurrently, there is development of resistance of insulin metabolic signaling in adipose and hepatic tissue. The development of systemic insulin resistance drives compensatory increases in pancreatic islet insulin secretion, but eventually there is pancreatic islet cell/ β -cell failure giving rise to overt diabetes [25].

Our focus for this paper is on the use of young rodent models to explore the ultrastructural abnormalities that are extant in the early stages of the CRS. With this focus in mind, we will review multiple, early ultrastructural cellular and extracellular remodeling changes found in the various organs responsible for the development of the CRS. We utilized young Zucker obese (ZO) rats (fa/fa) and obese db/db mice, both of which display a point mutation in the leptin receptor resulting in hyperphagia and obesity [23, 24]. Both of these rodent models progress through the CRS prior to development of diabetes. Thus, these are appro-

Table 1. Brief description of electron micrograph figures 1–6

Figure No.	Brief description	Model
1 Adipose	Illustrates adipocyte hypertrophy, interstitial inflammation, and early fibrosis in ZO omental adipose tissue	ZO
2 Muscle	Displays the loss of subsarcolemma, intermyofibrillar, and pericapillary mitochondria in the db/db soleus muscle	db/db
3 Islet	Demonstrates islet exocrine interface widening, pericyte hyperplasia, islet hypertrophy and depletion of ISGs with loss of electron-lucent bodies	ZO
4 Heart	Reveals increased intramyocardial lipid and abnormal mitochondrial deposition resulting in disorganization of cardiac myofibrils	db/db
5 Kidney	Obesity-related glomerular remodeling, hypertrophy, podocytopenia, and remaining synthetically active podocytes	ZO
6 Kidney	Loss of basolateral polarity in proximal tubule cells, mitochondrial fragmentation, decreased canalicular infoldings, thickening of the basement membrane, chronic interstitial inflammation, and tubulointerstitial fibrosis	ZO

ZO = Nine-week-old ZO (fa/fa) rat model; db/db = 12-week-old obesity mouse model.

priate models to demonstrate ultrastructural remodeling changes associated with the CRS at an early age. Importantly, these models potentially give us additional insight into the role of obesity in the progression of organ damage associated with the CRS that translates to childhood and adolescent youths. A brief summary of these ultrastructural changes is outlined in table 1.

Transmission electron microscopy (TEM) was utilized as a research tool to evaluate ultrastructural remodeling changes (quantitative and qualitative) in young CRS rodent models, the ZO rat and the db/db mouse. Briefly, appropriate tissues were thinly sliced and placed immediately in standard TEM fixative. Ultrathin sections (85 nm) were then prepared and stained with 5% uranyl acetate and Sato's triple-lead stain for TEM viewing. Herein, we describe ultrastructural observational-qualitative remodeling found in the young (pre-adolescent) male obesity models. All procedures were approved by the University of Missouri Animal Care Committees, and animals were housed in accordance with NIH Guidelines.

Omental Adipose Tissue as the Instigator/Driver of the CRS: Ultrastructural Morphological Remodeling and Functional Changes

Obesity is thought to be a major contributing factor in the development of skeletal muscle insulin resistance, which is a known early event in the CRS and plays a critical role in the development of T2DM [12–14, 23–26]. Importantly, we now know that adipocytes have properties of endocrine cells in addition to being a major storage site for energy via retention of free fatty acids (FFA) and glycerol. Indeed, the adipocyte is capable of secreting numerous peptide hormones, namely adiponectin, leptin, resistin, cytokines, and inflammatory factors, including TNF- α , IL-6, and tissue growth factor- β [12–14, 25, 26]. These endocrine qualities of adipocytes allow them to influence distant end organs, such as skeletal and cardiac muscles, the liver, pancreatic β -cells, and the kidney.

Fig. 1. Ultrastructure remodeling of omental adipose tissue in the ZO model. **b** Adipocyte hypertrophy, interstitial widening, and inflammatory infiltrate of macrophages (asterisks) in the ZO (**b**) and ZL (**a**) models. **c** Disorging of smaller lipid vesicles (arrows) and vacuoles (LV) into the large unilocular lipid granule (dashed arrows), which were not present in the ZL or ZO subcutaneous tissue models. Importantly, note the novel and unique findings of collagen adhering directly to the plasma membrane (PM; asterisk). **d** An interstitial fibrocyte and/or a differentiated pericyte synthesizing and secreting collagen fibrils that directly adhere to the adipocyte plasma membrane (PM) in the ZO omental tissues (Om.). These findings were only observed in omental ZO plasma membranes.

The omental adipose tissue in the young ZO model (fig. 1) depicts the following ultrastructural changes: adipocyte hypertrophy, macrophage infiltration, and widening of the interstitium between cells. In addition, the cytoplasm of these adipocytes is filled with disorging lipid droplets and vesicles, and early fibrosis with collagen fibrils adhering to adipocyte plasma membranes. These abnormalities are not observed in the subcutaneous depot of the ZO rat, or in omental or subcutaneous depots of Zucker lean (ZL) littermates. These changes are compatible with the inflammation and early fibrosis that we observe in omental fat tissue in humans. Inflammatory and fibrotic maladaptive changes in omental adipose tissue may result in increased stiffness and rigidity, which may contribute to the expansion and engorgement of lipids within the lipid granules. Additionally, there is the unique deposition of collagen type VI, in contrast to the usual type I and III collagen that are found in other organs like the kidney, heart, and liver [26]. This atypical collagen is unique for adipose omental tissue in this rodent model of the CRS.

Fig. 2. Obesity associated with loss of subsarcolemma, intermyofibrillar, and pericapillary mitochondria in the obese db/db mouse model. **c** Loss of subsarcolemma (open arrows – dotted line) and intermyofibrillar distribution (closed arrows) in the db/db model compared to the lean wild-type littermate (**a**; bars = 0.5 μm). **d** Loss of pericapillary mitochondria (dotted line; bar = 0.5 μm) compared to the lean wild-type (open arrows) littermate (**b**; bar = 1 μm).

Hyperlipidemia and Maladaptive Adipose Alterations in the CRS

The excessively engorged adipocytes associated with visceral and subcutaneous obesity are associated with the generation of excess FFA and triglycerides. This abnormality is felt to be due to the rapid metabolic turnover of fat due to lipolysis-instigating excessive intrahepatic and skeletal muscle lipid deposition, which results in insulin resistance in hepatic, skeletal, cardiac muscle and adipose tissue, creating a vicious cycle [25, 26]. Additionally, this excess lipid accumulation is thought to be responsible for supplying the substrates for oxidative stress and contributes to the generation of inflammatory adipokines such as TNF- α . These inflammatory factors and redox stress-related changes induce further production of adipokines and FFA, which in turn contribute to the development of insulin resistance in skeletal muscle tissue [27].

Fig. 3. Widening of the islet exocrine interface and depletion of ISGs in the ZO model. **c** Marked widening of the islet exocrine interface (IEI; double arrows) in the ZO model compared to the ZL model (**a**; single arrows). Bar = 2 μ m. **d** Decreased ISGs (electron-dense black dots) compared to the ZL model (**b**). Bar = 1 μ m. **b, d** Note the loss of electron-lucent Hayden-Sowers bodies (open arrow) in the exploded inset image of an ISG of the ZO model (**d**) compared to the inset of an ISG of the ZL model (**b**).

Skeletal Muscle as a Target Organ of Overweight and Obesity and Instigator of Systemic Insulin Resistance

In the early evolution of insulin resistance, there is a loss of subsarcolemma, intermyofibrillar, perinuclear, and pericapillary mitochondria in both the soleus (slow twitch) and gastrocnemius (fast twitch) muscles (fig. 2). Recently, our laboratory has been able to disclose an association between fundamental pericytopathy in both skeletal muscle and the pancreatic islet tissue, pointing to the important role of this abnormality in the pathogenesis of the CRS [28]. Mitochondrial pericyte and capillary loss may be related to skeletal muscle and pancreatic rarefaction [28]. Interestingly, there are emerging data suggesting that insulin resistance and the compensatory hyperinsulinemia may also contribute to obesity due to the direct metabolic effects of impaired insulin metabolic signaling and growth effects of hyperinsulinemia on adipocytes [29].

Increased tissue levels of FFA, TNF- α , and ROS are known to interfere with insulin signaling in skeletal muscles and this occurs due to the impairment in tyrosine phosphorylation

Fig. 4. Intermyofibrillar lipid and abnormal mitochondrial deposition in the db/db model. **b, d** Increased intermyofibrillar lipid droplets (open arrows) and mitochondria (Mt) compared to the wild-type lean littermates (WT; **a, c**). Bar = 5 (**a, b**) and 2 μ m (**c, d**). Note disordered-displaced striated myofibrils (MYF) in db/db (**b, d**) compared to WT animals (**a, c**). Note loss of mitochondrial cristae and matrix of db/db (**d**; inset) compared to the WT animals (**c**; inset). Bar = 200 nm. IMF = Intermyofibrillar.

of IRS-1 (insulin receptor substrate-1), which ultimately impairs GLUT-4 (glucose transporter type-4) translocation from the cytosol to the plasma membrane resulting in impaired glucose uptake [25]. Importantly, this impaired glucose uptake and the compensatory hyperinsulinemia results in insulin resistance and increased oxidative stress, which is a significant part of the multiple metabolic toxicities of the CRS.

Insulin Resistance Results in Compensatory Pancreatic β -Cell Hyperinsulinemia and Hyperamylinemia and Ultrastructural Islet Remodeling

The ultrastructural remodeling in the pancreatic islet consists of widening of the islet exocrine interface-peri-islet regions, insulin-secretory granule (ISG) loss/depletion, loss of electron-lucent Hayden-Sowers bodies (fig. 3), which normally indicate maturity of insulin and amylin synthesis in ISGs, islet hypertrophy-periductal islet (β -cell) neogenesis, peri-islet pericyte hyperplasia, and angiogenesis [28]. Compensatory hyperinsulinemia and hyper-

Fig. 5. Obesity-related glomerular remodeling in the young 9-week-old ZO model. **b, c** Glomerular hypertrophy, widened Bowman's space (BS), dilated glomerular capillaries (cap) reduced podocyte (P-POD) numbers, podocyte/endothelial cell (EC) ratio, and rouleaux formation (**b**: inset) compared to the ZL where podocytes predominate (**a**). Bar = 5 (**a, b**) and 2 μm (**c**). **d** Compensatory podocyte hypertrophy with increased synthetic organelles of rough endoplasmic reticulum (rER) and variable electron-dense proteinaceous inclusion storage granules and lysosomes (arrow in exploded inset). Bar = 1 μm .

amylinemia develop in obesity due to skeletal muscle insulin resistance and the compensatory pancreatic response. Importantly, these hormones contribute to the local and systemic activation of the renin-angiotensin-aldosterone system and sympathetic nervous system and consequent development of the CRS.

Myocardium as a Target Organ of Overweight and Obesity

The myocardium in models of the CRS demonstrates considerable end-organ ultrastructural remodeling in the ZO rat and consists of decreased insulin sensitivity, increased intermyofibrillar mitochondria (biogenesis) and disordered myocyte ultrastructure, pericapillary and interstitial fibrosis, abnormal swollen mitochondria with decreased crista and matrix electron density, and collapsed capillaries, which result in diastolic dysfunction typical of diabetic cardiomyopathy [23, 25, 30].

Fig. 6. Loss of basolateral polarity in ZO models. **b** Chaotic disorganization of the basolateral region of the proximal tubule cell, loss of basolateral polarity depicted by the loss of mitochondrial (Mt) elongation, termed Mt fragmentation, loss of elongated basilar canalicular membrane infoldings (arrows: **a**). Bar = 0.5 μm . **c** Loss of basolateral polarity, chronic tubulointerstitial infiltration of macrophages (M Φ ; inset), and a recently extruded monocyte (MC). BM = Basement membrane. Bar = 1 μm . **d** Microvascular and tubulointerstitial fibrosis in an older ZO (12-month-old) model with a mast cell, M Φ , and numerous pericyte foot processes (Pc-fp). Bar = 1 μm .

In the obese db/db mouse model, myocardial ultrastructural remodeling consists of increased lipid accumulation, fibrosis, and abnormal intermyofibrillar mitochondrial deposition with disordered myocyte ultrastructure (fig. 4).

Kidney as a Target Organ: CKD and End-Stage Kidney Failure

The young ZO rat model of insulin resistance and obesity develops microalbuminuria and therefore may serve as a good model of early renal disease in the CRS [20, 21, 23, 31–34]. The kidney glomerulus undergoes marked ultrastructural remodeling consisting of glomerular hypertrophy and widening of Bowman's space, inflammation, loss of podocytes (podocytopenia), and podocyte remodeling (fig. 5) changes that have been described in humans and are termed obesity-related glomerulopathy. Additionally, there is marked ultrastructural remodeling of the proximal tubule, which is similar to that in another rodent model of

insulin resistance and excess angiotensin II production (due to renin transfection with mouse renin gene) [32]. The kidney proximal tubule cells also undergo marked ultrastructural remodeling consisting of loss of basolateral polarity, loss of elongated mitochondria with spherical rounded mitochondria (mitochondrial fragmentation), abnormal mitochondrial cristae and matrix, decreased basilar invaginating canalicular infoldings, and chronic inflammation (fig. 6) [34]. In the young ZO rat model, we refer to these proximal tubule cell remodeling changes as obesity-related tubulopathy. These changes are precursors of the tubulointerstitial fibrosis that characterizes progressive renal disease in T2DM.

Pancreatic Islets and T2DM

In addition to the early islet compensatory remodeling associated with skeletal muscle and systemic insulin resistance, the pancreatic islets also undergo targeted end-organ remodeling consisting of advanced fibrosis, increasing β -cell dysfunction and apoptosis, islet amyloid deposition and β -cell failure with progressively diminished insulin secretion [35]. The progressive islet remodeling mimics that observed in humans [17–19] and may eventually result in the need for daily exogenous insulin therapy.

Conclusion

The childhood obesity epidemic appears to be the driving force behind the development of CRS in children, and associated organ ultrastructural changes are demonstrated in young rodent models. The progression of CRS to diabetes reflects the ability of obesity to engender reduced insulin metabolic signaling in skeletal muscles and later to involve the systemic end organs, including the liver, adipose depots, heart, kidney, and the islet cells of the pancreas. The compensatory increase in the β -cells in response to insulin resistance is associated with β -cell endoplasmic reticulum stress, insulin metabolic signaling defects with secretory impairments (β -cell fatigue), and eventually β -cell failure due largely to apoptosis and fibrosis. In this overview, we have attempted to demonstrate the earliest ultrastructural remodeling changes, which will provide the first opportunity to observe the critical cellular and extracellular remodeling that is occurring in the organs responsible for the development of the CRS. The complex orchestration of metabolic signaling involves the detrimental effects of inflammation and oxidative stress, as well as the marked ultrastructural cellular and tissue remodeling. While these representative remodeling changes have been demonstrated in adolescent rodent models, we speculate that similar changes may be concurrently occurring in young obese patients. The early cellular-extracellular remodeling changes will definitely give rise to a more fundamental understanding of the earlier structural abnormalities in involved end organs, such as the adipose depots, skeletal muscles, pancreatic islets, the myocardium, and the kidney. These ultrastructural changes allow us to better understand why young obese persons are at a much greater risk for developing the CRS and associated major end-organ disease. The ultrastructural images only provide a snapshot in time; however, they do provide evidence suggesting that obesity in young adolescent models leads to increased glomerular, proximal tubular, and cardiac remodeling. The current strategies to prevent childhood obesity center on lifestyle interventions, including proper diet and adequate exercise, for our childhood and adolescent youth.

Acknowledgments

The authors would like to acknowledge the Electron Microscopy Core Facility (University of Missouri) for the preparation and handling of specimens for viewing, and thank Brenda Hunter for editing the manuscript.

This research was supported by the National Institutes of Health (R01 HL73101-01A1), Veterans Affairs Merit System 0018 (J.R.S.).

Disclosure Statement

The authors have nothing to disclose.

References

- 1 Ogden CL, Carroll MD, Flegal KM: High body mass index for age among US children and adolescents, 2003–2006. *JAMA* 2008;299:2401–2405.
- 2 Allcock DM, Gardner MJ, Sowers JR: Relation between childhood obesity and adult cardiovascular risk. *Int J Pediatr Endocrinol* 2009;2009:108187.
- 3 Hampton T: Pediatric obesity guidelines released. *JAMA* 2008;300:2238.
- 4 Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S: Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350:2362–2374.
- 5 Caprio S: Insulin resistance in childhood obesity. *J Pediatr Endocrinol Metab* 2002;15(suppl 1):487–492.
- 6 Lee WWR: An overview of pediatric obesity. *Pediatr Diabetes* 2007;8(suppl 9):76–87.
- 7 Franks PW, Hanson RL, Knowler WC, Moffett C, Enos G, Infante AM, Krakoff J, Looker HC: Childhood predictors of young-onset type 2 diabetes. *Diabetes* 2007;56:2964–2972.
- 8 Chen W, Srinivasan SR, Li S, Xu J, Berenson GS: Metabolic syndrome variables at low levels in childhood are beneficially associated with adult cardiovascular risk: the Bogalusa Heart Study. *Diabetes Care* 2005;28:126–131.
- 9 Morrison JA, Friedman LA, Gray-McGuire C: Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. *Pediatrics* 2007;120:340–345.
- 10 Bates LM, Acevedo-Garcia D, Alegria M, Krieger N: Immigration and generational trends in body mass index and obesity in the United States: results of the National Latino and Asian American Survey, 2002–2003. *Am J Public Health* 2008;98:70–77.
- 11 Cook S, Auinger P, Li C, Ford ES: Metabolic syndrome rates in United States adolescents, from the National Health and Nutrition Examination Survey, 1999–2002. *J Pediatr* 2008;152:165–170.
- 12 Lee S, Bacha F, Gungor N, Arslanian S: Comparison of different definitions of pediatric metabolic syndrome: relation to abdominal adiposity, insulin resistance, adiponectin, and inflammatory biomarkers. *J Pediatr* 2008;152:177–184.
- 13 Sun SS, Liang R, Huang TT, Daniels SR, Arslanian S, Liu K, Grave GD, Siervogel RM: Childhood obesity predicts adult metabolic syndrome: the Fels Longitudinal Study. *J Pediatr* 2008;152:191–200.
- 14 Messiah SE, Arheart KL, Luke B, Lipshultz SE, Miller TL: Relationship between body mass index and metabolic syndrome risk factors among US 8- to 14-year-olds, 1999 to 2002. *J Pediatr* 2008;153:215–221.
- 15 Falkner B, Lurbe E, Schaefer F: High blood pressure in children: clinical and health policy implications. *J Clin Hypertens* 2010;12:261–276.
- 16 Whaley-Connell A, Pavey BS, McCullough PA, Saab G, Li S, McFarlane SI, Chen SC, Vassalotti JA, Collins AJ, Bakris G, Sowers JR, KEEP Investigators: Dysglycemia predicts cardiovascular and kidney disease in the Kidney Early Evaluation Program. *J Clin Hypertens* 2010;12:51–58.
- 17 Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, Shochat T, Kochba I, Rudich A, for the Israeli Diabetes Research Group: Normal plasma fasting glucose levels and type 2 diabetes in young men. *N Engl J Med* 2005;353:1454–1462.
- 18 Gungor N, Bacha F, Saad R, Janosky J, Arslanian S: Youth type 2 diabetes: insulin resistance, beta-cell failure, or both? *Diabetes Care* 2005;28:638–644.
- 19 Tfayli H, Lee S, Arslanian S: Declining beta-cell function relative to insulin sensitivity with increasing fasting glucose levels in the nondiabetic range in children. *Diabetes Care* 2010;33:2024–2030.
- 20 Sowers JR: Metabolic risk factors and renal disease. *Kidney Int* 2007;71:719–720.
- 21 Bakris G, Vassalotti J, Ritz E, Wanner C, Stergiou G, Molitch M, Nesto R, Kaysen GA, Sowers JR, for the CKD Consensus Working Group: National Kidney Foundation consensus conference on cardiovascular and kidney diseases and diabetes risk: an integrated therapeutic approach to reduce events. *Kidney Int* 2010;78:726–736.
- 22 Coppen AM, Risser JA, Vash PD: Metabolic syndrome resolution in children and adolescents after 10 weeks of weight loss. *J Cardiometab Syndr* 2008;3:205–210.

- 23 Whaley-Connell A, Pulakat L, DeMarco VG, Hayden MR, Habibi J, Henriksen EJ, Sowers JR: Overnutrition and the cardiorenal syndrome: use of a rodent model to examine mechanisms. *Cardiorenal Med* 2011;1:23–30.
- 24 Lindborg KA, Jacob S, Henriksen EJ: Effects of chronic antagonism of endocannabinoid-1 receptors on glucose tolerance and insulin action in skeletal muscles of lean and obese Zucker rats. *Cardiorenal Med* 2011;1:31–44.
- 25 Sowers JR, Whaley-Connell A, Hayden MR: The role of overweight and obesity in the cardiorenal syndrome. *Cardiorenal Med* 2011;1:5–12.
- 26 Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, Zhang BB, Bonaldo P, Chua S, Scherer PE: Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol Cell Biol* 2009;29:1575–1591.
- 27 Fabbrini E, deHaseth D, Deivanayagam S, Mohammed BS, Vitola BE, Klein S: Alterations in fatty acid kinetics in obese adolescents with increased intrahepatic triglyceride content. *Obesity (Silver Spring)* 2009;17:25–29.
- 28 Hayden MR, Yang Y, Habibi J, Bagree SV, Sowers JR: Pericytopathy: oxidative stress and impaired cellular longevity in the pancreas and skeletal muscle in metabolic syndrome and type 2 diabetes. *Oxid Med Cell Longev* 2010;3:290–303.
- 29 Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 2000;106:473–481.
- 30 Zhou X, Ma L, Habibi J, Whaley-Connell A, Hayden MR, Tilmon RD, Brown AN, Kim JA, Demarco VG, Sowers JR: Nebivolol improves diastolic dysfunction and myocardial remodeling through reductions in oxidative stress in the Zucker obese rat. *Hypertension* 2010;55:880–888.
- 31 Liese AD, Hense HW, Döring A, Stieber J, Keil U: Microalbuminuria, central adiposity and hypertension in the non-diabetic urban population of the MONICA Augsburg survey 1994/95. *J Hum Hypertens* 2001;15:799–804.
- 32 Hayden MR, Habibi J, Whaley-Connell A, Sowers D, Johnson M, Tilmon R, Jain D, Ferrario C, Sowers JR: Nebivolol attenuates maladaptive proximal tubule remodeling in transgenic rats. *Am J Nephrol* 2010;31:262–272.
- 33 Hayden MR, Whaley-Connell A, Sowers JR: Renal redox stress and remodeling in metabolic syndrome, type 2 diabetes mellitus, and diabetic nephropathy: paying homage to the podocyte. *Am J Nephrol* 2005;25:553–569.
- 34 Habibi J, Hayden MR, Sowers JR, Pulakat L, Tilmon RD, Manrique C, Lastra G, Demarco VG, Whaley-Connell A: Nebivolol attenuates redox-sensitive glomerular and tubular mediated proteinuria in obese rats. *Endocrinology* 2011;152:659–668.
- 35 Hayden MR, Tyagi SC: Islet redox stress: the manifold toxicities of insulin resistance, metabolic syndrome and amylin derived islet amyloid in type 2 diabetes mellitus. *JOP* 2002;3:86–108.