A high ratio of (n-3)/(n-6) polyunsaturated fatty acids in vivo is potentially beneficial in chronic kidney disease

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Abstract

Chronic kidney disease (CKD) has emerged as a global health problem of epidemic proportions in the past few years. One of the major progressive features seen in CKD is renal fibrosis, which is one of the largest challenges in nephrology because it ends in chronic renal failure (CRF). The mechanisms of progression of CKD are poorly understood. Epidemiologic studies suggest a strong genetic component, but the genes that contribute to the onset and progression of CKD are largely unknown. Many studies have shown that ω-3/n-3 polyunsaturated fatty acids (n-3 PUFAs) ameliorate renal ischemic injury according to functional and histologic criteria. This is associated with decreased activation of the genes for TGF-β and inducible nitric oxide synthase (iNOS). Fat-1 transgenic mice, in which an exogenous fat-1 gene from Caenorhabditis elegans has been inserted, can endogenously synthesize ω-3/n-3 polyunsaturated fatty acids (n-3 PUFAs) from ω-6/n-6 polyunsaturated fatty acids (n-6 PUFAs). It has long been realized that dietary supplementation with fish oils that contain large amounts of n-3 PUFAs can bring benefits in the treatment of CKD. Some clinical studies have focused on the effect of n-3 fatty acids from fish oil on blood pressure, and on the lipid profiles of dialysis patients, confirming the feasibility and safety of this treatment in human beings. In general, we hypothesis that a high ratio of (n-3)/(n-6) PUFAs in vivo may prevent progression of CKD, which is a novel approach to clinical treatment of this condition.

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Introduction

In the last few decades, chronic kidney disease (CKD) has emerged as a worldwide health problem [1]. The declaration of World Kidney Day, observed annually beginning in March 2006, sent a clear message to the public, government health officials, physicians, allied health professionals, patients, and families that ‘CKD is common, harmful, and treatable’ [2]. Tubulointerstitial fibrosis is the final common pathway in late-stage renal disease. The pathogenesis of kidney fibrosis is characterized by overproduction and deposition of extracellular matrix (ECM), which ultimately leads to fibrotic lesions and tissue scarring. Although the mechanisms of myofibroblast activation and fibrogenesis in various pathologic conditions are not completely understood, activation of multiple cytokines and growth factor receptors is involved in these processes [3]. Transforming growth factor-β (TGF-β), angiotensin II, nuclear factor-κB (NF-κB) and tumor necrosis factor-α (TNF-α) are growth factors and cytokines that have been implicated in animal models of obstructive renal injury [3].

Most patients with CKD need to receive long-term drug therapy. Inhibitors of the renin–angiotensin–aldosterone system, including angiotensin converting enzyme inhibitors and angiotensin II receptor blockers, are recommended as first-choice drugs for patients with CKD, according to several guidelines. However, poor long-term compliance with drug therapy is universally recognized as one of the major clinical issues in the management of chronic diseases, including renal diseases [4]. The n-3 polyunsaturated fatty acids (PUFAs), such as docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are precursors of anti-inflammatory compounds. Fish oils and plant seeds/vegetable oils are used to provide the required (n-3) and (n-6) fatty acids respectively [5]. Therefore, these may be potentially beneficial foods for patients with CKD.

A considerable number of ex vivo and in vivo studies have shown that dietary supplementation of n-3 PUFAs, but not n-6 PUFAs, ameliorates murine ischemic acute renal failure and prevents increases in mRNA of both TNF-α and inducible nitric oxide synthase (iNOS), and has the potential to be a promising drug therapy for CKD in humans [6]. Long-term dietary supplementation with DHA or DHA-containing fish oils decreases TNF-α production. Such diets also ameliorate ischemic ARF (Acute renal failure) in dogs [7-10]. In addition to decreasing TNF-α mRNA expression, increases in TGF-β mRNA are also consistently prevented by n-3 PUFAs after ischemia–reperfusion injury [11, 12].

Kang et al. generated the transgenic Fat-1 mouse on a C57/BL6 background by inserting the fat-1 gene from Caenorhabditis elegans. This gene encodes an n-3 desaturase enzyme (Figure 1) that can synthesize n-3 PUFAs from n-6 PUFAs [13]. Different tissues of Fat-1 mice show an increase in n-3 PUFAs and decrease in n-6 PUFAs, leading to a significant decrease in the ratio of (n-3)/(n-6) PUFAs. Thus, Fat-1 transgenic mice have an (n-3)/(n-6) PUFA ratio of 1:0.7–5.7; wild-type mice have a ratio of 1:16.5–
49 (Table 1) [13, 14]. Substantial research has shown that n-3 PUFAs exhibit anti-inflammatory effects in various acute and chronic inflammatory diseases, such as hepatic fibrosis [15], neuroinflammation [16], obesity [17], osteoporosis [18] and colitis [19]. Obesity has emerged as the largest pandemic in recent history, with important implications not only for cardiovascular disease (CVD) but also for CKD [20]. Colitis, chemical-induced hepatitis and osteoporosis are all due to enhanced production of cytokines IL-6 and TNF-α; these diseases are much less severe in the transgenic Fat-1 mouse. This indicates that the presence of enhanced amounts of n-3 fatty acids in the tissues and plasma has a dampening effect on inflammation.

Consequently, we hypothesize that directly elevating the ratio of (n-3)/(n-6) PUFAs in vivo may prevent progression of CKD. Fat-1 transgenic mice is not only show increased n-3 PUFAs, but also reduced n-6 PUFAs. However, the ratio of (n-3)/(n-6) PUFAs can reach a very high level by the use of transgenic techniques which conventional exogenous intake is incapable of reaching [5]. In the authors’ opinion, this explains why some studies have found that dietary n-3 PUFAs did not have a positive effect in inhibiting the progression of nephropathy [21]. It is necessary to explore the therapeutic effect, or even the toxic and side effects, of n-3 and n-6 PUFAs in the development of CKD, and our hypothesis may lead to a new clinical treatment for this disease.

**Hypothesis**

Our hypothesis is based on the crucial role of TNF-α, TGF-β and IL4 in the progress of CKD, and the fact that n-3 PUFAs inhibit activation of the above three cytokines; moreover, it has been reported that n-3 PUFAs not only reduce the production of IL-1 and TNF-α but also promote IGF-1 (Insulin-like growth factors 1) production [22-24]. We propose that increasing the dietary ratio of (n-3)/(n-6) PUFAs may prevent the progression of CKD, and we use the Fat-1 transgenic mice, which has a greatly increased ratio of (n-3)/(n-6) PUFAs, to verify our hypothesis.

**Evaluation of hypothesis**

Obesity, which is epidemic in modern society, carries a markedly increased risk for comorbid complications, such as type 2 diabetes, cancer, hypertension, dyslipidemia, cardiovascular disease, and sleep apnea [20, 25]. In addition, obesity increases the risk of CKD and its progression to ESRD (End-stage renal disease). Recent data from the United States indicate that the incidence and prevalence of obesity in patients on maintenance dialysis greatly exceed the corresponding figures in the general population [26]. Basic research shows that dietary ratios of (n-3)/(n-6) PUFAs can control markers of metabolic disorders, including obesity, insulin resistance (IR), inflammation, and lipid profiles, which are also presumed to be partly related to type 2 diabetes mellitus (T2DM) [27]. The increasing dominance of n-6 over n-3 PUFAs in
typical Western diets may be one key factor contributing to the increasing incidence of obesity and T2DM [28]. The n-3 PUFAs have been observed to increase the capacity for fatty acid oxidation (FAO) and to improve insulin sensitivity [29]. A recent study found that ingestion of fish oil decreases learning impairment in diabetic rats by blocking PI3K/AKT/nuclear factor-κB-mediated inflammatory pathways [30]. Cytokines and chemokines, including IL-1β, IL-6 and TNF-α, have been found to be increased in basic research on CKD. All of these mediators are therapeutic targets in CKD [6, 31], with anti-TNF-α treatment proving highly effective at controlling disease progression in a significant proportion of patients [31, 32]. The marine n-3 PUFAs exert a range of anti-inflammatory effects, including reduced production of eicosanoids, cytokines and adhesion molecules. These effects suggest that n-3 PUFAs may be useful therapies for patients with inflammatory conditions.

More importantly, genetic research has revealed that TGF-α alleles are involved in the susceptibility to CKD progression [6]. TGF-α is a crucial intermediate in angiotensin II-induced renal lesions [33]. Levels of TGF-α protein were markedly increased after nephron reduction in an animal study, and this increase preceded the development of renal lesions [6]. These data suggest that variable TGF-α expression may explain, in part, the genetic susceptibility to CKD progression [6]. TNF-α has been suggested to be associated with the reduction of glomerular filtration resulting from hemodynamic changes, and it may lead to useful dietary suggestions for patients with CKD. Furthermore, it may provide as well as altering endothelial permeability. It is a useful basis for the application of n-3 PUFAs
to clinical treatment.

**Verification of hypothesis**

The experimental design is briefly illustrated as follows:

1. Establishment of a proteinuria kidney disease model in 8-week-old male Fat-1 transgenic mice and littermate controls by feeding a diet containing 0.2% adenine.
2. The animals were maintained in a room at 22°C with a 12-h light–dark cycle with free access to food and water.
3. Assessments of the induction of CKD were conducted and body weights were recorded daily. The correlation between renal injury and serum (n-3)/(n-6) PUFA ratios was calculated; evaluation of serum (n-3)/(n-6) PUFA ratios, TNF-α synthesis and pro-inflammatory cytokine expression should also be included. Both kidneys were removed for histological and immunohistochemistry analysis.

   Our hypothesis was briefly verified, as shown in Figure 2.

\[
\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH} = \text{CH}_3\text{-CH}_2\text{-CH}_2\text{--COOH} \quad \omega-6 (18:2; 20:4; 22:4; 22:5) \\
\text{CH}_3\text{-CH} = \text{CH}_2\text{-CH}_2\text{-CH} = \text{CH}_2\text{-CH}_2\text{--COOH} \quad \omega-3 (18:3; 20:5; 22:5; 22:6)
\]

**Figure 1** | The fat-1 gene encodes a n-3 desaturase that converts n-6 to n-3 fatty acid.

**Figure 2** | Illustration of our hypothesis. Biochemical pathways of n-6 and n-3 fatty acids, Fat-1 mice can endogenously convert n-6 PUFAs to n-3 PUFAs and gain high ratio of n-3/n-6 PUFAs. N-6 and n-3 PUFAs pathways lead to their derived pro-inflammatory or anti-inflammatory and pro-resolution mediators.
Consequence of hypothesis

A high ratio of (n-3)/(n-6) PUFAs altered the TNF-α activity and pro-inflammatory microenvironment in vivo, which prevented progression of CKD as shown by proteinuria, renal fibrosis and apoptosis. This is a new method for studying the relationship between (n-3)/(n-6) PUFAs in vivo and the mechanism of CKD progression. Current studies have found that reducing the dietary (n-3)/(n-6) PUFA ratio gives a less healthy diet. Our research may supply a novel perspective on nutrition for patients with CKD. However, there is a long way to go before the hypothesis can be validated by clinical evaluation and further well-designed research.


Funding This work was supported by the National Natural Science Foundation of China (No. 81300155).

Conflict of interest No potential conflicts of interest relevant to this article are reported.

Ethics approval None.

Provenance and peer review Not commissioned; externally peer reviewed.

Acknowledgements None.

References
Hypothesis


Zhang, X.N. Xu, A.L. Ji, R. Cao, R.H. Yang, F. Wang, A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats, Nutrition research, 33 (2013) 849-858.

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