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Low-protein diet supplemented with ketoacids reduces the severity of renal disease in 5/6 nephrectomized rats: a role for KLF15

Xiang Gao[1,8], Lianghu Huang[2,3,8], Fabrizio Grosjean[3], Vittoria Esposito[3], Jianxiang Wu[4], Lili Fu[1], Huimin Hu[1], Jiangming Tan[2], Cijian He[5], Susan Gray[6], Mukesh K. Jain[7], Feng Zheng[2,3] and Changlin Mei[1]

[1]Department of Medicine, Kidney Institute of PLA, Changzheng Hospital, Second Military Medical University, Shanghai, People's Republic of China; [2]Department of Urology, Dongfang Hospital, Fuzhou, People's Republic of China; [3]Division of Experimental Diabetes and Aging, Department of Geriatrics, Mount Sinai School of Medicine, New York, New York, USA; [4]Division of Cardiology, Department of Medicine, Changzheng Hospital, Second Military Medical University, Shanghai, People's Republic of China; [5]Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA; [6]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA and [7]University Hospitals Harrington-McLaughlin Heart and Vascular Institute and Case Cardiovascular Research Institute, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

Dietary protein restriction is an important treatment for chronic kidney disease. Herein, we tested the effect of low-protein or low-protein plus ketoacids (KA) diet in a remnant kidney model. Rats with a remnant kidney were randomized to receive normal protein diet (22%), low-protein (6%) diet (LPD), or low-protein (5%) plus KA (1%) diet for 6 months. Protein restriction prevented proteinuria, decreased blood urea nitrogen levels, and renal lesions; however, the LPD retarded growth and decreased serum albumin levels. Supplementation with KA corrected these abnormalities and provided superior renal protection compared with protein restriction alone. The levels of Kruppel-like factor-15 (KLF15), a transcription factor shown to reduce cardiac fibrosis, were decreased in remnant kidneys. Protein restriction, which increased KLF15 levels in the normal kidney, partially recovered the levels of KLF15 in remnant kidney. The expression of KLF15 in mesangial cells was repressed by oxidative stress, transforming growth factor-β, and tumor necrosis factor (TNF)-α. The suppressive effect of TNF-α on KLF15 expression was mediated by TNF receptor-1 and nuclear factor-κB. Overexpression of KLF15 in mesangial and HEK293 cells significantly decreased fibronectin and type IV collagen mRNA levels. Furthermore, KLF15 knockout mice developed glomerulosclerosis following uninephrectomy. Thus, KLF15 may be an antifibrotic factor in the kidney, and its decreased expression may contribute to the progression of kidney disease.

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Restriction of dietary protein intake remains a common practice for patients with chronic renal failure, although the results from the MDRD (modification of diet in renal disease) study are inconclusive.[1] Overall, data from both clinical and experimental studies suggest a beneficial effect of a low-protein diet (LPD) on chronic kidney disease (CKD).[1–4] The mechanism(s) by which a LPD slows down the progression of CKD are unknown. However, it is clear that restriction of dietary protein intake reduces the metabolic burden, especially the levels of urea nitrogen.[4] Additionally, treatment of 5/6 nephrectomized rats, a model of progressive CKD, with a LPD has been shown to preserve the antioxidant capacity and decrease the levels of oxidative stress in the remnant kidney.[3] As decreased protein synthesis because of insufficient amino-acid supply and malnutrition is a concern for long-term protein restriction, ketoacids (KA), a nitrogen-free substitution for the essential amino acids, have been prescribed together with a LPD to patients with advanced CKD.[5,6] Nitrogen-free branched-chain KA are equally efficient as their counterpart of essential amino acids in protein synthesis.[7] Furthermore, branched-chain KA have been reported to inhibit renal gluconeogenesis and increase liver albumin synthesis.[7] In this study, we evaluated the effect of a LPD with or without supplementation of KA on a remnant kidney model.

Renal fibrosis, a result of extracellular matrix accumulation in glomeruli and tubulointerstitium, is a hallmark of progressive CKD.[8] Multiple factors, such as chronic inflammation, transforming growth factor-β (TGF-β),

Correspondence: *Changlin Mei, Department of Medicine, Kidney institute of PLA, Changzheng Hospital, Second Military Medical University, Shanghai 200003, People's Republic of China. E-mail: chlmei@hotmail.com or Feng Zheng, Department of Urology, Dongfang Hospital, Fuzhou 350025, People's Republic of China. Email: feng.zheng@mssm.edu*

[8]These authors contributed equally to this work.

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hemodynamic changes, and oxidative stress, have been implicated in the development and progression of renal fibrosis.[9,10] Kruppel-like factors (KLFs) are a subclass of the cys2/his2 zinc-finger family of transcriptional regulators.[11,12] Thus far, 17 members of KLF have been identified and shown to be key regulators in cell growth and differentiation, inflammation, and metabolism.[11,12] KLF15 was isolated and cloned independently by two groups.[13,14] KLF15 is expressed abundantly in the kidney and liver, and has thus far been identified as a regulator of gluconeogenesis, the cardiovascular response to stress, and adipocyte differentiation.[13–17] As the levels of type I collagen transcription and the activity of connective tissue growth factor promoter were decreased by KLF15, KLF15 may also regulate the expression of extracellular matrix.[13,15,18] Here, we examine the level of KLF15 expression in the remnant kidney. We found that KLF15 expression was significantly decreased in remnant versus normal kidney, and we explored the factor(s) that might contribute to reduced KLF15 expression. Finally, we examined the effects of altered KLF15 expression on the production of extracellular matrix in mesangial cells and on the development of renal fibrosis.

RESULTS

General

Body weight increased progressively in normal rats from 2 to 8 months of age (Figure 1a). There was no difference in the growth curve between normal rats and 5/6 nephrectomized rats fed with normal protein diet (NPD). Dietary protein restriction significantly slowed down animal growth. At the age of 8 months, the body weight of the rats in LPD group was 466.3 ± 35 g, which was lower than that of the animals in NPD group (664.8 ± 42 g, $P<0.01$). The loss of weight gain was partially corrected by KA supplementation.

Serum albumin levels were significantly decreased in 5/6 nephrectomized rats in NPD group, and also low in 5/6 nephrectomized rats in LPD group (Figure 1b). Addition of KA to the LPD prevented serum albumin from decreasing.

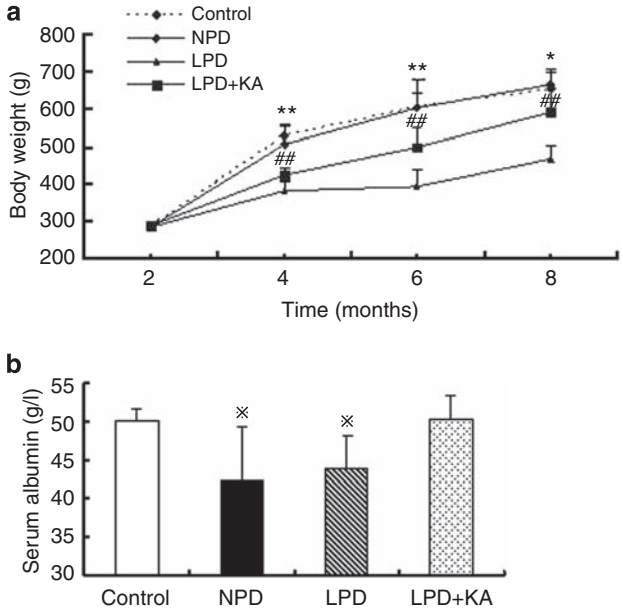

Figure 1 | General data of rats. Body weight (**a**) and serum albumin levels (**b**) of control and 5/6 nephrectomized rats fed with NPD, LPD, or LPD + KA. Data are expressed as means ± s.d. $^{*}P<0.05$, $^{**}P<0.01$ versus LPD, or LPD + KA; and $^{\#\#}P<0.01$ versus LPD; $\divideontimes P<0.05$ versus control or LPD + KA. LPD, low-protein diet; LPD + KA, low-protein diet supplemented with ketoacids; NPD, normal protein diet.

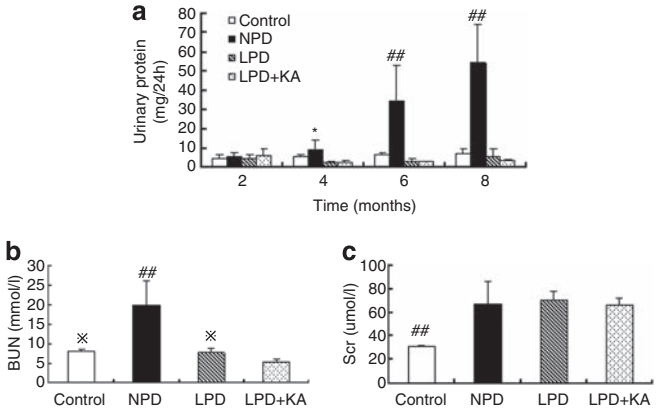

Figure 2 | Renal function. Proteinuria (**a**), BUN (**b**), and Scr (**c**) levels of control and 5/6 nephrectomized rats fed with NPD, LPD, or LPD + KA. Data are expressed as means ± s.d. $^{*}P<0.05$ versus LPD or LPD + KA; $^{\#\#}P<0.01$ versus the other three groups; and $\divideontimes P<0.05$ versus LPD + KA. BUN, blood urea nitrogen; LPD, low-protein diet; LPD + KA, low-protein diet supplemented with ketoacids; NPD, normal protein diet; Scr, serum creatinine.

Proteinuria and renal function

Urinary protein excretion increased progressively in 5/6 nephrectomized rats in NPD group (Figure 2a). Dietary protein restriction completely prevented the progression of proteinuria. The effect was retained in animals fed with a low-protein plus KA. Blood urea nitrogen levels were elevated in NPD-fed rats after 6 months of 5/6 nephrectomy, and was normal in LPD- and LPD supplemented with KA (LPD + KA)-fed 5/6 nephrectomized rats (Figure 2b). However, dietary protein restriction with or without KA did not reduce the increased serum creatinine levels in 5/6 nephrectomized rats (Figure 2c).

Renal histology and extracellular matrix

Renal histology was normal in age-matched normal controls (Figure 3a). There was a moderate expansion of mesangial area and an increase in thickness of membranes of Bowman's capsules in glomeruli of NPD-fed 5/6 nephrectomized rats. Severe tubulointerstitial lesions, including tubular atrophy, dilatation, increased thickness of tubular basement membranes, and fibrosis, were present in NPD-fed 5/6 nephrectomized rats. Masson's trichrome staining revealed intensive interstitial fibrosis and extensive inflammatory cell infiltration in this group (Figure 3b). Restriction of dietary protein intake decreased glomerular lesions and, more prominently,