CARDIOVASCULAR AND MENTAL HEALTH BENEFITS OF SOY CONSUMPTION: ROLE OF SOY ISOFLAVONES

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B Med Pharm Biotech (Hons)

A thesis submitted for the degree of Doctor of Philosophy

Discipline of Physiology

University of Adelaide

South Australia

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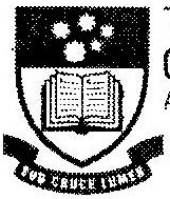
APPENDIX 2- Recruitment Material for Soy Food Intervention

Seeking Volunteers with High Cholesterol

If you're an adult with cholesterol over 5.5 mmol/L (not taking lipid lowering medication), the Nutritional Physiology Research Group seeks your participation in an 18-week dietary intervention trial.

Our study, approved by Adelaide Uni and UniSA, will investigate the palatability of foods containing soy and/or dairy ingredients and their ability to improve cholesterol levels and blood vessel function.

The trial commences in early July. To obtain more information and have your cholesterol checked, please call Alicia Thorp on 8302 2097.



THE UNIVERSITY OF ADELAIDE
AUSTRALIA

UniSA

CRICOS Provider No. 00121B

UNOFSO001001

SOY DAIRY HEALTH STUDY 2004

Recruitment Email:

The Nutritional Physiology Research Group is seeking individuals who have or suspect they have **high cholesterol** ($\geq$ 5.5 mmol/L) and are not receiving any lipid lowering medication for it to participate in a unique 18 week dietary intervention trial.

The study will require you to eat 3 serves of foods a day (range includes plain and flavoured milks, biscuits, snack bars, pasta, custard, pancake mix) that have been specially formulated to contain soy protein, dairy or a combination of the two. All foods will be supplied free of charge and their taste, appearance and texture will be matched so you wont be able to identify their protein content.

It's hoped that eating foods which can combine the taste of dairy with the health benefits of soy will help lower cholesterol as effectively as the choice of soy only products that are currently available to consumers.

If you are interested in participating, please contact Ms Alicia Thorp (8302 2097) or simply reply to this email so a brief screening interview can be arranged.

The anticipated starting date for the trial is mid July. Please find attached a copy of the Volunteer Information Sheet which outlines in more detail the objectives of the study and what your involvement as a participant would entail.

Your involvement in this study would be very much appreciated!

University of South Australia

Media Release

Educating Professionals • Creating and Applying Knowledge • Serving the Community

June 1 2004

Trial to beat cholesterol with soy and dairy combo

Researchers in the joint Adelaide Uni / UniSA Nutritional Physiology Research Group are looking for people to take part in a trial to see if combination low-fat dairy and soy foods will have an impact on reducing cholesterol and be more appealing to consumers.

Research Group leader Professor Peter Howe says evidence-based health claims in the US and the UK state that soy foods as part of a diet low in saturated fat may reduce the risk of heart disease.

"But what we know from consumer feedback is that many Australians don't like the taste of soy," Professor Howe said.

"With this trial we are looking at testing a potential solution by developing and trialling more palatable food products that combine low fat dairy foods with soy to see if they have the same health benefits as straight soy foods."

Researchers are looking for healthy participants who have mildly elevated cholesterol which is not being treated with medication.

Once recruited for the study subjects will engage in an 18 week dietary trial where they will eat test foods provided free as part of their normal diet. A range of foods has been specifically designed for the trial and includes plain and flavoured milks, biscuits, custards, snack bars, pasta and pancake mixes.

"In essence people will be put on a planned diet for 6 weeks eating dairy based, soy based or combination soy-dairy foods and at the six week mark they will cross over to an alternate food diet and this will happen again in another six weeks," Professor Howe said.

"By the end of the trial we hope to be able to get a clearer idea of the impact of soy products and the bioavailability of the beneficial components of soy when delivered in combination with dairy ingredients."

People involved in the trial will need to attend the Nutritional Physiology Research Facility at UniSA's City East campus on nine occasions. They will be asked to complete a dietary questionnaire three times during the study and be expected to eat the test foods provided for the 18 weeks of the study. The volunteers will also be asked to give blood and urine samples during the trial and will have be measured weighed and have general checks on blood pressure.

The trial is a collaborative research project between the Universities of South Australia, Adelaide, Wollongong, Western Australia and So Natural Foods.

"Apart from the important scientific questions being addressed, the study offers participants the opportunity to engage in some important personal health education", Prof Howe said. "It should help them beyond the life of the trial to remain informed and conscious about healthy diet and lifestyle decisions. They will also know they have made a worthwhile contribution to research into cardiovascular disease."

If you are interested in volunteering for the trial please telephone Alicia Thorp between 9am and 4 pm on (08) 83022097

Media contact: Michèle Nardelli on (08) 83020966 or 0418823673

Advertising feature

Soy theory put to test

ADELAIDE researchers are looking for people to take part in a trial to see if combined low-fat dairy and soy foods will have an impact on reducing cholesterol and appeal more to consumers.

The trial will be a joint project between Adelaide University and UniSA Nutritional Physiology Research Group.

Group leader Professor Peter Howe said evidence-based health claims in the US and the UK say soy foods, as part of a diet low in saturated fat, may reduce the risk of heart disease.

"But what we know from consumer feedback is that many Australians don't like the taste of soy," he said.

"With this trial, we are looking at testing a potential solution by developing and trialling more palatable food products that combine low-fat dairy foods with soy to see if they have the same health benefits as straight soy foods."

Researchers are looking for healthy participants who have mildly elevated cholesterol which is not being treated with medication.

Those recruited for the study will engage in an 18-week dietary trial where they will eat test foods provided free as part of their normal diet.

A range of foods designed for the trial includes plain and flavoured milks, biscuits, custards, snack bars, pasta and pancakes.

"In essence, people will be put on a planned diet for six weeks eating dairy-based, soy-based or combination, soy-dairy foods," Professor Howe said.

"And at the six-week mark, they will cross over to an alternate-food diet and this will happen again in another six weeks."

Professor Howe said by the end of the trial, the group hoped to get a clearer idea of the impact of soy products and the bio-availability of the beneficial components of soy when delivered in combination with dairy ingredients.

People involved in the trial will need to attend the Nutritional Physiology Research Facility at UniSA's City East campus nine times. They will be asked to complete a dietary questionnaire three times and are expected to eat the foods provided for the 18 weeks of the study.

Details: Alicia Thorp from 9am to 4pm on 8302 2097.

Nutritional Physiology Research Group

University of Adelaide & University of South Australia

Volunteers needed with High Cholesterol!!!

SOY DAIRY STUDY 2004

The Nutritional Physiology Research Group is conducting an 18-week dietary intervention trial to see whether eating foods combining soy with dairy can lower blood cholesterol.

If you know (or suspect) that your cholesterol is high (≥5.5 mmol/L) and is not being treated with drugs, we would like to hear from you!

To register for the study or obtain more information please contact Ms. Alicia Thorp on 8302 2097 or via email: alicia.thorp@unisa.edu.au

Soy Dairy Study
PH: 8302 2097 (9-4pm)
Email:alicia.thorp@unisa.edu

Nutritional Physiology Research Group

University of Adelaide and University of South Australia

Ms Alicia Thorp
Nutritional Physiology Research Facility
Room BJ1-61, Bonython-Jubilee Building
School of Health Sciences
University of South Australia
Adelaide, SA 5000
Ph: 8302 2097

June 1st, 2004

Dear

Thank you for expressing interest in the Soy Health Study being conducted by the Nutritional Physiology Research Group (a joint initiative of the University of South Australia and Adelaide University)

We are currently recruiting individuals who have elevated cholesterol levels ($\geq$ 5.5 mmol/L) and are not taking any lipid lowering medication, to participate in an 18 week dietary intervention trial commencing in early July. The study, which is being supported by So Natural and Freedom Foods, will be looking specifically at the cholesterol lowering effects associated with the consumption of foods containing soy and whether combining soy together with dairy, to make more palatable foods, provides comparable health benefits. Throughout the study period, participants will be asked to consume a variety of milks, dessert slices, biscuits, pasta and custards on a daily basis that contain soy, dairy or a combination of the two. All test foods will be supplied at no cost and will have an equivalent taste, texture and appearance to one another so you will not know whether they contain soy.

Please find enclosed an information sheet which explains in more detail the objectives and design of the study including the different assessments that will be performed.

If, after reading the information, you feel that you would be interested in participating, I would invite you to complete the attached diet and lifestyle questionnaire and return it in the enclosed reply paid envelope so that a screening interview can be arranged at your convenience.

If you have any questions or concerns regarding this study please don't hesitate to contact me on 8302 2097 as I will be more than happy to discuss them with you.

Yours sincerely,

Ms. Alicia Thorp (PhD Candidate)
for Prof Peter Howe
Head, Nutritional Physiology Research Group

Nutritional Physiology Research Group

University of Adelaide and University of South Australia

DIET AND LIFESTYLE QUESTIONNAIRE

Development and nutritional evaluation of novel soy based foods

In this questionnaire general questions are asked about your health, diet and some background information about yourself. All information will be kept strictly confidential. If you have any concerns about the questions in this form or have difficulty in answering any questions please do not hesitate to contact Ms. Alicia Thorp (8302 1822)

1. Title: Dr Mrs Ms Miss Mr **Date:** _____

2. Full name: _____

3. Address: _____

_____

4. Telephone No : **Home:** _____ **(Please tick best number to contact you on)**

Work: _____

Mobile: _____

5. Email: _____

6. Date of birth:_____day_____month_____year **7. Age:**_____

PLEASE COMPLETE IF KNOWN:

Weight:_____kg **Height:**_____cm **BMI:**_____

Total Cholesterol (if known) :_____ **Date last taken:** _____

PLEASE COMPLETE THE FOLLOWING DETAILS :

Doctors Name: _____

Name of Practice: _____

Address: _____

_____

Post Code: _____ **Telephone:** _____

Would you be happy for us to notify your doctor of your involvement in this study and if necessary contact them?

Yes ☐ **No** ☐

9. Have you had, or do you have :	Yes	No	Not sure
Arrhythmia	☐	☐	☐
High blood pressure	☐	☐	☐
Angina	☐	☐	☐
Heart attack	☐	☐	☐
Stroke	☐	☐	☐
Kidney Disease	☐	☐	☐
High cholesterol (>5.5mmol/L)	☐	☐	☐
High triglycerides (>2mmol/L)	☐	☐	☐
Type 1 or 2 Diabetes Mellitus (diagnosed)	☐	☐	☐
Gestational diabetes (if female)	☐	☐	☐
Peripheral vascular disease	☐	☐	☐
Cardiac valve abnormality	☐	☐	☐
Gastrointestinal disorder	☐	☐	☐

Any other medical condition (Please describe):

_____

_____

Are you undergoing treatment for any of the above conditions? Yes ☐ No ☐

If yes, please give details (and medication , if any):

_____

_____

10. Do you have a family history of :	Yes	No	Not Sure
Obesity	☐	☐	☐
Heart disease	☐	☐	☐
Stroke	☐	☐	☐
High blood pressure	☐	☐	☐
Diabetes (Type 1 or 2)	☐	☐	☐
Hyperlipidemia (high cholesterol)	☐	☐	☐
Kidney Disease	☐	☐	☐

11. Do you have a transferable blood borne virus such as HIV, Hepatitis A, B or C?

Yes ☐ No ☐

12. Have you ever been a smoker ?

Yes ☐ No ☐ (Go to Q 13)

If yes, how long ago did you give up:_____

OR

If you **currently still smoke** how many cigarettes approx. would you smoke in a day?_____

How many years have you been a smoker? _____

13. Do you take any medication regularly ?

Eg: do you regularly use lipid and/or blood pressure lowering drugs, drugs for diabetes, phosphodiesterase inhibitors eg. Viagra, Levitra etc?

Yes ☐ No ☐ (Go to Q14)

If yes, please give details:_____

_____

_____

14. Do you regularly take vitamin, mineral or other dietary supplements?

Eg. Evening primrose oil, fish oil capsules, vitamin C etc.

Yes ☐ No ☐ (Go to Q 15)

If yes, please give details of dose and frequency of consumption: _____

_____

_____

15. Do you drink alcohol?

Yes ☐ No ☐ (Go to Q 16)

If yes, what type (eg. red wine, beer, spirit)?

_____

Estimated average weekly consumption:

Less than 7 standard drinks ☐ 15-28 standard drinks ☐

8-14 standard drinks ☐ More than 28 standard drinks ☐

16. Do you exercise ?

Yes ☐ (Go to Q17) No ☐ (Go to Q18)

17. What sort of exercise do you do each week?

Please list type, how often, duration and intensity: _____

_____

_____

18. Do you eat soy products regularly ?

Yes ☐ No ☐ (Go to Q 19)

If yes, what type and approx. amount (eg, tofu, soy milk, soy powder, soy yoghurt, soybeans):

_____

_____

How often?

Daily ☐ A few days a week ☐

Once a week ☐ Occasionally ☐

Rarely or never ☐

If no, would you be prepared to consume soy products daily during the study period?

Yes ☐ No ☐

19. Do you eat dairy products regularly ?

Yes ☐ No ☐ (Go to Q 20)

If yes, what type and approx. amount (ie. milk, cheese, yoghurt, ice- cream)

N.B. Please state if full cream or low-fat (skim)

_____

_____

How often?

Daily ☐ A few days a week ☐

Once a week ☐ Occasionally ☐

Rarely or never ☐

20. Would you be prepared to consume low-fat dairy products daily during the study period?

Yes ☐ No ☐

21. Have you ever been a blood donor?

Yes ☐ No ☐

22. Do you have difficulty with blood taking?

Yes ☐ No ☐

If yes, please explain:

[eg. poor surface veins, fear of needles, thin- walled veins that tend to collapse]

_____

_____

If **FEMALE** please answer **Questions 23-26.** If **MALE** please go to **Question 27.**

23. Would you consider yourself to be postmenopausal?

Yes ☐ No ☐

24. Are you, or do you suspect that you may be pregnant?

Yes ☐ No ☐

25. Are you currently on hormone replacement therapy (HRT)? (eg. Oral oestrogen- Premarin, oestrogen patch)

Yes ☐ No ☐

26. Have you ever taken HRT?

Yes ☐ No ☐

If so, please indicate when you last used HRT (please tick)?

☐ less than a month ago ☐ 6-8 months ago ☐ more than 12 months ago

☐ 3-6 months ago ☐ 8-12 months ago

27. Would you be able to attend the Nutritional Physiology Research Clinic on 9 occasions?

Yes ☐ No ☐

29. Would you be able to attend the clinic for ½- 1.5 hour long visits between 7.00 am- 12 pm?

Yes ☐ No ☐

Thank you for your co-operation. We will be selecting a study group of approximately 50 people. Please do not be offended if you are not chosen, selection into the study is based on a set list of criteria that you may have not met for whatever reason. We will be notifying everyone in due course, whether they have been selected or not. Those selected will then be asked to attend The University of South Australia City Easy Campus (Bonython Jubilee Building)) to take part in the study.

Nutritional Physiology Research Group

University of Adelaide and University of South Australia

Nutritional Physiology Research Facility
Room BJ1-61, Bonython-Jubilee Building
School of Health Sciences
University of South Australia
Adelaide, SA 5000
Ph: 8302 2097

VOLUNTEER INFORMATION SHEET

Development and nutritional evaluation of novel soy based foods

Thank you for expressing interest in our study looking at the potential health benefits of eating a combination of soy and dairy foods. Foods containing soy ingredients are thought to offer a wide range of health benefits including being able to lower cholesterol levels, but not everyone likes the taste of soy. By combining soy with dairy to make more palatable foods, its possible similar health benefits will occur that consumption of soy alone has demonstrated. It is therefore the purpose of this study to assess if consumption of soy/dairy foods offer the same health benefits that consumption of soy alone does.

Research Plan

The study requires 45 individuals, to consume a variety of test foods (flavoured milks, biscuits, slices/dessert bars, pasta etc) supplied by our sponsor, So Natural. The study, which will run for a total of 18 weeks, will be conducted at the Nutritional Physiology Research Facility located at the University of South Australia (City East Campus) on Frome Road.

You will be assessed for your suitability to take part by completing a health and lifestyle questionnaire (which will require you to divulge information regarding personal medical conditions) and attending a brief screening visit where your height, weight, blood pressure and a small, fasting blood sample will be taken. If you are found to have mildly elevated cholesterol ($\geq$5.5mmol/L) which is not being treated with drugs and are considered to be otherwise healthy, you will be eligible to participate. If you do not meet this criteria and/or are on a diet high in soy or plant sterol enriched margarines, are unable to consume the test foods (ie. soy, lactose, cow milk protein intolerant), unable to comply with study protocol, receiving Hormone Replacement Therapy, taking phosphodiesterase inhibitors eg. Viagra, Levitra and/or suffer from a blood borne virus eg. Active Hepatitis C, Hepatitis B infection or HIV infection) you will be deemed ineligible for the study.

Once recruited, you will then be allocated at random (by chance) to eat either the dairy based foods, the soy based foods, or the combination of soy/dairy foods for a period of 6 weeks. After this time you will crossover to one of the alternate food diets for another 6 weeks and then change over again to the last alternative foods for the remaining 6 weeks.

At the very beginning of the study and at the end of each 6 week diet period (i.e. at 4 points in the study: 0, 6, 12, 18 weeks), you will need to attend the clinic on two consecutive days. During these visits you will need to be fasted (i.e. no food or drink after 10pm the night before) and a blood sample will be taken (approximately two tablespoons). In addition, your height (first visit only) and weight will be measured as well as your blood pressure. Non-invasive tests that assess the elasticity of the arteries and their ability to dilate from induced blood flow will also be performed.

Assessment of arterial compliance (elasticity) is a simple, non-invasive test performed lying down that causes no discomfort. A small sensor is placed over the pulse in the wrist and a computer (which is linked to the sensor) uses the pulse signal to calculate your arterial compliance. Blood pressure will also be measured at this time. To assess arterial dilatation, you will be asked to lie on your back while an ultrasound probe takes an image of the large artery in your upper right arm (brachial artery). A blood pressure cuff will be placed around the right forearm and inflated to a high pressure for 5 minutes. It will then be released and a second image of the artery will be taken to measure the change in the artery's diameter in response to the increased flow of blood to the forearm. You will then be asked to place half a nitroglycerine tablet (300µg) underneath your tongue for 3 minutes. Nitroglycerine causes blood vessels to dilate, and an image of the artery will be recorded by the probe to measure any change that occurs to the artery's diameter.

You will also be asked to collect your urine for 24 hours prior to the first of the two scheduled consecutive visits (i.e. 6, 12 and 18 weeks). A dietary questionnaire will also be administered during each of the 3 dietary phases (i.e. 0-6, 6-12 and 12-18 weeks).

We will supply all of the test foods, both soy and dairy, and the combination of soy/dairy that you will need for the study. The study is scheduled to commence in July and will be completed before Christmas. You will be asked to substitute the test foods for your normal foods. For example, soy/dairy milk will be substituted for normal milk and a snack bar will be substituted for another snack, like biscuits or muesli bar.

Potential Risks and Benefits

Nitroglycerin is a non-prescription drug, which can be purchased over the counter at any pharmacy. It is commonly used to prevent or relieve chest pain and works by relaxing the blood vessels that feed the heart, so blood flow and oxygen supply to it is increased. Like any drug, there may be some potential side effects that occur from taking the nitroglycerin, with the most common complaint being a headache in 60% of people. Other reported effects are a rash, dizziness, upset stomach, flushing (feeling of warmth), blurred vision, dry mouth, chest pain and fainting. It is also advisable you do not drive a car, drink alcohol or operate heavy machinery a couple of hours after taking the nitroglycerin.

As you will only be taking half the recommended minimum dose, you are unlikely to experience any side effects from taking the drug.

During the study, only personal who have been trained in the technique of venipuncture will collect blood samples. However in order for you to make an informed decision, the risks associated with venipuncture are set out below.

- **Infection-** although all of the needles will be sterile and all reasonable precautions will be taken; in any situation involving penetration of the skin these is a slight risk of infection.
- **Bruising-** it is possible you may experience slight bruising around the area where the needle was inserted. This is nothing to worry about as any such bruising should clear up within a few days.

As a precaution, blood thinning agents such as aspirin and warfarin, should not be taken 3 days prior to giving your blood sample.

In terms of your general health and weight management over the study period, soy foods have been a major component of traditional Asian diets and present no health risks to adults. The dairy products to be consumed will have low saturated fat content to match that of the soy products and should not adversely affect cholesterol levels. The soy/dairy combination foods will not have a greater fat content than either the soy alone or dairy alone foods.

As a participant in this study, you will have an opportunity to try high quality soy/dairy foods provided by the sponsor before those in the general community and will be privy to regular cardiovascular risk assessments not normally available through your local doctor. You will also be informed of the overall outcomes of the study as well as receiving a summary of your individual assessments.

Confidentiality

Details of your participation in this study will remain confidential at all times. Once enrolled, you will be identified on records by a coded number and your information will be filed in hard copy and electronic format in a locked data storage facility at the University of South Australia,(School of Health Sciences) for a period of seven years. While the overall outcomes of the study will be reported to the sponsor and submitted for publication in scientific journals, at no stage will your identity be disclosed. At the end of the intervention period you will receive a copy of your personal results from the tests that will be performed and the corresponding treatment you where on during the three dietary periods.

In summary, you would be asked to:-

- Attend a research clinic at the University on 9 occasions
- Provide information about your diet by completing the dietary questionnaire on 3 occasions.
- Eat the test foods provided for 18 weeks
- Provide a blood sample after an overnight fast on 9 separate occasions
- Provide 24 hour urine collections on three separate occasions
- Undergo other laboratory measures including height (on the first occasion), weight, arterial compliance, arterial dilatation and blood pressure.

Your participation in the study is entirely voluntary and you would be free to refuse to participate or withdraw at any time. If you would like to participate or have any enquiries about the research, please contact either of the following:

Ms. Alicia Thorp
PhD Candidate in Nutritional Physiology
University of Adelaide
Phone: 8302 2097
Email: alicia.thorp@unisa.edu.au

Professor Peter Howe
Professorial Research Fellow in Nutritional Physiology
University of Adelaide & University of South Australia
Phone: 8303 4157
Email: peter.howe@adelaide.edu.au

If you, or any member of your family, would like to discuss any ethical aspects of the study or the rights of participants with a person not directly involved in its conduct, please feel free to contact:

Ms Vicki Allen
Executive Officer of the University of South Australia Human Research Ethics Committee
Phone: 8302 3118
Email: vicki.allen@unisa.edu.au