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

Melvin R. Hayden a, c James R. Sowers a–d

a Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, and b Department of Medical Pharmacology and Physiology, University of Missouri-Columbia School of Medicine, c Diabetes and Cardiovascular Center, and d Harry S. Truman VA Medical Center, Columbia, Mo., USA

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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-secretory 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.

M.R. Hayden, MD
D109 HSC Diabetes Center
One Hospital Drive, Columbia, MO 65212 (USA)
Tel. +1 573 884 0769, E-Mail mrh29@usmo.com
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/omentumal 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-
appropriate 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.

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

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

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.
The omental adipose tissue in the young ZO model (fig. 1) depicts the following ultrastructural changes: adipocyte hypertrophy, interstitial widening, and inflammatory infiltrate of macrophages (asterisks) in the ZO (b) and ZL (a) models. c Disgorging 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 disgorging 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) littersmates. 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.
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].
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...
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-
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].
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 (podoctopenia), 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

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**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 (Mϕ; 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, Mϕ, and numerous pericyte foot processes (Pc-fp). Bar = 1 μm.
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.
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Disclosure Statement

The authors have nothing to disclose.

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