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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>A randomized controlled trial of whole soy diet in place of red/processed meat and high fat dairy products on features of metabolic syndrome in postmenopausal women: Study protocol</th>
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<tr>
<td>AUTHORS</td>
<td>Liu, Zhao-min; Ho, Suzanne; Hao, Yuantao; Chen, Yu-ming; Woo, J; Wong, Samuel; He, Qi-Qiang; Xie, Yao Jie; Tse, LA; Chen, Bailing; Su, Xuefen; Lao, XQ; Wong, Carmen; Chan, Ruth; Ling, Wenhua</td>
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VERSION 1 - REVIEW

REVIEWER
David J A Jenkins
University of Toronto, Canada

I have received funding for research and honorarium from the soy industry & the food industry in general. Please see my ICMJE declaration and my most recent COI (Jenkins DJ et al. Nutr Metab Cardiovasc Dis. 2015 Dec;25(12):1132-9)

REVIEW RETURNED
16-Jun-2016

GENERAL COMMENTS
This trial is worthwhile and the rationale well argued in the introduction. I have no major points to add or concerns since this group of investigators have a track record in this area of research and on which they base their power calculations. My one substantial question is whether the original FDA health claim of 25g of soy protein is sufficient? Might 35g/day plus be more certain to capture the effect? Although I would agree we do not have good dose response data for soy intake and serum cholesterol reduction, 25g soy protein/day may be the ideal.

I was also surprised at the level of meat intakes in Hong Kong in 2009 of 130g/d, presumably providing 22-30 g protein per day (nearer 30g if pork is the meat source). If one aims at allowing complete replacement, even though this goal is unlikely to be achieved by the majority, than one would require more soy protein than 25g/d.

In this respect might it be useful to instruct participants not simply that you wish to increase soy intakes by 25g protein per day but that you wish to reduce/replace meat intake with soy protein foods. Perhaps the way you present the exchange to participants may be important.

Minor points
1) Do you wish to include women with thyroid dysfunction who are on stable doses of thyroxine?

2) Given some concerns that soy may increase iodine requirements is it worth measuring TSH at the start and end of your study.

3) Will you include Tempeh and miso? As part of your soy food
advice, or are these forms of soy not consumed much in Hong Kong?

4) Page 14 will glucose be measured enzymatically.

5) Will your primary outcome be number of metabolic syndrome characteristic that are reduced for a maximum of 5 (waist, BP, glucose, TG, HDL-C)?

6) Page 8 under "participants" it is probably worth repeating at the beginning that these will be "women, aged ….. to … etc."

REVIEWER
Jean-Philippe Drouin-Chartier
Institute on Nutrition and Functional Foods,
Université Laval,
Québec, Canada

Recipient of doctoral scholarships from the Fonds de Recherche du Québec - Santé and from the Canadian Institute of Health Research. Reviewer declares no competing interests related to this protocol.

REVIEW RETURNED
12-Jul-2016

GENERAL COMMENTS
The protocol entitled “A randomized controlled trial of whole soy diet in place of red/processed meat and high fat dairy products on metabolic features in postmenopausal women – study protocol” (bmjopen-2016-012741) by Liu et al. presents an original study design that is likely answer key questions related to health effects of soy consumption. The study will be a 12-month randomized, single-blind, parallel controlled trial among 208 postmenopausal women with high risk of early MetS.

Rationale: Authors should clarify why they aimed to decrease high-fat dairy product consumption in addition to red meat consumption in the whole soy groups. The association between high-fat dairy product consumption and MetS risk is uncertain according to available data.

Study design, study aim and statistical analyses: The study is a 12-month randomized, single-blind, parallel controlled trial. Authors mentioned several times in the protocol that the study aim to examine the effect of whole soy foods in replacement of red or processed meat and high-fat dairy products. However, I believe that the study is mainly designed to compare the effects of a diet rich in whole soy foods and poor in meat/high-fat dairy products vs. a diet poor in whole soy foods and rich in meat/high-fat dairy products. The parallel design does not allow to evaluate the effect of a "replacement" per se. To evaluate a replacement per se, authors should have designed a crossover study. Nonetheless, authors will be able to evaluate the effect of the replacement of red meat/high fat dairy by whole soy foods by evaluating the difference in metabolic features of post- vs. pre-intervention in the soy group. However, this analysis may be inappropriate. Authors should modify study aim and hypothesis statements according to this issue. Please see Bland JM, Altman DG. Best (but oft forgotten) practices: testing for treatment effects in randomized trials by separate analyses of changes from baseline in each group is a misleading approach. Am J Clin Nutr. 2015;102:991-994
The hypothesis statement in the abstract differs from the statement in the methods. In the abstract, authors mentioned “that whole soy substitution diet will notably decrease the risk of MetS”. However, the statement in the methods is more appropriate: whole soy replacement diet will significantly improve metabolic features in postmenopausal women with risk of MetS. Authors should modify the abstract accordingly.

Methods: Authors should clarify why women with MetS are included in the study. The inclusion of women with MetS in addition to women at risk of MetS is not in accordance with study aim and hypothesis. It is also likely to affect study results as well as interpretation of the results.

Sample size planning: Authors should clarify on what outcome was calculated the sample size planning.

Minor comment: Title: Is it possible that the word “syndrome” is missing between “metabolic” and “features”?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
David J A Jenkins
Institution and Country
University of Toronto, Canada
Please state any competing interests or state 'None declared':
I have received funding for research and honorarium from the soy industry & the food industry in general. Please see my ICMJE declaration and my most recent COI (Jenkins DJ et. al. Nutr Metab Cardiovasc Dis. 2015 Dec;25(12):1132-9)

Please leave your comments for the authors below
This trial is worthwhile and the rationale well argued in the introduction. I have no major points to add or concerns since this group of investigators have a track record in this area of research and on which they base their power calculations.
R: We greatly appreciate the Reviewer’s positive comment on our protocol.

My one substantial question is whether the original FDA health claim of 25g of soy protein is sufficient? Might 35g/day plus be more certain to capture the effect? Although I would agree we do not have good dose response data for soy intake and serum cholesterol reduction, 25g soy protein/day may be the ideal.
R: Thanks a lot for the Reviewer’s comments.

The FDA in 1999 approved the health claim that 25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease (1). The amount of soy protein 25 g/d was established as the threshold intake for cholesterol reduction based on the reviewed research (1). In 2006, the AHA suggested that the health claim was not justified because, in their estimation, soy protein lowered LDL cholesterol only by 3% (2). However, several meta-analyses since 2005 reported a larger LDL-c reduction by soy protein by 4-6% (3, 4). A systemic review(5) using a predictive model further indicated that soy consumption had an additional LDL-c reduction effect due to displacement of saturated fat and cholesterol from animal foods. The combined intrinsic and extrinsic effects of soy foods on LDL-c ranged from 7.9 to 10.3%. Furthermore, in addition to soy protein, many other soy components are beneficial to cardiovascular and overall health because of
their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat. This is also the reason why we propose the trial to use whole soy foods, which are minimally processed relative to soy extracts, as a replacement diet.

Soy intake recommendation is based on several considerations: Asian soy intake, clinical and epidemiologic studies assessing the health consequences of soy consumption as well as general principles of dietary practice (6). We agree with the Reviewer’s point of view, current research is lack of a good dose–response relationship between soy protein intake and health outcomes. According to the National Health and Nutrition Survey in China, Chinese adults consume average 70 g/day total protein, and around 40% of that comes from animal sources and 5% from dairy products and 7-10% from soy protein or other legumes (7). The proposed 25g soy protein accounts for around 35% total protein, which have exceeded the soy protein intake of at least 90% of the Japanese and Shanghai (China) populations although this intake level is still within the dietary range (8, 9). In addition, findings from several clinical studies showed that fewer than 25 g/day soy protein can also lower cholesterol (10, 11). Higher dosage than 25g/d may not be dietary practical and may affect the participants’ compliance. The Chinese Dietary Guideline 2015 also suggests 30-50g soy foods consumption per day, equal to 10-18g soy protein. Thus, we think 25g/d soy protein may be an appropriate dosage. According to the Reviewer’s comment, we revised the ‘Rationale of whole soy dosage’ as below (see page 11, last paragraph):

“Soy intake recommendation is based on three considerations: Asian soy intake, clinical and epidemiologic studies assessing the health consequences of soy consumption as well as general principles of dietary practice (6). Existing epidemiologic studies and clinical trials on soy and health indicate the optimal adult soy intake is two to four servings per day(12). The proposed 25g soy protein exceeds the soy protein intake of at least 90% of the Japanese and Shanghai (China) populations but it is still within the dietary range(8, 9). Higher dosage may not be practical and may affect the participants’ compliance.”

I was also surprised at the level of meat intakes in Hong Kong in 2009 of 130g/d, presumably providing 22-30 g protein per day (nearer 30g if pork is the meat source). If one aims at allowing complete replacement, even though this goal is unlikely to be achieved by the majority, than one would require more soy protein than 25g/d.

In this respect might it be useful to instruct participants not simply that you wish to increase soy intakes by 25g protein per day but that you wish to reduce/replace meat intake with soy protein foods. Perhaps the way you present the exchange to participants may be important.

R: Thanks for the Reviewers’ advice. We agree with the Reviewer’s comment that the complete replacement is impractical and unlikely to be achieved by the majority. However, according to Hong Kong dietary survey data(13), the habitual soy protein intake was around 7-8 g/d, around one third to one fourth amount of animal protein (22-30g). Our participants have already had a basal soy protein intake around 7-8 g/d(13). In addition, a certain portion of the replacement could be made from full-fat dairy products (around 10% total protein from dairy products). It thus may not be a complete replacement.

As suggested, we will instruct participants to reduce/replace their meat intake with soy foods, not simply increase soy intake. A detailed and practical booklet and DVD on exchange of soy foods for animal foods (red/processed meat and high fat dairy products) will be well-prepared for participants before study initiation.

We thus revised our manuscript in methods as (see page 11 paragraph 2):

“Practical techniques to incorporate of 4 servings of whole soy foods (equivalent to 25g soy protein) into their daily diet and reduce/replace high saturated fat and cholesterol rich animal foods (including red/processed meat and full-fat dairy products) based on their prior 7-day dietary record during run-in;

“as well as a detailed and practical soy food exchange/replacement list applied in daily meal schedules."
Minor points
1) Do you wish to include women with thyroid dysfunction who are on stable doses of thyroxine?
R: We will not include women with thyroid dysfunction since soy intake may increase the risk of developing clinical hypothyroidism in patients with thyroid disease(14). Thanks for the reminding. We have made the revision on the inclusion and exclusion criteria accordingly (see page 9 last paragraph):
“Women will be excluded if they are on use of medications known to affect body weight, lipids and glucose within past 3-month such as hypoglycemic or hypocholesterolemic or weigh reduction agents or hormone therapy; medical history or presence of severe systemic or endocrine diseases such as thyroid disease, stroke, cardiac infarction, severe liver and renal dysfunction, gout; present or history of breast, endometrial or ovarian cancer, abnormal uterine bleeding after menopause; on prescribed or vegetarian diet and known soy allergy.”

2) Given some concerns that soy may increase iodine requirements is it worth measuring TSH at the start and end of your study.
R: Thanks for the suggestion. We have added the TSH measurement in the outcomes in the protocol, please see page 15, the last paragraph:
“4) Serum thyroid stimulating hormone (TSH): Given that soy may increase iodine requirements, serum TSH level will be measured at the baseline and end of the trial. Serum TSH will be measured by a standardized immunoassay.”

3) Will you include Tempeh and miso? As part of your soy food advice, or are these forms of soy not consumed much in Hong Kong?
R: We will not include temphe and miso as the whole soy diet since the fermented soy products are not commonly consumed in Hong Kong and China (15). In addition, these fermented soy products may contain high salt content which may increase the risk of hypertension. Thanks.

4) Page 14 will glucose be measured enzymatically.
R: Thanks. We have added the method of glucose measurement accordingly in the part of Data collection/outcome measures (see page 15).

5) Will your primary outcome be number of metabolic syndrome characteristic that are reduced for a maximum of 5 (waist, BP, glucose, TG, HDL-C)?
R: Thanks for Reviewer’s suggestion. Yes, we have added this outcome to our primary outcome measures (page 15 paragraph 2):
“Number of metabolic syndrome characteristics: The number of metabolic characteristics (a maximum 5 for WC, BP, glucose, TG and HDL-C) will be counted at baseline and final of the trial.”

6) Page 8 under “participants” it is probably worth repeating at the beginning that these will be "women, aged ... to ... etc.”
R: Yes, thanks. Have revised as (see page 9):
“Women aged 45~70y will be recruited...”.

Reviewer 2
Reviewer Name
Jean-Philippe Drouin-Chartier
Institution and Country
Institute on Nutrition and Functional Foods, Université Laval, Québec, Canada
Please state any competing interests or state ‘None declared’:
Recipient of doctoral scholarships from the Fonds de Recherche du Québec - Santé and from the Canadian Institute of Health Research. Reviewer declares no competing interests related to this protocol.
Please leave your comments for the authors below

The protocol entitled “A randomized controlled trial of whole soy diet in place of red/processed meat and high fat dairy products on metabolic features in postmenopausal women – study protocol” (bmjopen-2016-012741) by Liu et al. presents an original study design that is likely answer key questions related to health effects of soy consumption. The study will be a 12-month randomized, single-blind, parallel controlled trial among 208 postmenopausal women with high risk of early MetS.

Rationale: Authors should clarify why they aimed to decrease high-fat dairy product consumption in addition to red meat consumption in the whole soy groups. The association between high-fat dairy product consumption and MetS risk is uncertain according to available data.

R: Thanks a lot for the Reviewers’ comments. We agree with the Reviewer’s point of view that the association of dairy products consumption and MetS risk is still uncertain. However, studies have shown that high intakes of saturated fat and total energy will increase cardio-metabolic risk (16). The major sources of saturated fats are meat and full-fat dairy products. Although the relationship of dairy intakes and MetS is inconclusive, most of studies indicated the low-fat dairy products are beneficial in improving glycemic control and insulin sensitivity, and reducing the risk of diabetes or MetS.

We thus revised the introduction as (page 2 paragraph 2):

“Studies have shown a diet that includes less saturated fats (16), more unsaturated fats (17), dietary fiber (10) and low-fat dairy products (18) will benefit patients with MetS (19). Red meat and full-fat dairy products are among the main sources of saturated fat in diets. Most of epidemiological studies reported that the consumption of low-fat instead of high-fat dairy products are favorably in improving glycemic control (17, 20, 21) and decreasing the risk of MetS (19, 21, 22) and diabetes (23-25).”

Study design, study aim and statistical analyses: The study is a 12-month randomized, single-blind, parallel controlled trial. Authors mentioned several times in the protocol that the study aim to examine the effect of whole soy foods in replacement of red or processed meat and high-fat dairy products. However, I believe that the study is mainly designed to compare the effects of a diet rich in whole soy foods and poor in meat/high-fat dairy products vs. a diet poor in whole soy foods and rich in meat/high-fat dairy products. The parallel design does not allowed to evaluate the effect of a “replacement” per se. To evaluate a replacement per se, authors should have designed a crossover study.

R: Thanks for the Reviewers’ comments. The study is not designed to compare the effects of a diet rich in whole soy foods and poor in meat/high-fat dairy products vs. a diet poor in whole soy foods and rich in meat/high-fat dairy products. As indicated in our protocol (see page 11), participants who are assigned to the control group will remain their usual diet and take only a 5~10 min conventional lifestyle education on MetS by a research staff. In contrast, participants who are allocated to whole soy group will receive an intensive training by a dietitian on replacing whole soy foods for red/process meat or high fat dairy products. A detailed and practical pamphlet and DVD will be prepared for participants on further instructions for the whole soy replacement strategy.

There are a number of published dietary replacement studies used parallel study design (26-29), not cross-over design. In addition, our protocol is a 12-month RCT. It seems impractical to apply a cross-over design due to the increased study period and wash-time period. Although cross-over design has advantages in decreasing confounding variables and requiring fewer participants compared with those of parallel design, the cross-over design may not be optimal for a possible curative treatments. This is because certain participants who are at risk of MetS may have improved metabolic risk factors even recover to normal after one year whole soy replacement diet. In addition, there may be a learning effect due to a long-term modification of dietary habits, which may possibly bias our results.

Nonetheless, authors will be able to evaluate the effect of the replacement of red meat/high fat dairy by whole soy foods by evaluating the difference in metabolic features of post- vs. pre-intervention in the soy group. However, this analysis may be inappropriate. Authors should modify study aim and
hypothesis statements according to this issue. Please see Bland JM, Altman DG. Best (but oft forgotten) practices: testing for treatment effects in randomized trials by separate analyses of changes from baseline in each group is a misleading approach. Am J Clin Nutr. 2015;102:991-994

R: Thanks for the Reviewer’s suggestion. We have revised the statistical analysis as (see page 16 paragraph 1):

“Comparisons of means of outcome measures (MetS components) at 6 and 12 months between groups will be made using both repeated-measures analysis of variance and analysis of covariance (ANCOVA) with baseline data as covariate.”

The hypothesis statement in the abstract differs from the statement in the methods. In the abstract, authors mentioned “that whole soy substitution diet will notably decrease the risk of MetS”. However, the statement in the methods is more appropriate: whole soy replacement diet will significantly improve metabolic features in postmenopausal women with risk of MetS. Authors should modify the abstract accordingly.

R: Thanks, we have revised the abstract accordingly:

“We hypothesize that whole soy substitution diet will notably improve metabolic features in postmenopausal women with risk of MetS or early MetS.”

Methods: Authors should clarify why women with MetS are included in the study. The inclusion of women with MetS in addition to women at risk of MetS is not in accordance with study aim and hypothesis. It is also likely to affect study results as well as interpretation of the results.

R: In our study protocol (the first submitted version), we have included both women at risk of MetS and women of early MetS as our participants. They are consistent in both abstract, introduction and methods.

1) In Abstract: “This will be a 12-month randomized, single-blind, parallel controlled trial among 208 postmenopausal women with high risk or early MetS.”

2) In introduction: “Thus, we propose a 12-month randomized controlled trial (RCT) among Hong Kong postmenopausal women at risk of MetS or early MetS to examine …”

3) In methods: “Participants who meet 2 or more of the following items will be enrolled (i) waist circumference (WC) ≥80 cm; (ii) triglyceride concentration ≥1.7 mmol/l; (iii) HDL-c <50 mg/dl (1.29 mmol/l); (iv) SBP/DBP ≥130/85 mm Hg; (v) fasting glucose ≥5.6 mmol/l.”

We are sorry for the possible confusion we have made in the inclusion criteria. As suggested, we revised the inclusion criteria as: “Participants will be recruited if they are aged 45~70y within 15 years after menopause; women at risk of MetS or early MetS will be identified based on a modified National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) criteria”.

Sample size planning: Authors should clarify on what outcome was calculated the sample size planning.

R: Thanks for the suggestion. As suggested, we have revised the sample size planning as (see page 12 paragraph 2):

“…Based on the change and SD of change of fasting glucose or body weight (the largest ratio of SD/change among above outcomes), 90 subjects per group will yield at least 80% power at 5% level of significance (2-side) to detect a difference in above four metabolic components.”

Minor comment:

Title: Is it possible that the word “syndrome” is missing between “metabolic” and “features”?

R: Thanks. We have revised the ‘metabolic features’ as ‘features of metabolic syndrome’ or ‘features of MetS’ in several sites including the title.

References


| REVIEWER | David J A Jenkins  
University of Toronto, CANADA |
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<td>REVIEW RETURNED</td>
<td>25-Aug-2016</td>
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**GENERAL COMMENTS** All my questions are answered. I still think that 25g Soy Protein may be too little but I understand and accept the authors response.

| REVIEWER | Jean-Philippe Drouin-Chartier  
Institute on Nutrition and Functional Foods,  
Université Laval,  
Québec, Canada |
|---|---|
| Recipient of doctoral scholarships from  
the Fonds de Recherche du Québec - Santé and  
from the Canadian Institute of Health Research.  
Reviewer declares no competing interests related to this protocol. I received speaker honoraria from Dairy Farmers of Canada in 2016. |
| REVIEW RETURNED | 30-Aug-2016 |
I am glad to read that authors agree with the recommendations proposed by the reviewers. I remain concerned about how authors are planning to interpret the results. As mentioned in the statistical analyses section, authors will compare the features of the metabolic syndrome between the two groups after 6 and 12 months. In the experimental group, subjects will exhibit a diet rich in soy foods, low in meat and low in high-fat dairy foods that will be achieved by replacing meat and high-fat dairy by soy-foods at baseline. The control group will likely consume a diet that will be richer in meat and high-fat dairy foods than the experimental group. In this context, statistical analyses that will compare the impact of a diet rich in soy foods/poor in meat and high-fat dairy product (experimental group) to the impact of a regular diet rich in meat, high fat dairy/poor in soy foods on features of the metabolic syndrome. In this context, I am reiterating that the use of the terminology “in place” or “replacement” does not reflect the actual experimental design. Even if many RCTs have been previously published using this terminology, authors should address this concern. Nonetheless, the protocol is of sufficient quality and interest to be published.
Randomised controlled trial of effect of whole soy replacement diet on features of metabolic syndrome in postmenopausal women: study protocol

Zhao-min Liu, Suzanne Ho, Yuan-tao Hao, Yu-ming Chen, Jean Woo, Samuel Yeung-shan Wong, Qiqiang He, Yao Jie Xie, Lap Ah Tse, Bailing Chen, Xue-fen Su, Xiang-qian Lao, Carmen Wong, Ruth Chan and Wen-hua Ling

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