Obesity-induced changes in kidney mitochondria and endoplasmic reticulum in the presence or absence of leptin

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Submitted 4 May 2015; accepted in final form 13 August 2015

Obesity has emerged as a global health problem and is a major risk factor for cardiovascular diseases, such as heart failure (9, 10) and liver disease, such as non-alcoholic fatty liver disease (32, 35). Obesity may also be a risk factor for end-stage renal disease (43, 46), even after adjustment for hypertension and diabetes, and multiple studies in experimental animals (53, 61, 67, 84, 85) and humans (2, 4, 41, 91, 92) strongly implicate obesity in the development of chronic kidney disease.

With obesity, tissues are presented with elevated levels of fatty acids. Two consequences of this are 1) increased oxidation of fatty acids by the mitochondria, and 2) increased production of reactive oxygen species (ROS; superoxide, hydrogen peroxide, hydroxyl radical, lipid peroxides) in mitochondria (5, 77). Increased ROS can damage mitochondrial proteins, leading to further ROS production, and ultimately to mitochondrial dysfunction. Mitochondrial dysfunction presents in various forms, presumably due to variables such as the tissue source of the mitochondria and obesity-associated pathophysiological conditions, such as hypertension and diabetes. In addition, several protective events may be induced, including increased antioxidant defense (73, 95), activation of uncoupling proteins (54, 77), and promotion of mitochondrial biogenesis (47) and mitophagy (82).

Information on kidney mitochondrial function during obesity is limited. In mice fed a high-fat diet, kidney mitochondria respiring succinate produced more H2O2 and showed evidence of oxidative stress, while respiration and coupling appeared normal (73). However, the ability of the kidney mitochondria to oxidize fatty acids was not examined.

Obesity may also impair the protein-folding machinery within the endoplasmic reticulum (ER), a condition known as ER stress (11, 52). Disruption of the resident ER chaperone BiP/glucose-regulated protein 78 (GRP78) aggravates renal tubular injury (51). Experimentally induced ER stress in renal tubules (50) and podocytes (45) has been demonstrated to cause autophagy and result in cell injury, respectively. A recent study in obese Zucker rats, a rodent model of obesity with hypertension and metabolic syndrome, revealed activation of ER stress in the kidneys (90). However, the effect of obesity, in the absence of hypertension, on kidney ER has not, to our knowledge, been previously reported.

Obesity increases levels of the adipocyte-derived hormone leptin, which acts in the brain to regulate food intake (59). Leptin also stimulates β-oxidation of fatty acids, as well as export of lipids from tissues through signaling events involving both central (brain) and peripheral receptors (7, 13, 17, 68, 93, 99). However, the effects of leptin on kidney mitochondrial function during obesity have not, to our knowledge, been reported.

One objective of this study was to determine obesity-associated changes in triglyceride accumulation, mitochondrial function, ER stress, and kidney function in the absence of hypertension. A second objective was to test whether obesity affects the functions of renal mitochondria and ER differently in mice with high levels of leptin compared with obese mice with leptin deficiency. To accomplish these objectives, we used two genetic mouse models of obesity: 1) ob/ob mice which are obese because they do not synthesize leptin (66, 97) and 2) hyperleptinemic melanocortin-4 receptor deficient mice (LoxTB MC4R−/−) (87). The melanocortin-4 receptor (MC4R) signaling pathway also plays a central role in regu-
lating appetite and energy homeostasis in humans and other mammals (76). Mutations in the MC4R gene are the most common genetic causes of early onset, morbid obesity in humans (24) and are associated with increased adiposity and weight gain in mice (56). Unlike genetic mutations in leptin or leptin receptors, alterations in the MC4R gene are associated with high plasma leptin levels (20, 87).

An important advantage of these obesity models, besides the fact that they have very different levels of leptin, is that they do not have increased sympathetic nervous system activity and hypertension that are usually associated with obesity. Therefore, the use of MC4R−/− and ob/ob mice permitted us to examine the impact of obesity on mitochondrial function, ER stress, and kidney function in the absence of confounding effects of hypertension. Our results show that obesity in the absence of hypertension is linked to glomerular hyperfiltration and renal dysfunction associated with increased albumin excretion. Both ob/ob and MC4R−/− mice showed reduced ATP levels in kidney tissue and mitochondrial dysfunction, although the mitochondrial alterations seen in the absence of leptin differed from those seen in the presence of the hormone. The kidneys of obese mice with high plasma leptin levels exhibited mitochondrial oxidative stress and evidence of ER stress, while the kidneys of obese mice lacking leptin did not.

**MATERIALS AND METHODS**

All the experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi Medical Center (Jackson, MS). Mice were placed in a 12:12-h dark-light cycle and given free access to food and water throughout the period of study.

**Blood Pressure Recording and Urine, Blood, and Tissue Collection**

Thirty-six to forty-five-wk-old C57BL/6J wild-type (WT), ob/ob, LoxTB MC4R−/−, and their age-matched WT littermate control mice (n = 7/group) were used in the study. C57BL/6J WT mice were used as controls for ob/ob mice, whereas the LoxTB MC4R−/− mice that were raised in a B6 mixed background were compared against their WT littermates (WT-LoxTB) from the breeding colony. LoxTB MC4R−/− mice have a loxP-flanked transcriptional blocking (loxTB) sequence inserted between the transcriptional start site and the start codon of MC4R, which prevents the transcription of the MC4R gene (8).

Mice were implanted with telemetric blood pressure transmitters (model TA11PAC10, Data Sciences) as described previously (87). Ten days after recovery from surgery, mean arterial pressure (MAP) and heart rate (HR) were measured by telemetry continuously, 24 h/day, for 5 consecutive days.

Following blood pressure recording, mice were acclimatized in metabolic cages for 48 h before measurement of food intake and urine collection for 3 consecutive days to determine albumin and creatinine excretion. On the fifth day, mice were anesthetized with isoflurane and transcardially perfused with 0.1% PBS, pH 7.4. The brain, heart, liver, kidneys, perirenal, mesenteric, and epididymal fat were collected and weighed. Mitochondria were isolated from the right kidney as described below. The left kidney was divided into two portions: one portion was frozen in liquid nitrogen for biochemical and proteome measurements, and the other portion was fixed in 10% buffered formalin to assess histological changes in the kidney.

**Blood and Urine Biochemistry**

Fasting blood glucose levels were measured with a glucometer (Optima ultrafast glucose strips). Urine albumin, plasma insulin, and leptin levels were determined by ELISA kits using Exocell/Albuwell M, Linco Insulin, and R&D Leptin, respectively. Urine and plasma creatinine levels were determined using HPLC. The glomerular filtration rate was estimated from creatinine clearance (ml/min) calculated from the following formula: [urine volume (μl/24 h) × urine creatinine (mg/dl)]/[plasma creatinine (mg/dl) × 1.140 min].

**Histological Assessments**

Coronal sections ~5 μm thick of the kidneys were stained for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) to analyze renal morphology and hyalination. Renal macrophage infiltration was assessed by immunostaining for F4/80 antigen, a cell surface marker widely expressed in mouse macrophages, using anti-F4/80 antibody (Abd Serotec) as described previously (20).

**Measurement of Renal Triglyceride Levels, Renal ATP Content, and Mitochondrial Protein Carbonyl Levels**

Renal triglycerides were extracted using the chloroform: methanol method described by Folch et al. (26) and measured using a colorimetric triglyceride assay kit (Wako Diagnostics). ATP levels were determined in whole kidney homogenates using a bioluminescent ATP determination kit (Molecular Probes). Mitochondrial protein carbonyl levels were assayed spectrophotometrically by derivatization with 2,4-dinitrophenyl hydrazine using a protein carbonyl assay kit (Cayman Chemical).

**Mitochondrial Isolation and Respiration Measurements**

Immediately after harvest, right kidneys from mice were washed with ice-cold PBS, pH 7.4, and thoroughly minced with a sharp scalpel. The contents were then transferred to a 5-ml capacity teflon-glass homogenizer and homogenized in 1.5 ml of ice-cold mitochondrial extraction buffer [250 mM sucrose, 10 mM HEPES, 1 mM EDTA, 1 mg/ml BSA, pH 7.4, with complete protease inhibitor and PhosStop phosphatase inhibitor cocktail (Roche Diagnostics)] by performing 8–10 strokes. Homogenates were centrifuged at 600 g for 10 min at 4°C to remove debris, and the resulting supernatants were centrifuged at 10,000 g for 10 min at 4°C to pellet the mitochondrial fraction. The pellets were resuspended in 0.5 ml of extraction buffer and centrifuged again at 10,000 g for 10 min at 4°C. The final pellets containing mitochondria were resuspended in 50 μl of mitochondrial extraction buffer, and the protein concentrations were determined by bichinchoninic acid assay (Pierce).

Mitochondrial respiration measurements were performed polarographically using a Clark-type O2 electrode [Yellow Spring Instruments (YSI)] at 25°C in a 1.7-ml water-jacketed chamber (Gilson) and a YSI 5300A biological oxygen monitor. The analog output from the YSI monitor was ported to an 18-bit analog-to-digital converter in a Hansatech Oxygraph unit, and the O2 consumption measurements (mV vs. time) were collected as ASCII data using Hansatech software and transferred to Kaleidograph (Synergy Software) for extraction of the rates of O2 consumption. The reaction buffer included 250 mM sucrose, 8 mM potassium phosphate, 4 mM magnesium chloride, 1 mM EDTA, and 100 mM Tris buffer, pH 7.2. O2 consumption was initiated by the addition of respiratory substrates such as succinate (5 mM), glutamate (10 mM), malate (5 mM), palmitoyl-1-carnitine (5 μM), or palmitoyl-CoA (5 μM) with 1-carnitine (1 mM). The rate of O2 consumption during state-3 respiration was measured after the addition of 0.2 mM ADP. State-4 respiration was induced by adding 2 μg/ml oligomycin, to inhibit mitochondrial ATP synthase. To
OBESITY AND KIDNEY MITOCHONDRIAL DYSFUNCTION

Table 1. Anthropometric measurements of transgenic and wild-type mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT (n = 7)</th>
<th>ob/ob (n = 7)</th>
<th>WT-LoxTB (n = 7)</th>
<th>LoxTB MC4R−/− (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>36–45</td>
<td>36–45</td>
<td>36–45</td>
<td>36–45</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>34.7 ± 1.0</td>
<td>80.4 ± 3.9†</td>
<td>38.0 ± 2.3</td>
<td>56.9 ± 2.8†</td>
</tr>
<tr>
<td>Body length, cm</td>
<td>10.2 ± 0.1</td>
<td>10.6 ± 0.1*</td>
<td>10.2 ± 0.2</td>
<td>10.4 ± 0.1</td>
</tr>
<tr>
<td>Liver, g</td>
<td>1.81 ± 0.08</td>
<td>4.74 ± 0.37*</td>
<td>1.70 ± 0.16</td>
<td>4.41 ± 0.75†</td>
</tr>
<tr>
<td>Kidney, g</td>
<td>0.24 ± 0.01</td>
<td>0.28 ± 0.01*</td>
<td>0.25 ± 0.02</td>
<td>0.28 ± 0.01†</td>
</tr>
<tr>
<td>Brain, g</td>
<td>0.45 ± 0.01</td>
<td>0.41 ± 0.02*</td>
<td>0.48 ± 0.01</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>Heart, g</td>
<td>0.16 ± 0.01</td>
<td>0.23 ± 0.02*</td>
<td>0.16 ± 0.01</td>
<td>0.20 ± 0.01†</td>
</tr>
<tr>
<td>Epididymal fat, g</td>
<td>0.99 ± 0.21</td>
<td>1.36 ± 0.08*</td>
<td>0.93 ± 0.18</td>
<td>1.54 ± 0.16†</td>
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<tr>
<td>Brown adipose tissue, g</td>
<td>0.30 ± 0.03</td>
<td>0.28 ± 0.04*</td>
<td>0.36 ± 0.03</td>
<td>0.80 ± 0.06†</td>
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<tr>
<td>Visceral fat, g</td>
<td>0.35 ± 0.06</td>
<td>1.96 ± 0.18‡</td>
<td>0.58 ± 0.11</td>
<td>1.16 ± 0.14‡</td>
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<tr>
<td>Perirenal fat, g</td>
<td>0.41 ± 0.08</td>
<td>3.58 ± 0.02‡</td>
<td>0.54 ± 0.09</td>
<td>1.11 ± 0.07‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. WT, wild-type. *P < 0.05 compared with WT. †P < 0.05 compared with WT-LoxTB. ‡P < 0.05 compared with LoxTB MC4R−/−.

correct for changes in the mitochondrial content, citrate synthase (CS) activities were determined spectrophotometrically as described by Srere et al. (83). State-3 respiration rates are presented as nanomoles O2 consumed per minute per unit CS activity.

To measure complex IV (cytochrome c oxidase) activity, an aliquot of mitochondria was solubilized in 0.2% dodecylmaltoside. Oxygen consumption by complex IV was measured in the apparatus described above by methods described previously (89), using ascorbate plus N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) to supply electrons to horse heart cytochrome c. The activities were expressed as nanomoles of electrons per minute per milligram mitochondrial protein.

Markers of ER Stress

Fifty micrograms of protein from whole kidney homogenates were separated on SDS-PAGE and transferred onto a nitrocellulose membrane. Membranes were blocked with Odyssey blocking buffer (LI-COR) for 1 h and then probed for ER chaperone protein GRP78, C/EBP homologous protein (CHOP) and X-box binding protein-1 (XBP-1) using commercial antibodies (anti-rabbit polyclonal GRP78, 1:1,000 dilution, Cell Signaling Technology; anti-mouse monoclonal CHOP, 1:1,000 dilution, Cell Signaling Technology; and anti-rabbit polyclonal XBP-1, 1:200 dilution, Santa Cruz Biotechnology). Blots were imaged using an Odyssey imaging system, and densitometry analyses were performed to quantitate protein levels.

Statistical Analysis

Data are expressed as means ± SE. One-way ANOVA followed by Tukey’s post hoc test was performed to compare between groups using GraphPad Prism 6. A P value <0.05 was considered to be statistically significant.

RESULTS

Anthropometric, Metabolic, and Cardiovascular Characteristics

The body weights of ob/ob and LoxTB MC4R−/− mice were 2.3- and 1.5-fold greater than their respective lean controls (Table 1). The wet weights of the liver, heart, and fat depots, such as visceral fat and perirenal fat, were significantly increased in both obese mouse models (Table 1). Although the brown adipose tissue depots in ob/ob mice were smaller than those of LoxTB MC4R−/− mice, the visceral and perirenal fat depots were significantly larger.

Both ob/ob and LoxTB MC4R−/− mice exhibited elevated fasting blood glucose levels compared with their control groups, despite large increases in plasma insulin levels (Table 2). As expected (87), plasma leptin levels were markedly elevated in the LoxTB MC4R−/− mice (49.1 ± 9.8 ng/ml) compared with their littermate control mice (7.8 ± 3.0 ng/ml). In contrast, plasma leptin levels were undetectable in ob/ob mice plasma compared with WT control mice (4.9 ± 2.1 ng/ml). Despite marked obesity, ob/ob and LoxTB MC4R−/− mice were normotensive and showed normal heart rates compared with their controls (Table 2).

In summary, consistent with our previous studies (20, 21, 87), the melanocortin-4 receptor deficient LoxTB MC4R−/− mice as well as the ob/ob mice displayed hyperglycemia and hyperinsulinemia but normal blood pressure and heart rate. The models differ in that the LoxTB MC4R−/− mice have high plasma levels of leptin (49.1 ± 9.8 ng/ml) while the ob/ob mice have no detectable plasma leptin.

Renal Function and Architecture

Obesity caused by leptin deficiency or MC4R deficiency resulted in glomerular hyperfiltration, as shown by increases in creatinine clearance rates (Fig. 1A). Creatinine clearance in ob/ob mice was increased by 30% compared with lean controls (693 ± 61 vs. 534 ± 32 μl/min). Glomerular hyperfiltration was more pronounced in MC4R-deficient mice, in which there was a 50% increase in creatinine clearance rate compared with controls (752 ± 51 vs. 489 ± 81 μl/min). Similarly, the urine albumin excretion was also increased in both genetic mouse
models of obesity (Fig. 1B). The ob/ob mice excreted about fivefold more albumin than lean controls, while LoxTB MC4R−/− mice excreted about fourfold more albumin than their lean controls.

Despite the increases in creatinine clearance and urine albumin excretion in these two models of normotensive obesity, only subtle changes in glomerular and renal tubular architecture were observed. This was assessed by PAS staining of renal sections (Fig. 2A) as well as H&E staining (data not shown). F4/80 antigen staining, the cell surface marker for murine macrophages, did not show any major changes in macrophage infiltration in the kidneys of these normotensive obese mice compared with their lean counterparts (Fig. 2B).

Renal Triglycerides, ATP Levels, and Oxidative Stress

Renal triglyceride levels. The kidneys of ob/ob mice had a 68% greater triglyceride (TG) content compared with WT mice (8.1 ± 0.8 in ob/ob mice vs. 4.8 ± 0.2 mg TG/g kidney weight in WT mice) (Fig. 3A). Renal TG levels were 77% greater in LoxTB MC4R−/− than in their littermate lean controls (3.9 ± 0.2 vs. 2.2 ± 0.3 mg TG/g kidney wt). Both models contain elevated kidney TG compared with their respective controls.

Renal ATP levels. Renal ATP content was significantly lower in kidneys from both obese models compared with their lean controls (Fig. 3B). The ATP levels observed in ob/ob mice kidneys (5.95 ± 0.34 pmol/mg) were 75% of that measured in WT controls (7.86 ± 0.35 pmol/mg), whereas ATP levels in LoxTB MC4R−/− mice kidneys (4.99 ± 0.17 pmol/mg) were ~62% of that in WT-LoxTB controls (8.00 ± 1.11 pmol/mg).

Oxidative stress. Protein carbonylation results from the irreversible oxidation of protein side chains by hydrogen peroxide or hydroxyl radicals, both downstream products of superoxide produced by the electron transfer chain of oxidative phosphorylation (34). Hence, carbonylation provides a relative measure of the extent of oxidative damage from mitochondrial ROS. Protein carbonyl levels were increased by 20% in homogenates of LoxTB MC4R−/− kidney mitochondria (13.41 ± 0.92 nmol/mg) compared with lean controls (11.14 ± 0.18 nmol/mg) (Fig. 3C). However, there was no significant difference in the carbonylation of kidney mitochondria protein in ob/ob mice vs. lean controls.

Mitochondrial Function

Since the kidney is a highly oxidative tissue, the decreased kidney ATP levels imply that renal mitochondria in obese animals may not be meeting the ATP demands of the cells. For this reason, we examined isolated kidney mitochondria for obesity-related changes in oxidative phosphorylation.

Ob/ob mice kidney mitochondria. compared with lean controls, no significant changes in the rate of state 3 respiration (+ADP) were observed with mitochondria isolated from ob/ob mice kidneys, using succinate, glutamate-malate, palmitoyl-carnitine, or palmitoyl-CoA plus carnitine as substrates (Fig. 4A). The finding argues against significant obesity-related declines in the activities of complex I, complex II, carnitine palmitoyl transferase I, and the dehydrogenases of the β oxidation pathway in kidney mitochondria of ob/ob mice. There was, however, an increase in the rate of state 4 respiration, compared with the lean controls (Fig. 4B). This increase is most clearly observed for state 4 respiration supported by succinate (i.e., succinate complex II complex III complex IV complex IV → O2), probably because succinate allowed the most rapid rate of O2 consumption. State 4 was induced by the addition of oligomycin to block H+ transfer through ATP synthase, from the outer surface of the inner mitochondrial membrane to the inner surface. When H+ transfer through ATP synthase is blocked, the proton gradient created by the electron transfer-driven proton pumps increases, which slows both proton pumping and electron transfer; this is seen as a decreased rate of O2 consumption compared with state 3 respiration. Electron transfer slows but does not cease in the absence of proton flow through ATP synthase because secondary pathways, such as uncoupling proteins, allow protons to flow across the inner mitochondrial membrane, albeit at a slower rate. The greater rate of state 4 respiration observed in the ob/ob kidney mitochondria reflects a greater capacity for these secondary pathways of H+ flow in these mitochondria. If these secondary pathways are also in operation when proton flow through ATP synthase is operational (i.e., during state 3 respiration), some of the protons that would normally flow through ATP synthase will instead flow through the secondary pathways. This diversion of proton flow away from ATP synthase, termed partial uncoupling, leads to a slower rate of ATP synthesis, which should contribute to the decrease in kidney ATP levels in the ob/ob mice. We cannot claim that partial uncoupling fully accounts for the decrease in tissue ATP
levels because we have no measure of the rate of cellular ATP consumption.

The degree to which electron transfer is coupled to ATP synthesis in isolated mitochondria is typically presented as the respiratory control ratio (RCR), the ratio of the state 3 and state 4 respiration rates. Hence, the lower RCR values observed for respiration supported by succinate and by glutamate-malate (Fig. 4C) provide another indication that electron transfer is partially uncoupled from ATP synthesis in mitochondria isolated from the kidneys of ob/ob mice. A lower RCR was not apparent using fatty acids as a substrate, perhaps because of the relatively low rates of state 3 respiration obtained with these substrates.

**LoxTB MC4R−/− mice kidney mitochondria.** compared with lean controls, mitochondria isolated from the kidneys of obese LoxTB MC4R−/− mice showed significantly decreased (11–43%) rates of state 3 respiration supported by substrates that produce NADH and must use the pathway of complex I

![Representative images of periodic acid-Schiff (PAS)-stained (A) and F4/80-stained (B) kidney sections of ob/ob and LoxTB MC4R−/− mice compared with their respective WT controls under ×400 magnification.](image-url)
We thought that the apparent decline in complex I activity in LoxTB MC4R−/− mice could be part of a general decrease in the components of oxidative phosphorylation, as has been observed in some studies (28). To test this, we measured the activity of complex IV (cytochrome c oxidase) (Fig. 4D). Instead of a decline, we found that the mitochondria in the kidneys of high-leptin LoxTB MC4R−/− mice contained 40% greater complex IV activity, relative to control (Fig. 4D). In contrast, no significant changes in complex IV activity were observed in kidneys of leptin-deficient ob/ob mice (Fig. 4D). This result rules out a general decrease in all complexes of oxidative phosphorylation in the kidneys of high-leptin LoxTB MC4R−/− mice. However, the mechanisms underlying the apparent decline in complex I activity as well as the increase in the amount of complex IV remain to be determined.

Markers of ER Stress

ER stress frequently accompanies mitochondrial dysfunction. Immunoblots of ob/ob and LoxTB MC4R−/− mice kidney homogenates were analyzed by densitometry for obesity-related changes in the expression of ER stress markers. We observed a fourfold induction of the proapoptotic protein CHOP (63) in LoxTB MC4R−/− mice kidneys (Fig. 5A), suggesting long-term ER stress. In terms of common markers of the cellular response to ER stress, a small increase was noted in the expression of the spliced form of X-box binding protein 1 (s-XBP-1) (75) in these kidneys (Fig. 5B), suggesting activation of inflammatory markers in the kidney. However, emerging evidence from animal models of diet-induced obesity strongly support the idea that obesity impairs renal function but not major renal injury. Previous studies in experimental animals and in humans have shown that obesity is associated with the development of chronic kidney injury, although the pathogenic mechanisms remain unclear. Metabolic, hormonal, and hemodynamic factors have all been proposed to contribute to renal injury in obese subjects. Hemodynamic changes include increased arterial pressure and glomerular hypertension associated with glomerular hyperfiltration (4, 38, 39).

DISCUSSION

Obesity, in the absence of hypertension, is associated with altered kidney function but not major renal injury. Previous studies in experimental animals and in humans have shown that obesity is associated with the development of chronic kidney injury, although the pathogenic mechanisms remain unclear. Metabolic, hormonal, and hemodynamic factors have all been proposed to contribute to renal injury in obese subjects. Hemodynamic changes include increased arterial pressure and glomerular hypertension associated with glomerular hyperfiltration (4, 38, 39).

Emerging evidence from animal models of diet-induced obesity strongly support the idea that obesity impairs renal architecture and function (18, 19, 73). For example, studies by Ruggiero et al. (73) showed that obesity induced by a high-fat diet (HFD) feeding in mice is associated with glomerular hypertrophy, mesangial expansion, and renal fibrosis. Similarly, studies conducted in HFD-fed obese mice by Decleves et al. (18, 19) also demonstrated that diet-induced obesity induces renal hypertrophy, alterations in renal lipid metabolism, and activation of inflammatory markers in the kidney. However,
the obesity models used in these HFD studies also present with elevated arterial pressure (23, 36, 40), a well-recognized risk factor for the development of renal disease (39). This leads to the question of how obesity itself, with its associated metabolic changes, causes alterations in renal architecture and function, compared with obesity plus increased blood pressure (19, 72, 73).

In the present study, we used two genetic models of obesity, hyperleptinemic MC4R-deficient mice and leptin-deficient ob/ob mice. Both models have normal or slightly reduced blood pressures despite severe obesity and multiple metabolic abnormalities including insulin resistance, hyperinsulinemia, and dyslipidemia. Studies have shown that a functional central nervous system (CNS) leptin-melanocortin pathway is essential for obesity to cause sympathetic nervous system activation and hypertension in rodents as well as in humans (33, 37, 79). Therefore, LoxTB MC4R−/− and ob/ob mice provide an opportunity to study the impact of obesity and associated metabolic abnormalities on kidney function in the absence of hypertension (Table 3).

Our results indicate that obesity, even when blood pressure is not elevated, leads to glomerular hyperfiltration and increased albumin excretion in ob/ob and LoxTB MC4R−/− mice. However, we found no major macrophage infiltration or substantial structural changes in the kidneys of LoxTB MC4R−/− or ob/ob mice. Therefore, a surprising finding is that severe obesity with its associated metabolic abnormalities in mice may not cause major renal injury in the absence of sympathetic activation and hypertension. Increased sympathetic nervous system activity and hypertension, interacting synergistically with obesity-induced metabolic changes, may be required for obesity to cause major renal injury. This explanation is consistent with the finding that tight pharmacological control of hypertension appears to protect against development and progression of chronic kidney disease in obese type 2 diabetic patients even when metabolic abnormalities and obesity are poorly controlled (14).

Obesity promotes renal TG accumulation. Previous studies in leptin-deficient ob/ob mice have documented excessive fat accumulation in visceral organs (94, 96) and pancreatic islets (99). However, administration of leptin attenuates fat accumulation in isolated rodent hearts (7) and skeletal muscle (58).

Thus we initially hypothesized that the high leptin levels in leptin-deficient ob/ob mice would protect the kidneys from excessive fat deposition despite obesity. This hypothesis turned out to be incorrect. Both the leptin-deficient and the leptin-abundant mouse models of obesity showed significant hypertrophy of the kidney (and also of the liver) plus an expansion of fat pads surrounding the peritoneum and kidney. In fact, both obese mouse models showed similar increases in kidney fat (~1.7-fold increase for the LoxTB MC4R−/− mice and ~1.8-fold increase for the ob/ob mice compared with their respective lean controls). It is interesting to note that in both

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**Fig. 4.** Mitochondrial respiration studies showed impaired mitochondrial state 3 respiration in LoxTB MC4R−/− mice kidneys under complex I substrate glutamate (a glucose substrate), palmitoyl-l-carnitine (an esterified fatty acid), and nonesterified fatty acid palmitoyl CoA+L-carnitine (A). Similar impairments to mitochondrial state 4 respiration were observed in LoxTB MC4R−/− mice kidneys under fatty acid substrates palmitoyl-l-carnitine and palmitoyl CoA+L-carnitine (B). In contrast, LoxTB MC4R−/− mice kidneys demonstrate an increased respiratory control ratio (RCR) under complex II glucose substrate succinate (C) and increase in complex IV (cytochrome c oxidase) activity (D). Although no changes in state 3 respiration or complex IV activity was observed, the ob/ob mice renal mitochondria show increased state 4 respiration under complex II glucose substrate succinate (B) and diminished RCR values under glucose substrates succinate and glutamate (C) but not under fatty acid substrates. State 3 and state 4 respiration values were normalized for citrate synthase (CS) activity in renal mitochondria. Values are means ± SE. *P < 0.05 compared with WT Control. †P < 0.05 compared with WT-LoxTB mice.
Mitochondria isolated from kidneys of leptin-deficient ob/ob mice respired both fatty acid and non-fatty acid substrates at rates similar to lean controls (Fig. 4). However, electron transfer was partially uncoupled from ATP synthesis in the ob/ob mice, as explained in RESULTS. The partial uncoupling of oxidative phosphorylation likely contributes to the observed 25% decline in whole kidney ATP levels, also as explained in RESULTS. Partial uncoupling has also been observed in mitochondria isolated from the livers of ob/ob mice (57, 80).

In contrast to ob/ob mice, the RCR values measured for kidney mitochondria of LoxTB MC4R−/− mice indicated normal coupling of electron transfer to ATP synthesis (Fig. 4). In addition, mitochondria isolated from kidneys of hyperleptinemic LoxTB MC4R−/− mice showed normal rates of respiration of succinate, but decreased respiration rates of substrates that require complex I activity, such as glutamate-malate and fatty acids (Fig. 4). A deficiency in complex I activity could decrease mitochondrial ATP production and thereby contribute to the decrease in kidney ATP levels in the LoxTB MC4R−/− mice (Fig. 3B). It is also likely that the increased oxidative damage observed in these mitochondria may impair mitochondrial ATP production by lowering the activity of mitochondrial enzymes and complexes.

The kidney mitochondria of the obese mouse models in this study show dysfunctions that can at least partially explain the finding of lower tissue ATP levels. However, the types of mitochondrial dysfunction seen with high leptin and leptin deficiency are distinctly different (Table 3).

Mitochondria of obese mice with sustained high levels of leptin, comparable to those found in LoxTB MC4R−/− mice, have not previously been studied. However, mice fed a HFD for an extended time do show a sustained increase in plasma leptin, although the absolute amount of leptin is far lower than that of LoxTB MC4R−/− mice (3). Ruggiero et al. (73) analyzed kidney mitochondria isolated from male C57BL/6 mice fed a HFD for 12–16 wk. Their findings are more similar to what we have found for the hyperleptinemic LoxTB MC4R−/− mice than for the leptin-deficient ob/ob mice (Table 3). In the HFD-fed mice of Ruggiero et al. (73), the RCR measured during the respiration of succinate was either slightly elevated or unchanged, indicating retention of normal coupling. This is similar to the hyperleptinemic LoxTB MC4R−/− mice (Fig. 4). After 16 wk, the expression of complex I in the HFD mice was ~20% less than in the low-fat diet mice, although the result was not significant (74). This is similar to our finding of a loss of complex I activity in the kidney mitochondria of hyperleptinemic mice.

High leptin levels are associated with an increase in oxidized protein in kidney mitochondria. Previous studies have supported a role for oxidative stress in promoting renal injury during obesity hypertension (22, 62, 73, 74, 81). However, the role of leptin in obesity-related renal oxidative stress is less clear. In this study, we found that mitochondria from the kidneys of leptin-deficient ob/ob mice showed no significant elevation in protein carbonylation, indicating that the mitochondria were not producing ROS in excess of mitochondrial antioxidant systems. The lack of increased oxidative damage in ob/ob mitochondria is likely due to the observed partial uncoupling, which lessens the production of superoxide and downstream ROS during respiration (6). Livers of ob/ob mice have indications of oxidative damage, but the principal finding

mouse models of genetic obesity, the changes in kidney function, including increased creatinine clearance and albumin excretion, occurred in proportion to the percent increase in intrarenal fat deposition. This observation suggests, but does not prove, a causal link between the renal fat accumulation and altered renal function.

Obesity is associated with decreased kidney ATP levels and altered mitochondrial function. Both obese mouse models exhibited significantly decreased kidney ATP levels, compared with their lean controls, which implies less efficient oxidative phosphorylation in kidney mitochondria. This was tested by examining the respiratory activity of isolated kidney mitochondria.

![Western blot and densitometry analysis](http://ajprenal.physiology.org/)
in these studies is increased protein nitrilation, possibly due to increased mitochondrial nitric oxide production (25, 28, 29). Increased protein nitrilation is not solely induced by low leptin levels, however, since it also occurs in the high-leptin environment produced by a HFD (73).

In hyperleptinemic LoxTB MC4R−/− mice, the carbonylation of kidney mitochondrial proteins was increased, indicating the production of ROS in excess of antioxidant capacity. Obese animal models in which leptin levels are elevated have generally shown increased production of mitochondrial ROS (1, 15, 16, 69, 71, 73, 86). This is often attributed to the increased oxidation of fatty acids, which is promoted by leptin (7, 13, 17, 68, 93, 99). Consistent with our finding of increased proton carbonylation, kidney mitochondria of mice made obese by a HFD release more hydrogen peroxide during state 3 respiration than mitochondria from mice fed a control diet (73).

Obesity in the presence of high leptin levels is associated with ER/mitochondrial stress. Increased ROS and depletion of energy slow protein folding in the ER, a condition known as ER stress (44). Chronic ER stress leads to apoptosis via the expression of proapoptotic ER stress proteins, such as CHOP, and loss of cells contributes to tissue dysfunction (44, 50). A response to ER stress, termed the unfolded protein response (UPR), involves increased expression of protein-folding agents, such as the ER chaperone termed GRP78 (11). Studies in obese animals have demonstrated activation of this ER stress response in the liver (65), brain (64), and kidney (78). In most of these studies, however, it has been difficult to separate the effects of obesity on ER stress from the effects of hypertension, which can increase ER stress (64, 78). In this study, we examined the effect of obesity on kidney ER stress without the complications of hypertension in two mouse models with very different levels of leptin.

The apparent lack of ER stress in kidneys of leptin-deficient ob/ob mice is consistent with the lack of mitochondrial protein carbonylation, since mitochondrial ROS are primary contributors to both ER stress and protein carbonylation. In contrast, the kidneys of hyperleptinemic LoxTB MC4R−/− mice showed a marked increase in the levels of CHOP, a strong indication of advanced ER stress (63). Consistent with our findings, induction of the ER stress marker CHOP has also been documented in vitro (48, 49) and in vivo (78, 90) models of obesity-induced nephropathy. Together, these findings unveil a potential role of ER stress in obesity-induced renal dysfunction during MC4R deficiency.

In terms of a response to ER stress, only a slight increase in the spliced form of XBP-1 (s-XBP-1) was observed, and there was no increase in the expression of ER chaperone GRP78. This unique pattern of the UPR, evidenced by a solitary increase in CHOP, in conjunction with increased mitochondrial protein carbonylation in the high-leptin MC4R-deficient mice kidneys, suggests the involvement of mitochondrial stress (42, 98), a condition similar to ER stress. In support of this possibility, elevated mitochondrial ROS has been shown to increase the expression of CHOP in adipocytes (12). Similarly, reduced complex I activity, another key finding in the LoxTB MC4R−/− mice kidneys, induces the expression of CHOP in cybrid cells (27). Therefore, the increased levels of CHOP in MC4R-deficient mice kidneys could be a response to one or more of the following: 1) ER UPR, 2) mitochondrial UPR, 3) mitochondrial ROS, or 4) decreased complex I activity. However, our results appear to indicate that high leptin levels in the kidney promote apoptosis or cell cycle arrest via induction of CHOP more than high leptin promotes the UPR in the ER.

One potential criticism of our studies is that LoxTB MC4R−/− mice are not only obese and hyperleptinemic, but also have MC4R deficiency which could have effects on kidney function independently of obesity and increased leptin levels. However, in adult mammals MC4R is expressed mainly in the CNS, especially in the hypothalamus, brain stem, and spinal cord intermediolateral nucleus (88). Although MC4R may be expressed in several peripheral tissues during fetal development, current evidence from Northern blotting, in situ hybridization, and radioligand binding studies suggests that MC4R is not expressed, or expressed at very low levels, in the kidneys of adult rodents (88). Therefore, the effects of MC4R deficiency on kidney function are likely to be related to indirect effects caused by altered CNS function. As discussed previ-

### Table 3. Summary of the findings from ob/ob and LoxTB MC4R−/− mice models compared with the findings from diet-induced obese animal models reported in the literature

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ob/ob Mice Model</th>
<th>LoxTB MC4R−/− Mice Model</th>
<th>Diet-Induced Obesity (Based on Literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin levels</td>
<td>None</td>
<td>Very high</td>
<td>Higher than normal (3)</td>
</tr>
<tr>
<td>Leptin CNS sympathetic effects</td>
<td>No</td>
<td>No</td>
<td>Yes (23, 79)</td>
</tr>
<tr>
<td>Leptin peripheral effects</td>
<td>No</td>
<td>Yes (15, 16)</td>
<td>Yes (23, 39, 40)</td>
</tr>
<tr>
<td>Sympathetic activation</td>
<td>No</td>
<td>Normal</td>
<td>High (23, 37, 40)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Normal</td>
<td>Normal</td>
<td>Yes (19)</td>
</tr>
<tr>
<td>Renal lipid accumulation</td>
<td>Yes</td>
<td>Yes (5, 18, 55, 95)</td>
<td>Yes (18, 19, 73)</td>
</tr>
<tr>
<td>Altered renal function</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Renal injury</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney mitochondrial dysfunction</td>
<td>Normal respiration rates but partial uncoupling, which may lower ROS production as well as ATP levels</td>
<td>Decreased respiration of substrates that require complex I may lower ATP levels; normal coupling</td>
<td>Normal respiration from succinate; normal coupling (73)</td>
</tr>
<tr>
<td>Kidney ATP levels</td>
<td>Lower than normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial oxidative stress</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proapoptotic signaling in kidney</td>
<td>No</td>
<td>Yes (increased CHOP expression)</td>
<td>Yes (increased CHOP expression) (78, 90)</td>
</tr>
</tbody>
</table>

CNS, central nervous system; ROS, reactive oxygen species.
Oursly, MC4R deficiency in the CNS causes hyperphagia, obesity, and hyperleptinemia, which are not associated with increased sympathetic activity or hypertension. Abnormalities of kidney function observed in LoxTB MC4R−/− mice may therefore be caused mainly by obesity and associated metabolic effects, including hyperleptinemia. However, we cannot completely rule out the possibility that MC4R deficiency in the kidneys during fetal development may have contributed to some of the renal dysfunction observed in LoxTB MC4R−/− mice.

Summary and Perspectives

Using two genetic mice models of obesity, MC4R-deficient (hyperleptinemic) mice and leptin-deficient ob/ob mice, we demonstrated that obesity, in the absence of hypertension, is associated with glomerular hyperfiltration and increased albumin excretion, but not with major renal injury. Our findings also help to define which obesity-related kidney changes are associated with leptin signaling and which are not. For example, obesity-related changes in renal TG accumulation and renal function were similar in the presence or absence of leptin. Similarly, mitochondrial dysfunction and a decline in renal ATP were present in both obesity models, regardless of the presence or absence of leptin; however, the type of mitochondrial dysfunction differed significantly depending on the leptin status. In mitochondria from kidneys of hyperleptinemic LoxTB MC4R−/− mice, oxidative phosphorylation and respiration of fatty acids are impaired by an apparent deficiency in complex I, while in the leptin-deficient kidneys of ob/ob mice oxidative phosphorylation is impaired by partial uncoupling of electron transfer from ATP synthesis. Another major difference was that mitochondrial oxidative stress and ER/mitochondrial stress in obese mice were evident only in the presence of leptin. Nevertheless, the absence of the MC4R in hyperleptinemic LoxTB MC4R−/− mice must also be taken into consideration when our results are analyzed.

Table 3 compares our findings from the ob/ob and LoxTB MC4R−/− mice models with findings from diet-induced obese animal models, which have elevated leptin. There are similarities and differences. As noted, mice made obese by a HFD are hypertensive. All of the obese models show altered renal function, but only the hypertensive obese mice exhibit substantial renal injury. In high-leptin mice fed a HFD and hyperleptinemic LoxTB MC4R−/− mice, kidney oxidative phosphorylation remains coupled, while in leptin-deficient ob/ob mice it is partially uncoupled. The kidneys of both groups of mice with high levels of leptin show evidence of oxidative stress in mitochondria as well as increased expression of the proapoptotic protein CHOP. Neither of these abnormalities occurs in obese ob/ob mice with leptin deficiency.

Resistance to overt renal injury in these two normotensive genetic models of obesity may also be due, in part, to other factors besides lack of increased blood pressure. Previous studies have shown that blockade of nitric oxide synthesis, for example, renders mice much more susceptible to diabetes-induced nephropathy (60). However, blockade of nitric oxide synthesis also increased arterial pressure in these previous studies, making it difficult to assess the contribution of hemodynamic changes to the renal injury. Further studies are needed to determine whether increases in arterial pressure may interact synergistically with metabolic changes to mediate obesity-induced ER stress, mitochondrial dysfunction, oxidative stress, and other changes that may contribute to renal injury in obese subjects.

Overall, our findings indicate that obesity, independently of leptin signaling, can cause kidney mitochondrial dysfunction even in the absence of hypertension, although increased leptin associated with MC4R deficiency may contribute to oxidative stress and a proapoptotic response via ER or mitochondrial stress.
endoplasmic reticulum and can be rescued to the cell surface by a chemical chaperone. Mol Endocrinol 24: 1805–1821, 2010.


production in ob/ob mice is not stimulated by massive de novo lipogenesis but is less sensitive to the suppressive effects of insulin. *Diabetes* 52: 1081–1089, 2003.


