Proinflammatory and Metabolic Changes Facilitate Renal Crystal Deposition in an Obese Mouse Model of Metabolic Syndrome

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Purpose: To clarify metabolic syndrome induced stone formation mechanisms we investigated the metabolic and immunohistochemical characteristics associated with renal crystal deposition using a model of mice with metabolic syndrome administered a high fat diet and ethylene glycol.

Materials and Methods: Ob/Ob mice with *Leptin* gene deficiencies and metabolic syndrome related characteristics were compared with wild heterozygous lean mice. Four study groups were fed standard food and water (control group), a high fat diet and normal water (high fat diet group), 1% ethylene glycol and standard food (ethylene glycol group) or a high fat diet and 1% ethylene glycol (high fat diet plus ethylene glycol group). Blood, urine and kidney samples were taken after 14 days.

Results: Ob/Ob mice in the high fat diet plus ethylene glycol group showed diffuse renal crystal depositions. Lean and Ob/Ob mice in the high fat diet plus ethylene glycol group showed significant excretion of urinary calcium oxalate crystals. Ob/Ob mice had significant hypercalciuria, hyperphosphaturia and hyperlipidemia, massive lipid fragments in tubular lumina and fat droplets in renal tubular cells. Ob/Ob mice in the high fat diet plus ethylene glycol group had markedly increased expression of osteopontin, monocyte chemoattractant protein-1, interleukin-6 and tumor necrosis factor-α. In Ob/Ob mice the number of proinflammatory macrophages was considerably elevated.

Conclusions: We induced renal crystal deposition in mice with metabolic syndrome using a high fat diet and ethylene glycol. Increases in luminal mineral and lipid density, and proinflammatory adipocytokines and macrophages facilitated renal crystal formation in mice with metabolic syndrome.

Key Words: kidney, nephrolithiasis, metabolic syndrome X, adipokines, macrophages

Abbreviations and Acronyms

APN = adiponectin
CaOx = calcium oxalate
CD = cluster of differentiation
Ctl = control
EG = ethylene glycol
Emr1 = epidermal growth factor-like module-containing mucin-like hormone receptor-like 1
HFD = high fat diet
IL = interleukin
Il = interleukin
MCP-1 = monocyte chemoattractant protein-1
MetS = metabolic syndrome
OPN = osteopontin
PCR = polymerase chain reaction
RT-PCR = reverse transcriptase-PCR
TEM = transmission electron microscopy
Tnf = tumor necrosis factor
TNF = tumor necrosis factor

METABOLIC syndrome is associated with the presence and severity of kidney stones.[1,2] In the United States it is estimated that 34% of the adult population has MetS. Some studies show that obesity, weight gain and higher calorie diets increase the risk of kidney stone formation (relative risk 1.5 to 2.0).[3,4] One report suggests that the prevalence of kidney stones will increase from 8.8% in 2007 to 9.5% in 2030, which will be associated with increases in the obese and diabetic populations.[5] The report

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concludes that the estimated additional cost associated with urolithiasis will be $1.24 billion per year by 2030. These studies indicate that resolution of the negative cycle between urolithiasis and MetS is anticipated globally.

Obesity is associated with inflammatory cell activation, including macrophage and leukocyte activation. Positive correlations between crystal formation and macrophage infiltration have been demonstrated.[6,7] Inflammation caused by obesity, which involves macrophage infiltration and reactive oxygen species, is a key factor in stone formation.[7] We previously reported that interactions among renal tubular cells, adipocytes and macrophages promote kidney stone formation.[8,9] In those series the paracrine mechanisms involving these 3 cell types produced massive excretions of inflammatory cytokines, including OPN, MCP-1, IL-6 and TNF-α, which facilitated adherence of CaOx monohydrate crystals to renal tubular cells. Although these results indicate the involvement of MetS in stone formation via paracrine mechanisms involving renal tubular cells, adipocytes and macrophages, translational studies using animal models are necessary to understand why MetS causes crystal development in kidneys.

To clarify the MetS induced stone formation mechanism we investigated metabolic and immunohistochemical characteristics that may be associated with renal crystal deposition. We used a model of MetS mice, which have bulimia caused by *Leptin* deficiency, that were given HFD and EG.

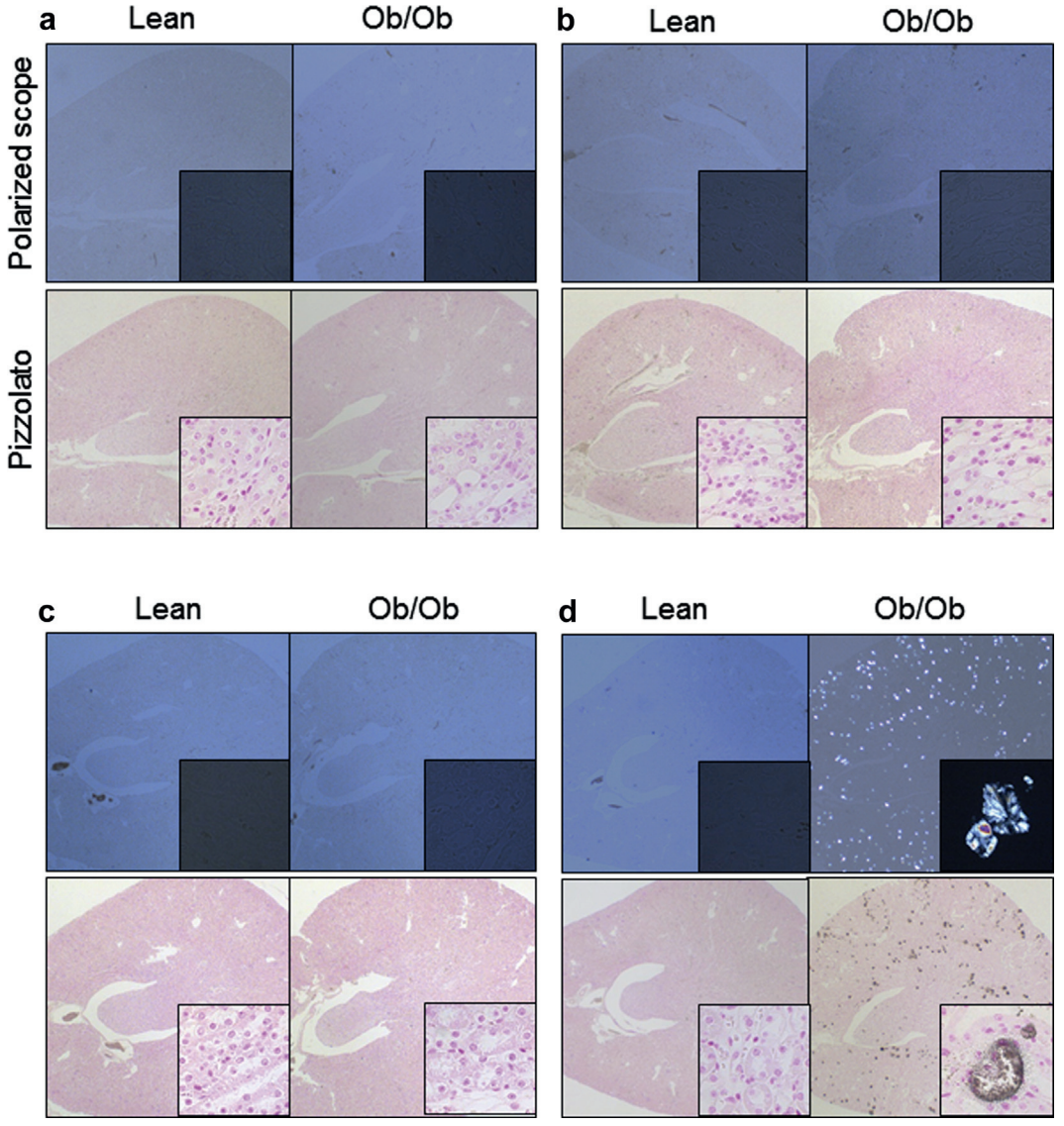

Figure 1. Representative micrographs obtained on day 14 reveal morphological distribution of renal CaOx crystal deposits detected by polarized light optical microscopy and Pizzolato staining. *a*, Ctl. *b*, HFD. *c*, EG. *d*, HFD plus EG. Reduced from ×20, Insets, reduced from ×400.