Author’s response to reviews

Title: Dietary patterns in relation to testosterone levels and severity of impaired kidney function among middle-aged and elderly men in Taiwan: a cross-sectional study

Authors:

Adi Kurniawan (adilukaskurniawan@yahoo.com)
Chien-Yeh Hsu (cyhsu@ntunhs.edu.tw)
Hsiao-Hsien Rau (hh.rau@jct.org.tw)
Li-Yin Lin (jlin11025@gmail.com)
Jane Chao (chenjui@tmu.edu.tw)

Version: 1 Date: 20 May 2019

Author’s response to reviews:

RESPONS TO THE EDITOR AND REVIEWERS

Editor: Please revise the language. In particular, avoid at all cost causal terms such as "protective effect" or the word "decline" which refers to a change over time, when it is a cross-sectional study. Moreover, the English needs revision by a native English speaker too. Watch the tenses. One recurrent error is the inadequate use of "the", eg. l.104 "had the errors" should be "had errors"; l.184 "had the mean of eGFR..." should be "had a mean eGFR of...". The cross-sectional design is explicit from the title but should also be mentioned in the abstract, in the objective section at the end of the introduction.

REPLY: We have revised accordingly. Moreover, we have asked someone who is fluent in English revising the language throughout the manuscript.

Reviewer #1: It is an interesting article, highlighting the dietary link to kidney function.

1. The number of subjects included in this analysis with readings on levels of testosterone is very low, only 256. And it severely affects the outcome/ findings. Thus it should not highlight levels of testosterone as the main area of study. Better to limit the article to describe the finding with dietary patterns and kidney function levels.
REPLY: We understood that the numbers (n = 256) of subjects who had testosterone levels were limited after excluding subjects who were less than 40 years old (n = 78), because serum testosterone level was not included in the basic health screening package in MJ Institute. Testosterone and other sex hormones were included in additional items and the members needed to pay extra fee for these particular items. However, we still considered adding testosterone findings as one part of the present study because no published literature had assesses the effect of dietary patterns on male testosterone levels in subjects with decreased kidney function. Hence, our study could become a pioneer study to assess these phenomena. Additionally, we mentioned the limitation of the study included: “Moreover, only a few of subjects had serum testosterone levels in this study” (lines 388-389).

2. Why the CKD was defined as associated with low testosterone levels? not clear.

REPLY: We have mentioned in the discussion section about the possible mechanisms for the association between CKD and low testosterone levels.

The possible mechanisms for the association between decreased kidney function and low testosterone levels are multi-factorial including uremia and increased prolactin levels. Comorbid conditions such as hypertension and diabetes may also contribute to the low testosterone levels. Uremia, commonly seen in subjects with decreased kidney function, could also impair the hypothalamic-pituitary-gonadal axis and further contribute to low testosterone levels in men. In addition, prolactin levels were inversely associated with kidney function (eGFR) due to the reduction of prolactin clearance by the kidney. Both uremia and prolactin negatively affected gonadal function by inhibiting the secretion of gonadotropin-releasing hormone and luteinizing hormone for testosterone production in Leydig cells (lines 294-303).

3. The selection criteria is limited to people with kidney disease (with proteinuria), thus the level of eGFR may varies according to the underlying cause / primary illness (ex-NCDs etc.) Therefore, care must be taken to attribute the association to dietary patterns and it is generalizable to people with chronic kidney disease only. Ideal is to include people without proteinuria to find the real association between diet and kidney function.

REPLY: In Taiwan, diabetes mellitus (DM) (43.2%), chronic glomerulonephritis (CGN) (25.1%), hypertension (8.3%), and chronic interstitial nephritis (2.8%) are four major underlying renal disease in 2007 [1]. The present study mainly focused on subjects with decreased kidney function. Thus, the present study on the association between dietary patterns, severity of decreased kidney function, and proteinuria selected the subjects with mildly decreased kidney function (eGFR 60–89 mL/min/1.73 m2) and one plus (+1) urinary protein as references. Moreover, we have added in our limitation as follows: “A temporal relationship may occur as we
observed in the present study, and which could be affected by the dietary advices given to the subjects due to their disease conditions” (lines 382-384); “Therefore, our results may not necessarily reflect the clinically diagnosed CKD subjects, and not generalize to those with decreased kidney function and without clinical proteinuria” (lines 405-407).

4. It is useful to include a discussion point to highlight the limitation in the temporal association with the diet pattern and the kidney function decline. As the diet we observe in this population could be affected by the dietary advises given due to the underlying conditions (DM, CVDs, etc)

REPLY: Thanks for reviewer’s suggestion. We have revised accordingly in our limitation as follows: “First, the cross-sectional study design cannot establish the causal relationship between dietary patterns and severity of decreased kidney function. A temporal relationship may occur as we observed in the present study, and which could be affected by the dietary advices given to the subjects due to their disease conditions” (line 380-384).

Reference


Reviewer #3: Manuscript number: NUTJ-D-19-00063

Title: Dietary patterns in relation to testosterone levels and severity of kidney function decline among middle-aged and elderly men in Taiwan: a cross-sectional study. Although the topic is well interesting, the paper needs some revisions.

1. In general, moderate English changes are required. I invite the authors to revise carefully the paper taking into account the recommended corrections and comments.

REPLY: We have asked someone who is fluent in English revising the language throughout the manuscript.
Abstract

1. The abstract should be improved, as for example specifying the effective number of subjects (n=256) assessed for their testosterone level and the used methodology for the dietary assessment (FFQ).

REPLY: We have revised accordingly. The abstract was revised as follows: “Among all the subjects, 256 men with testosterone levels were also included in the analysis. Dietary assessment was conducted using food frequency questionnaire, and three dietary patterns (fried-processed, vege-seafood, and dairy-grain dietary patterns) were identified using principal component analysis.” (lines 34-37).

Introduction section

The introduction is well written, there are few remarks at this level:

1. Line 73, 75 and 85, please add references.

REPLY: We have added the references as follows: “Dietary patterns have been linked to many risks of developing chronic disease [8]. However, most of the previous studies examining the effects of dietary patterns in subjects with decreased kidney function were conducted in Western countries and mostly in women [9-11]” (lines 76-79); “Additionally, to our knowledge, few studies have described the relationship between diet and testosterone levels [13-15]. Notably, the previous study found significant increases in serum testosterone levels in overweight or obese men on a low-fat diet for 12 weeks and weight maintenance for another 40 weeks [13]. However, only a few studies have reported specific dietary patterns with testosterone levels. Two small cross-sectional studies showed that there was no association between vegetarian diets and testosterone levels in men [14, 15]” (lines 89-94). Additionally, the references cited in the text were also added in the reference list (pages 20 #8, 21 #13-15).

2. Add some information about the association of diets and testosterone level

REPLY: We have added some information about the association of diets and testosterone levels.

Additionally, to our knowledge, few studies have described the relationship between diet and testosterone levels. Notably, the previous study found significant increases in serum testosterone levels in overweight or obese men on a low-fat diet for 12 weeks and weight maintenance for another 40 weeks. However, only a few studies have reported specific dietary patterns with testosterone levels. Two small cross-sectional studies showed that there was no association between vegetarian diets and testosterone levels in men (lines 89-94).
Methods section

1. Please indicate the rationale behind: i) the selection of subject aged above 40 y old; ii) and only men.

REPLY: The reason behind the selection of subject aged above 40 years old were: (1) prospective cohort study based on 462,293 adults in Taiwan reported that the mean age of people who had prolonged kidney disease was 41.8 years old, and (2) the prevalence of CKD started to increase at age 40-44 years old (7.4%) and doubled between ages 55-59 years (13.1%) and 60-64 years (26.1%) in Taiwan [2] (lines 59-61). Therefore, the subjects aged above 40 years old, which is a crucial period for increasing the prevalence of CKD, were selected in the present study.

We selected only men because Taiwanese men had a higher rate of kidney disease than women [2] (lines 82-83), and to our knowledge, no published literatures investigated the effect of dietary patterns on kidney function exclusively in men. Previous studies have investigated this effect in women. Most of the previous studies examining the effects of dietary patterns in subjects with decreased kidney function were conducted in Western countries and mostly in women [3-5] (lines 77-78).

2. Line 100 - 107: in order to help readers to understand the selection process, it is better to sum-up this paragraph in a figure entitled 'subjects selection process'.

REPLY: We have summarized the paragraph into the figure (Figure 1). The text was revised as follows: “After excluding some criteria in Figure 1, a total of 21,376 men were recruited for analysis” (lines 113-114).

3. Line 112: give the details relative to the auto-anthropometers.

REPLY: Body weight and height were measured by an auto-anthropometer (Nakamura KN-5000A, Nakamura, Tokyo, Japan) (lines 118-119).

4. Line 113: please specify the precautions and the conditions relative to the measure of the blood pressure.

REPLY: Subjects were asked to rest for 5 minutes prior to the measurement of blood pressure, and blood pressure was measured twice at 10 min intervals on the right arm in a sitting position by a standardized sphygmomanometer (lines 120-122).
5. Please, inverse the order of the both sentences "Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters" (Line 113-114) and the next one "hypertension was defined …. By a physician" (Line 114-117).

REPLY: We have revised accordingly (lines 125-126).

6. Line 118-122: please specify the apparatus used to quantify all biochemical parameters.

REPLY: Fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), C-reactive protein (CRP), and blood urea nitrogen (BUN) were analyzed (Toshiba C8000 auto-analyzers, Tokyo, Japan) in each subject after fasting for at least 8 hours (lines 127-130).

Serum creatinine levels were analyzed by uncompensated Jaffe method with alkaline picrate kinetic test. The Roche Miditron M semi-automated computer-assisted urinalysis system (Combur-10 test M dipstick, Basel, Switzerland) was used to measure urinary protein (line 135-138).

7. Line 118-122: indicate the used quality control samples and the assay reproducibility of all biochemical parameters.

REPLY: All specimens were analyzed at MJ Health Screening Central Laboratory. The internal and external quality control techniques were performed by the laboratory and the calibrations were supplied by the manufacturer (lines 139-142). Additionally, a cited reference in the text was added in the reference list accordingly (page 22 #18).

8. Line 122: it is unclear if authors quantified free or total testosterone and the used technique (RIA, CLIA …)?

REPLY: Serum testosterone was measured by chemiluminescent microparticle immunoassay (CMIA, Architect System, Abbott, IL, USA). The Architect testosterone assay measures total testosterone (lines 132-134).


REPLY: We have revised accordingly (lines 162-165).
10. Line 148: authors should give more details about the assessment of the physical activity? if they used a validated questionnaire?

REPLY: All subjects completed validated self-reported questionnaire authorized by the MJ Health Institute. The standardized and validated self-administered semi-quantitative food frequency questionnaire with 22 food groups was used to assess dietary habits in the subjects (lines 153-154). In the present study, we used validated and standardized semi-quantitative food frequency questionnaire that has been published elsewhere (lines 394-396). The questions regarding physical activity were the frequency and time for exercise during the past two weeks. The frequency of exercise had five response options ranged from none or rarely until 2 to 3 times per day, while the exercise time had four response options ranged from less than 30 minutes until more than 2 hours. We defined physical activity as yes if the subject engaged in exercises for more than one hour in a week and no if otherwise (lines 165-170).

11. Line 165-169: please explain more the rationale behind the food groups weighing by their respective loading factors. Add a reference if necessary.

REPLY: Factor loadings are equivalent to simple correlation between the food items and the extracted factor or pattern. Higher factor loadings indicate that the food shares more variance with that pattern. The factor score for each pattern was calculated by summing intake of food items or groups weighed by their factor loading, and thus each subject received a factor score for each identified dietary pattern [6] (lines 185-190).

12. Statistical analysis: how did the researchers deal with missing data?

REPLY: We have excluded subjects with missing data on dietary habit and blood biomarkers (Figure 1 in the manuscript). Subjects with missing data on demographic and lifestyle variables (education, marital status, income, smoking, alcohol, sleeping, and physical activity status) were not included for the statistical analysis, but their blood biomarkers and dietary habits data were used to analyze the association between dietary patterns and kidney function.

Results

This section in general is well written

Discussion

1. Discussion is somewhat long and not concise enough. Please add subheadings to make this section easier to read.
REPLY: We have added subheadings in the discussion section.

2. Line 303-312: Please improve the discussion about the association of diet composition (or nutrients intake) or diet quality and testosterone level.

REPLY: We have revised accordingly.

Previous prospective studies have described the association between low caloric diets and testosterone levels. Plasma total testosterone levels have been reported to be significantly increased after 8-week low caloric diet (850-900 kcal/d) in obese nondiabetic men. In a clinical trial of overweight or obese men on low fat and energy-restricted diet (~1600 kcal/d) for 52 weeks, significant improvements in serum testosterone levels were found. Limited published articles have investigated specific dietary patterns with male testosterone levels. A recent cross-sectional study in 125 Taiwanese men reported that higher adherence to a typical Western diet with high-calorie foods was associated with decreased testosterone level by 0.87 ng/mL. However, another study found that men consuming vegetarian diets had no association with serum testosterone levels. Consistently, our study showed there was no association between vege-seafood dietary patterns and testosterone levels (lines 319-330). Additionally, the references cited in the text were also added in the reference list accordingly (page 24 #37, #38).

3. Please try to discuss in depth the physiological links between the fried-processed dietary pattern and testosterone level among subjects affected by CKD.

REPLY: We have added some information about the links between the dietary pattern and testosterone level.

Increases in testosterone levels seem to occur in overweight or obese men following a low-fat or low-calorie diet, presumably due to weight loss and less aromatization of testosterone to estradiol. However, the potential links between diet patterns and testosterone levels are unclear, but this phenomenon might be due to Western diet-related insulin resistance. Insulin stimulates testosterone production, and insulin resistance may impair this process in Leydig cells (lines 330-335). Additionally, the references cited in the text were also added in the reference list accordingly (page 24 #38-#40).
References


