Renal lipid accumulation, oxidative stress and uric acid handling in a rodent model of obesity and metabolic syndrome

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ABSTRACT

Hyperuricemia is more prevalent among people with obesity and metabolic syndrome, and is associated with adverse clinical outcomes. We hypothesized that increased renal reabsorption of uric acid (UA) in obesity and metabolic syndrome may be an adaptive response of the kidney when faced with fatty acid-induced oxidative stress. To test this hypothesis, we examined lipid accumulation, markers of oxidative stress, and renal UA handling in Zucker diabetic fatty (ZDF) rats, and in matched lean control animals. Rats were randomized to either normal rodent chow or a diet supplemented with antioxidants (α-tocopheryl acetate, sodium selenite, zinc sulfate, and ascorbic acid), and were followed up for either 4 or 20 weeks after randomization. Dietary antioxidant supplementation had no significant effects in lean control rats but led to partial improvement in markers of elevated oxidative stress in the kidney of ZDF rats. Renal UA handling was not affected by antioxidant supplementation. We observed robust correlations between renal lipid content and oxidative stress markers in the pooled experimental groups, particularly in older animals after 20 weeks on the study diets. Dietary antioxidant supplementation did not prevent the gradual decline in renal function observed in older ZDF rats. These findings suggest that hyperuricemia in the ZDF rat model of obesity and the metabolic syndrome is not caused by renal oxidative stress, that there may be a pathophysiological link between lipid accumulation and oxidative stress in the kidney, and that antioxidant supplementation does not prevent age-related decline in renal function in ZDF rats.

Significance of this study

What is already known about this subject?

- People with obesity and the metabolic syndrome have a higher prevalence of hyperuricemia, in part because of increased renal reabsorption of uric acid (UA). Hyperuricemia, even when asymptomatic, is associated with multiple adverse clinical outcomes.
- Obesity and the metabolic syndrome are associated with excess renal lipid accumulation in both humans and animal models. Excess lipid accumulation in non-adipose tissues has toxic effects (lipotoxicity), caused in part by increased oxidative stress.
- UA is an antioxidant in vitro, but whether increased reabsorption of UA in obesity and the metabolic syndrome might be an adaptive response of the kidney when faced with increased oxidative stress is unknown.

What are the new findings?

- Dietary antioxidant supplementation led to partial improvement in renal oxidative stress markers in Zucker diabetic fatty (ZDF) rats, a rodent model of obesity and the metabolic syndrome, but did not affect renal UA handling. Increased renal UA reabsorption does not appear to be an adaptive response of the kidney when faced with increased oxidative stress.
- Dietary antioxidant supplementation did not prevent the gradual decline in renal function observed in older ZDF rats, in apparent contradiction with previous findings in ZDF rats subjected to pharmacological antioxidant therapy.

INTRODUCTION

Numerous clinical studies have demonstrated an independent association between hyperuricemia (with or without gout) and adverse clinical outcomes, including greater incidence and worse prognosis of hypertension, cardiovascular disease, diabetes, and chronic kidney disease (CKD).[1 2] In turn, hyperuricemia and gout are more prevalent among people with obesity and the metabolic syndrome because of a combination of increased metabolic production of uric acid (UA) and increased renal reabsorption of UA as urate anion.[3–7] The underlying cause of increased renal reabsorption of UA in these conditions is unknown.

We have previously shown that obesity and the metabolic syndrome are associated with renal lipid accumulation in both humans and animal models, affecting primarily the

Significance of this study

How might these results change the focus of research or clinical practice?

- These findings suggest that hyperuricemia in obesity and the metabolic syndrome is not an adaptive response to lipid-induced oxidative stress in the kidney, and that dietary antioxidant supplementation may not be a viable strategy for treating hyperuricemia in this context.
- Contrary to other published research in animals, but consistent with data from human trials, this study suggests that dietary antioxidant supplementation may notprevent renal function decline in obesity and the metabolic syndrome.

epithelial cells of the proximal tubule.[8–10] The proximal tubule is also the primary site of renal UA reabsorption.[11] Lipid accumulation in non-adipose tissues is indicative of metabolic disturbances associated with cellular fatty acid overload, which may have a host of other deleterious effects on cellular and organ functions. These effects are collectively termed lipotoxicity and are mediated in large part by increased production of reactive oxygen species (ROS), resulting in oxidative stress.[12–17]

Since UA can act as an effective antioxidant,[18–20] we hypothesized that increased reabsorption of UA in obesity and metabolic syndrome may be an adaptive response of the kidney when faced with fatty acid-induced oxidative stress in proximal tubule cells. The aim of the present study was to test whether a diet supplemented with pharmacological doses of antioxidants (α-tocopheryl acetate, sodium selenite, zinc sulfate, and ascorbic acid) would decrease UA reabsorption in rats with obesity, insulin resistance and renal lipid accumulation, but not in normal animals. As renal function declines and renal lipid accumulation increases with age in obese, insulin-resistant animals, we conducted separate experiments in younger and older animals.

MATERIALS AND METHODS

Animals

All animal experiments were performed in strict accordance with a protocol approved by the Institutional Animal Care and Use Committee for a total of 48 animals. Twenty-four male leptin receptor-deficient Zucker diabetic fatty (ZDF) rats and 24 male wild-type littermates, 8 weeks of age, were purchased from Charles River Laboratories (Wilmington, Massachusetts, USA). ZDF rats are an established rodent model of obesity and the metabolic syndrome,[21] and male ZDF rats have been shown to develop renal steatosis.[8 22] In contrast, female ZDF rats do not develop detectable renal steatosis, which is why female rats were not included in this study. All animals were acclimatized for 2 weeks on standard rodent chow prior to the beginning of the experiments and were kept on a 12-hour light–dark cycle with free access to food and water throughout the study.

Experimental diets

After acclimatization, an equal number of ZDF and control rats were randomly assigned to either normal rodent chow (FormuLab 5008; LabDiet, St. Louis, Missouri, USA) or to an antioxidant-enriched custom diet (5AKZ; TestDiet, St. Louis, Missouri, USA). The antioxidant diet was based on the FormuLab 5008 normal chow, enriched (by weight) with 0.98% α-tocopheryl acetate, 0.04% sodium selenite, 0.12% zinc sulfate, and 0.05% ascorbic acid. In separate experiments, animals were followed up for either 4 or 20 weeks on the study diets.

Serum and urine analyses

Blood samples were collected from the tail vein to measure pH, sodium, potassium, chloride, UA, blood urea nitrogen (BUN), and creatinine levels (Vitros Chemistry; Ortho Clinical Diagnostics, Markham, Ontario, Canada). For urine chemistry, animals were individually housed in rat metabolic cages (Tecniplast, West Chester, Pennsylvania, USA), and 24 hours urine samples were collected according to manufacturer instructions in 50 mL conical tubes containing thymol crystals to prevent bacterial growth. Measurements included total volume, urine creatinine and UA (Vitros Chemistry, Ortho Clinical Diagnostics).

Euthanasia, renal triglyceride content and oxidative stress markers

Animals were euthanized under anesthesia with ketamine/xylazine/acepromazine (100/10/1 mg/kg intraperitoneally), followed by bilateral thoracotomy. Renal triglyceride content was measured as described previously.[22] Briefly, fresh rat kidneys were dissected on ice and cortical tissue was homogenized (Polytron; Brinkmann Instruments, Westbury, New York, USA) in 1:20 weight:volume of isolation buffer [300 mM mannitol, 18 mM 4-(2-hydroxyethyl)-1-piperazin eethanesulfonic acid (HEPES), 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), pH 7.5], followed by lipid extraction and measurement using a triglyceride determination kit (Sigma, St. Louis, Missouri, USA). Oxidative stress was assessed using the malondialdehyde (MDA) assay in homogenized tissues (lipid peroxidation assay kit; Abcam, Cambridge, Massachusetts, USA) according to the manufacturer's instructions. Total antioxidant capacity in Trolox equivalents was measured using a total antioxidant capacity assay kit (Sigma) according to the manufacturer's instructions.

Calculations

Creatinine clearance was calculated according to the formula $C_{Cr}=[U_{Cr}\times(U_{Vol}/1440]/P_{Cr}$, where C_{Cr} is creatinine clearance (expressed in mL/min), U_{Cr} is the concentration of creatinine in a 24-hour urine collection (mg/dL); U_{Vol} is the total volume of the 24-hour urine collection divided by 1440 to obtain urine excretion rate per minute (mL/min), and P_{Cr} is plasma creatinine (mg/dL). Fractional excretion of uric acid (FE_{UA}, expressed as percentage) was calculated according to the formula $FE_{UA}=(U_{UA}\times P_{Cr})/(U_{Cr}\times P_{UA})\times 100$, where U_{UA} and P_{UA} are the concentrations of UA in urine and plasma, respectively, and U_{Cr} and P_{Cr} are the concentrations of creatinine in urine and plasma, respectively.

Table 1 Body mass, food intake, serum chemistry and markers of kidney function in ZDF and lean control rats fed normal chow (control) or AntiOx-enriched diets for 4 weeks

	Lean control	Lean AntiOx	ZDF control	ZDF AntiOx
Body mass (g)	271±21	267±27	361±20*	353±25*
Average daily food intake (g)	19.2	18.7	24.5*	23.2*
Blood pH	7.37±0.02	7.37±0.01	7.36±0.02	7.37±0.03
Na^+ (meq/L)	142.9±1.6	143.5±2.0	141.1±2.2	142.9±2.5
K^+ (meq/L)	4.67±0.25	4.68±0.3	4.98±0.43	5.04±0.59
Cl^- (meq/L)	105.8±2.3	104.7±4.3	103.7±4.3	101.5±3.6
Uric acid (mg/dL)	1.28±0.28	1.22±0.21	1.87±0.31†	1.97±0.45†
BUN (mg/dL)	20.3±0.2	21.3±2.1	23.2±2.6	24.7±3.6
Creatinine (mL/dL)	1.19±0.16	1.37±0.23	1.41±0.4	1.56±0.31
Creatinine clearance (mL/min)	4.16±0.69	3.43±0.79	3.31±0.79	3.03±0.62

*P values ZDF versus lean animals fed the same diet: p<0.001.
†P values ZDF versus lean animals fed the same diet: p=0.05.
AntiOx, antioxidant; BUN, blood urea nitrogen; ZDF, Zucker diabetic fatty.

Statistical analyses

Data are presented as mean and SD or as individual data points. We used one-way analysis of variance and two-tailed between-subjects t-tests to compare continuous variables between groups and Pearson product-moment correlation coefficients to examine correlations. The threshold for significance was $\alpha=0.05$. Statistical analyses were performed using SAS V.9.4.

RESULTS

Body mass, food intake, serum chemistry and markers of renal function in ZDF and lean control rats fed normal chow or antioxidant-enriched diets for 4 or 20 weeks are shown in tables 1 and 2, respectively.

ZDF rats had greater body mass, greater food intake, and higher serum UA levels than lean animals throughout the experiments, and these differences were not affected by antioxidant supplementation. The ratio of food intake per body mass was not significantly different between groups at any time point. Blood pH and serum electrolyte levels were similar across all experimental groups. After 4 weeks on the study diets (14 weeks of age), there were minimal differences between groups in BUN, serum creatinine, or creatinine clearance, indicative of preserved renal function in ZDF rats. In separate experiments, serum creatinine and creatinine clearance were indicative of a significant decline in kidney function in older ZDF rats compared with lean controls after 20 weeks on the study diets (30 weeks of age). Average BUN was also numerically higher in ZDF rats at this latter time point, although within-group variability prevented these differences from reaching the customary p=0.05 threshold for statistical significance. Antioxidant supplementation did not affect BUN, serum creatinine, or creatinine clearance at any experimental time point.

Table 2 Body mass, food intake, serum chemistry and markers of kidney function in ZDF and lean control rats fed normal chow (control) or AntiOx-enriched (AntiOx) diets for 20 weeks

	Lean control	Lean AntiOx	ZDF control	ZDF AntiOx
Body mass (g)	309±23	301±29	391±26*	387±24*
Average daily food intake (g)	26.8	23.9	36.6*	35.6*
Blood pH	7.37±0.04	7.3±0.02	7.35±0.07	7.33±0.06
Na^+ (meq/L)	143.8±1.9	142.1±2.0	141.2±2.2	141.5±3.1
K^+ (meq/L)	4.53±0.33	4.49±0.34	4.97±0.48	5.12±0.73
Cl^- (meq/L)	105.5±3.6	105.3±4.5	103.0±4.3	102.7±5.7
Uric acid (mg/dL)	1.4±0.37	1.0±0.25	2.82±0.66 †	3.2±0.73 *
BUN (mg/dL)	22.5±3.7	21.3±2.7	29.0±6.0	30.8±9.8
Creatinine (mL/dL)	1.47±0.29	1.72±0.46	2.53±0.39 *	2.57±0.59†
Creatinine clearance (mL/min)	3.65±0.57	3.49±1.1	2.36±0.79 †	2.08±0.96†

*P values ZDF versus lean animals fed the same diet: p<0.001.
†P values ZDF versus lean animals fed the same diet: p=0.05.
AntiOx, antioxidant; BUN, blood urea nitrogen; ZDF, Zucker diabetic fatty.

UA excretion rates were similar across groups in both younger and older animals (figure 1A,B). Younger ZDF rats had lower FE_{UA} compared with lean rats, and these differences were not affected by 4 weeks of antioxidant supplementation (figure 1C). Qualitatively similar findings were observed in older animals after 20 weeks on the study diets, with numerically lower average FE_{UA} in ZDF compared with lean rats not affected by antioxidant supplementation (figure 1D).

When compared with lean animals, both younger and older ZDF rats had higher renal cortical triglyceride content, irrespective of dietary group (figure 2A,B). Both younger and older ZDF rats fed normal control chow showed evidence of renal oxidative stress, as assessed by the MDA lipid peroxidation assay (figure 2C,D), and decreased total antioxidant capacity (figure 2E,F). Antioxidant supplementation for 4 weeks did not appear to affect lipid peroxidation (figure 2C) but improved total antioxidant capacity in ZDF rats (figure 2E), while there was no effect in lean animals. Antioxidant supplementation for 20 weeks significantly improved both lipid peroxidation (figure 2D) and total antioxidant capacity in ZDF rats, but not in lean rats (figure 2F).

We next sought to examine the relationship between renal lipid accumulation and oxidative stress markers in individual animals, taking advantage of the relatively wide range of values across all ZDF and lean rats in the study, regardless of diet. There were strong positive correlations between lipid peroxidation and cortical triglyceride content in both younger animals (14 weeks of age) and older animals (30 weeks of age) (figure 3A,B). A correlation between total antioxidant capacity and cortical triglyceride content was not apparent in younger animals (figure 3C) but was observed in older animals (figure 3D).

DISCUSSION

There are three key findings from this study. First, dietary antioxidant supplementation led to partial improvement in oxidative stress markers in the kidney of ZDF rats but had no effect on renal UA handling. These findings do not support our initial

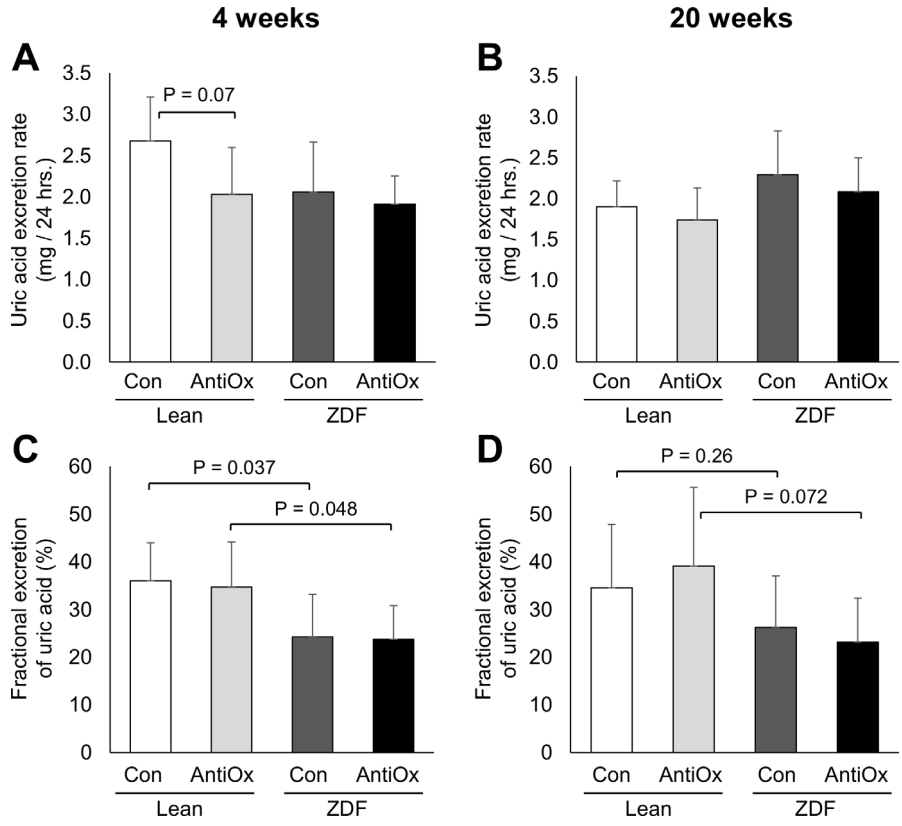

Figure 1 Renal UA handling. UA excretion rate in ZDF versus lean rats fed normal chow (Con) or an AntiOx-enriched diet for (A) 4 weeks and (B) 20 weeks, and fractional excretion of UA in ZDF versus lean rats fed normal chow (Con) or an AntiOx-enriched diet for (C) 4 weeks and (D) 20 weeks. AntiOx, antioxidant; Con, control; ZDF, UA, uric acid; ZDF, Zucker diabetic fatty.

hypothesis that increased reabsorption of UA in obesity and metabolic syndrome may be an adaptive response of the kidney when faced with fatty acid-induced oxidative stress in proximal tubule cells. Second, we observed robust correlations between renal lipid content and oxidative stress markers, particularly in older animals. While correlation does not imply causation, these findings are compatible with previous in vitro studies showing that fatty acid overload in non-adipose cells leads to both lipid accumulation and oxidative stress.[13 23] Third, dietary antioxidant supplementation did not prevent the gradual decline in renal function observed in older ZDF rats. This is in apparent contradiction with previous findings in ZDF rats subjected to pharmacological antioxidant therapy.[24]

Oxidative stress and renal UA handling

UA is the final product of purine catabolism in humans and other apes but is further oxidized to allantoin by the enzyme uricase (urate oxidase) in most other mammals. The uricase gene suffered multiple independent mutations that decreased its activity in the lineage of the family Hominidae (including orangutans, gorillas, chimpanzees, bonobos and humans), culminating with complete gene silencing due to a nonsense mutation in the coding region, estimated to have occurred approximately 15 million years ago.[25] A separate inactivating mutation in the uricase gene occurred in the lineage of the family Hylobatidae (gibbons) approximately 9 million years ago.

These data suggest that a significant evolutionary selection pressure favored the loss of uricase among hominoid ancestors, but the identity of this selection pressure is unclear. Multiple theories have been proposed, including putative roles for higher circulating levels of UA in the evolution of intelligence, maintenance of blood pressure under conditions of low salt intake, modulation of innate immunity, and antioxidant action.[11 20 26–28] In support of the latter hypothesis, although the antioxidant capacity of UA is smaller than that of other antioxidants such as ascorbate,[18] UA is approximately 100-fold more concentrated than ascorbate in human plasma. Furthermore, UA appears to be more effective than ascorbate at scavenging powerful oxidants such as peroxynitrite.[19]

UA is freely filtered at the glomerulus and is reabsorbed primarily in the proximal tubule.[11] If the antioxidant effect of UA was the evolutionary driving force behind uricase loss in hominoids, then it is conceivable that a state of increased oxidative stress would activate renal reabsorption mechanisms to maximize UA preservation. In this case, reducing oxidative stress with exogenous antioxidants would obviate the need for increased UA reabsorption. While enticing, this hypothesis was not supported by the results of our experiments, in which dietary antioxidant supplementation reduced oxidative stress in the kidneys of ZDF rats but did not affect renal UA handling. This suggests that renal UA retention and hyperuricemia in ZDF rats are not caused by oxidative stress in the kidney.

Oxidative stress and renal lipid accumulation

Accumulation of neutral lipids (triglycerides) in non-adipose tissues is not considered harmful per se but is an easily measurable indicator of dysregulated cellular lipid metabolism, usually occurring when the uptake of long-chain non-esterified fatty

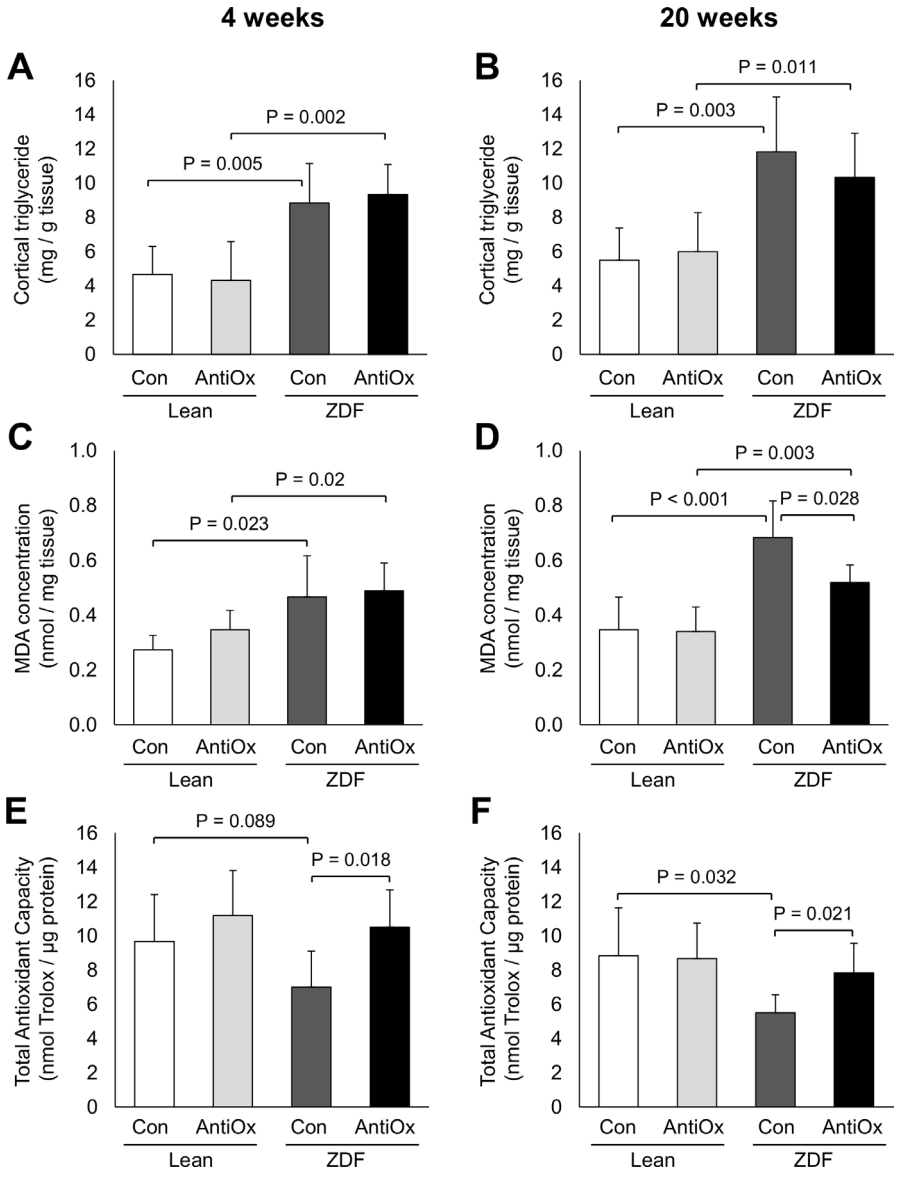

Figure 2 Renal lipid accumulation and markers of oxidative stress. Triglyceride content in the kidney cortex of ZDF versus lean rats fed normal chow (Con) or an AntiOx-enriched diet for (A) 4 weeks and (B) 20 weeks, lipid peroxidation in the kidney cortex of ZDF versus lean rats fed normal chow (Con) or an AntiOx-enriched diet for (C) 4 weeks and (D) 20 weeks, and total AntiOx capacity in the kidney cortex of ZDF versus lean rats fed normal chow (Con) or an AntiOx-enriched diet for (E) 4 weeks and (F) 20 weeks. AntiOx, antioxidant; Con, control; MDA, malondialdehyde; ZDF, Zucker diabetic fatty.

acids from the extracellular environment exceeds mitochondrial β-oxidative capacity.[17] In addition to lipid accumulation, intracellular fatty acid excess results in the production of toxic metabolites and activation of toxic pathways leading to lipid-mediated cellular damage or lipotoxicity.[17] One key mechanism of lipotoxicity is increased mitochondrial generation of ROS, triggered primarily by excess saturated fatty acids such as palmitate.[29] Increased mitochondrial ROS production results in oxidative stress, with multiple deleterious effects on cellular processes and components. These include mitochondrial dysfunction and decreased β-oxidative capacity, resulting in a vicious cycle that further exacerbates lipid accumulation and lipotoxicity.[30]

While these processes have been studied extensively in vitro in various types of cultured cells and, to a lesser extent, in organs such as the liver, heart and pancreas, there is a paucity of experimental evidence on the mechanisms and consequences of lipotoxicity in vivo in the kidney. The tight correlation between tissue lipid content and oxidative stress markers in the present study is compatible with a pathophysiological link between lipid accumulation and oxidative stress in the kidney, similar to that described in cell culture studies,[13 23] but further research is required to explore causal relationships. The fact that dietary antioxidant supplementation reduced oxidative stress but did not affect lipid accumulation in ZDF rat kidneys might suggest that oxidative stress is not causally upstream of lipid accumulation; however, given the complex interplay between these phenomena,[30] additional experiments would be required to make any mechanistic inferences.

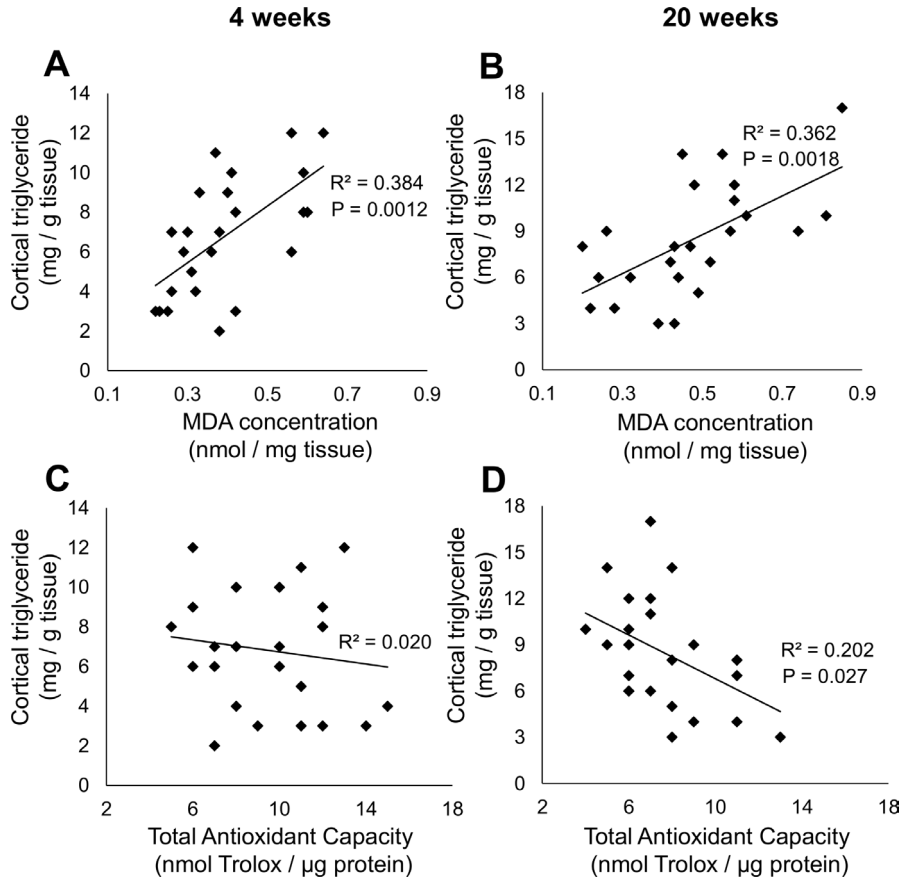

Figure 3 Relationship between markers of oxidative stress and renal cortical triglyceride content in individual animals. Scatterplots of individual animal data are shown, including all experimental animals, ZDF and lean, regardless of diet. The correlations between cortical triglyceride content and lipid peroxidation are shown at (A) 4 weeks and (B) 20 weeks after the beginning of the experiment, and the correlations between cortical triglyceride content and total antioxidant capacity are shown at (C) 4 weeks and (D) 20 weeks after the beginning of the experiment. MDA, malondialdehyde; ZDF, Zucker diabetic fatty.

Oxidative stress and renal function

Renal oxidative stress has been associated with both diabetic nephropathy and CKD,[31–33] but it is still unclear whether oxidative stress is a cause or a consequence of renal dysfunction in these conditions.[34 35] Antioxidant treatment in animal models of diabetes or CKD prevented renal function decline in some studies[24] but not in others.[36] In a prior study of ZDF rats, chronic 14 week therapy with an organoselenium compound (ebselen) with antioxidant properties resulted in partial prevention of age-related decrease in creatinine clearance.[24] In contrast, chronic therapy with a combination of antioxidants in the present study did not affect renal function decline in ZDF rats, in spite of a beneficial effect on reducing oxidative stress. One potential explanation for this apparent discrepancy is that ebselen partially prevented renal function decline by acting through mechanisms other than oxidative stress. This is compatible with the fact that multiple clinical trials of antioxidant supplementation failed to demonstrate beneficial effects on renal function among patients with diabetic nephropathy or early stages of CKD.[37 38]

Study limitations

The use of rats to study renal UA handling is a major limitation of this study, as rats have a functional uricase gene, lower levels of circulating UA than humans, and thus lower flux of renal UA reabsorption. There are important differences between UA transport mechanisms in the human and rodent kidney, and even differences between various rodent strains.[11] While access to a primate model of obesity and metabolic syndrome would have been ideal for this research, this was not possible because of both logistical and ethical considerations. Similarly, a study that included biochemical measurements in kidney tissue could not be performed in humans. Another potential limitation of this study is that the dose of antioxidants used in rats was several orders of magnitude higher that the recommended daily intakes of the respective compounds in humans, when normalized to body weight. This was done to ensure antioxidation in the kidney, but it is theoretically possible that using lower doses of antioxidants could have yielded different results. Finally, we did not examine the effect of antioxidant supplementation on other potentially relevant variables, such as serum glucose and insulin levels.

CONCLUSIONS

Our findings suggest that hyperuricemia in the ZDF rat model of obesity and the metabolic syndrome is not caused by renal oxidative stress, that there may be a pathophysiological link between lipid accumulation and oxidative stress in the kidney, and that antioxidant supplementation does not prevent age-related decline in renal function in ZDF rats. Further research is

required to test whether these findings in rodents can be extrapolated to human pathophysiology.

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Contributors Conceived and designed experiments: IAB (guarantor). Performed experiments: TRR, SKP, LMP and IAB. Analyzed the data: TRR, SKP, LMP and IAB. Interpreted results: TRR, SKP, SKa, SKh, LMP and IAB. Drafted manuscript: SKa, SKh, LMP and IAB. Edited and revised the manuscript: SKa, SKh, LMP and IAB. Approved the final version: TRR, SKP, SKa, SKh, LMP and IAB.

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