Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com


# Dietary n-3 polyunsaturated fatty acids or soy protein isolate did not attenuate disease progression in a female rat model of autosomal recessive polycystic kidney disease

*Kaitlin H. Maditz*[a], *Chris Oldaker*[a], *Nainika Nanda*[a], *Vagner Benedito*[b], *Ryan Livengood*[c], *Janet C. Tou*[a,*]

[a] *Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV 26506*
[b] *School of Medicine, West Virginia University, Morgantown, WV 26506*
[c] *Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506*

ARTICLE INFO

*Article history:*
Received 19 February 2014
Revised 14 April 2014
Accepted 6 May 2014

*Keywords:*
Rat
Polycystic kidney disease
n-3 PUFAs
Soy protein
Diet therapy

ABSTRACT

Polycystic kidney disease (PKD) is an incurable genetic disorder that is characterized by multiple benign cysts. As PKD advances, cyst growth increases kidney volume, decreases renal function, and may lead to end-stage renal disease; however, in a PKD rat model, feeding soy protein isolate (SPI) reduced cyst proliferation and growth. The n-3 polyunsaturated fatty acids (PUFAs) are noted for their anti-inflammatory actions. Therefore, diet therapy could offer a potentially efficacious, safe, and cost-effective strategy for treating PKD. The objective of this study was to investigate the role of soy protein and/or n-3 PUFAs on PKD progression and severity in the rat model of autosomal recessive PKD. We hypothesized that the antiproliferative and anti-inflammatory actions associated with soy protein and n-3 PUFA supplementation will attenuate PKD progression in female PCK rats. For 12 weeks, young (age, 28 days) female PCK rats were randomly assigned (n = 12/group) to 4 different diets: casein ± corn oil, casein ± soybean oil, SPI ± soybean oil, or SPI ± 1:1 soybean/salmon oil (SPI ± SB). The feeding of the different protein and lipid sources had no significant effect on relative kidney weight. Histologic evaluation showed no significant differences in cortical or medullary cyst size, interstitial inflammation, and fibrosis among diet groups. However, rats fed SPI ± SB diet had cortical cyst obstruction and the highest ($P < .01$) serum blood urea nitrogen concentration. Rats fed SPI ± SB diet had the highest ($P < .001$) renal docosahexaeonic acid, but there were no significant differences in renal tissue inflammation and proliferation gene expression among the diet groups. Based on these results, dietary soy protein and/or n-3 PUFAs did not attenuate disease progression or severity in the female PCK rat model of autosomal recessive PKD.

Published by Elsevier Inc.

*Abbreviations:* AA, arachidonic acid; ADPKD, autosomal dominant polycystic kidney disease; ALA, α-linolenic acid; ARPKD, autosomal recessive polycystic kidney disease; BUN, blood urea nitrogen; CO, corn oil; COX-2, cyclooxygenase; DHA, docosahexaeonic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MapK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PKD, polycystic kidney disease; PPARγ, peroxisome proliferator–activated receptor γ; SB, soybean oil/salmon oil blend; SEM, standard error of the mean; SO, soybean oil; SPI, soy protein isolate.

* *Corresponding author*. Tel.: +1 304 293 1919; fax: +1 304 293 2232.
E-mail address: janet.tou@mail.wvu.edu (J.C. Tou).

http://dx.doi.org/10.1016/j.nutres.2014.05.001
0271-5317/Published by Elsevier Inc.

## 1. Introduction

Polycystic kidney disease (PKD) is a genetic disorder characterized by multiple benign cysts derived from the epithelial lining of the nephron [1]. As PKD progresses, cyst growth increases kidney volume, reduces renal function, and eventually leads to end-stage renal disease [2]. In 2008, at a cost of 39.46 billion dollars, 547 982 patients in the United States were diagnosed as having kidney disease and received treatment (ie, dialysis or kidney transplant) for end-stage renal disease [3]. Of those patients, approximately 22% of all end-stage renal disease cases were due to PKD [3].

The 2 main forms of PKD are autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). In ADPKD, renal disease onset occurs in early adulthood, whereas ARPKD has infantile renal disease onset [4]. The poor prognosis associated with PKD has resulted in studies investigating various therapeutic strategies to attenuate disease progression. Diet therapy offers a potentially efficacious, safe, and cost-effective strategy for treating PKD. Studies have examined the role of a variety of protein and lipid sources as diet strategies for decreasing PKD progression and severity using the Hans:SPRD-cy rat, a non-orthologous animal model of ADPKD [5,6]. In concordance with reports that omega-3 polyunsaturated fatty acids (n-3 PUFAs) have anti-inflammatory actions mediated through alterations in membrane composition, gene expression, and eicosanoid production [7,8], male Han:SPRD-cy rats fed diets supplemented with n-3 PUFAs significantly decreased renal inflammation and damage [9].

High-protein consumption has been suggested to negatively affect PKD by causing higher renal perfusion and kidney hyperfiltration of the already compromised nephron [10]. However, protein restriction may be associated with risk of protein malnutrition, particularly for ARPKD characterized by early onset that includes rapid growth stages. The absence of a defined amount of protein that delays disease progression while meeting protein requirements has led to greater interest in probing the source of protein on PKD [6]. Feeding soy protein isolate (SPI) has been shown to reduce cyst proliferation and to decrease cyst growth in PKD animal models [11,12]. Feeding male Han:SPRD-cy rats soy protein slowed PKD progression by decreasing inflammation indicated by decreased renal cyclooxygenase-2 (COX-2) enzyme activity and renal prostanoids [12].

Although the rate of PKD progression and severity is affected by sex, most studies have only been conducted in male animal models [13]. In research conducted by Aukema and Housini [14], feeding soy protein reduced kidney cysts and improved renal functional measurements in male, but not female, Han:SPRD-cy rats. Currently, no rodent model is genetically orthologous while expressing phenotypes typical of human ADPKD or ARPKD. Therefore, Torres and Harris [15] suggested that several PKD rodent models be used to investigate experimental therapies. The PCK rat, an orthologous model of human ARPKD, has a phenotype that resembles a slow progressing form of ARPKD [16]. To our knowledge, no studies have examined diet therapy using the PCK rat model. We hypothesized that the antiproliferative and anti-inflammatory actions associated with soy protein and n-3 PUFA supplementation will attenuate PKD progression in female PCK rats. Therefore, the objective of this study was to investigate the role of these bioactive ingredients on PKD progression by randomly assigning female PCK rats to diets containing SPI and/or n-3 PUFAs. Because of the absence of human clinical trials including dietary intervention for PKD, human patients must rely on the evidence gained from animal models [6]. By examining various animal PKD models and their reaction to various dietary components, researchers can provide PKD patients with a better dietary treatment plan.

## 2. Methods and materials

### 2.1. *Animals and diets*

All animal procedures performed in this study were conducted in accordance with the National Research Council for the Care and Use of Laboratory Animals Guidelines [17] and were approved by the Animal Care and Use Committee at West Virginia University. Female, 28-day-old PCK rats were purchased from Charles River Laboratories (Wilmington, MA, USA). All rats were individually caged and maintained in a room kept at 21 °C, with a 12-hour light/dark cycle throughout the 12-week study. After a 7-day acclimation period, animals were randomly assigned (n = 12 rats/group) to different experimental diets. The experimental diets were based on the American Institute of Nutrition-93G (AIN-93 G) diet. The AIN-93G is a standard diet consisting of purified ingredients and formulated to meet all the nutritional requirements for growing rats, as defined by the National Research Council [18]. Diet ingredients and fatty acid composition are provided in Table 1. All diets were isocaloric and had similar ingredients, except for the lipid and protein source. The protein sources consisted of a 200-g/kg diet as casein or SPI. Both protein sources consisted of 87% crude protein by proximate analysis. Soy protein isolate was generously provided by DuPont Nutrition and Health (St Louis, MO, USA). The lipid sources consisted of a 70-g/kg diet as either corn oil (CO; which is low in n-3 PUFAs), soybean oil (SO; which contains the essential fatty acids linoleic acid [LA; 18:2n-6]), and α-linolenic acid (ALA; 18:3n-3) or salmon oil (which is rich in the long-chain n-3 PUFAs, eicosapentaenoic [EPA; 20:5n-3], and docosahexaenoic acid [DHA; 22:6n-3]) as a 1:1 SO ± salmon oil blend. Salmon oil was purchased from Jedwards International Inc (Quincy, MA, USA). Our 4 experimental diets consisted of the following: (1) casein ± CO, (2) casein ± SO, (3) SPI ± SO, or (4) SPI ± 1:1 soybean/salmon oil (SPI ± SB).

To prevent variability in food intake, rats were restricted to 15 ± 2 g of powdered diet daily. This was based on previous studies showing that this amount supported growth in young Sprague-Dawley female rats consuming ~15 g powdered diet/d [19]. All diets were kept at –20 °C until fed to the rats. Food intake was measured daily and replaced with fresh diet daily. Rats were provided deonized distilled water ($ddH_2O$) ad libitum. Body weights, water consumption, and urine outputs were recorded weekly, throughout the study. At the end of the 12-week feeding study, rats were euthanized by $CO_2$ inhalation. Blood was collected by aortic puncture and