The hypocholesterolaemic Effect of chronic Soy Consumption may be linked to Equol

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Introduction

Cardiovascular disease is the biggest killer in the Western world (NHF 2001). Diet plays a key role in determining plasma lipid levels that can be a major risk factor for CVD when elevated. It is well accepted that an increased intake of saturated fat increases circulating low-density lipoprotein (LDL) and that replacing saturated fat with either monounsaturated or polyunsaturated fat decreases LDL cholesterol.

Other dietary factors that are beneficial in terms of lipid lowering include the consumption of soy. Numerous studies purport the cholesterol lowering benefit of soy. A meta-analysis conducted by Anderson et al (1995) of 38 clinical trials found significant reductions in total cholesterol (9.3%), LDL cholesterol (12.9%) and plasma triglycerides (10.5%) as a result of soy supplementation. These findings were recognised in 1999 with approval by the US Food and Drug Administration of a specific health claim for soy protein. It states that "25 gof soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of (Name of food) supplies ___ grams of soy protein" (Stein 2000).

In Australia, the food industry responded to the health benefits of soy with the introduction of a wide range of soy and linseed breads to the market place. We assessed the potential health benefits from consuming soy and linseed based foods in 20 mildly hypercholesterolaemic ,postmenopausal women in an eight week intervention (Ridges et al., 2001). The study participants were asked to consume soy and linseed enriched foods including 2 slices of bread, a muffin, a muesli bar, an oatcake and 15 gcanola margarine and 5 g canola oil which provided approximately 45 mg isoflavones, 32 mg lignan and 6 g α-linolenic acid (LNA) per day. Significant decreases in concentrations of plasma total cholesterol (10%), LDL-cholesterol (12.5%) and non-HDL cholesterol (12%) were observed within 3 weeks of the intervention. The decreases in total and non-HDL cholesterol were still significant after 8 weeks of intervention (5% and 6.5% respectively). These reductions in cholesterol were associated with significant increases in urinary isoflavones. This study suggests that regular consumption of soy and linseed foods may improve plasma cholesterol in people with mild hypercholesterolaemia (Ridges et al., 2001).

However, there is controversy in the literature as to identity of the active component

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of soy that mediates the cholesterol lowering benefit. It is still unclear whether soy protein or the soy isoflavones, genistein and daidzein, or a combination of soy protein and isoflavones are responsible for the lipid lowering benefit.

Recently, new evidence has emerged which demonstrates that the health benefits are associated with equol, a metabolite of daidzein. For example, in women, there appears to be a correlation between the ability to produce equol and a steroid hormone profile associated with the lower risk of breast cancer (Duncan et al., 2000). In addition, premenopausal female equol producers have a greater lengthening of the oestrogen dependent follicular phase in their menstrual cycle as a result of soy supplementation than women not producing equol (Lampe et al 2001).

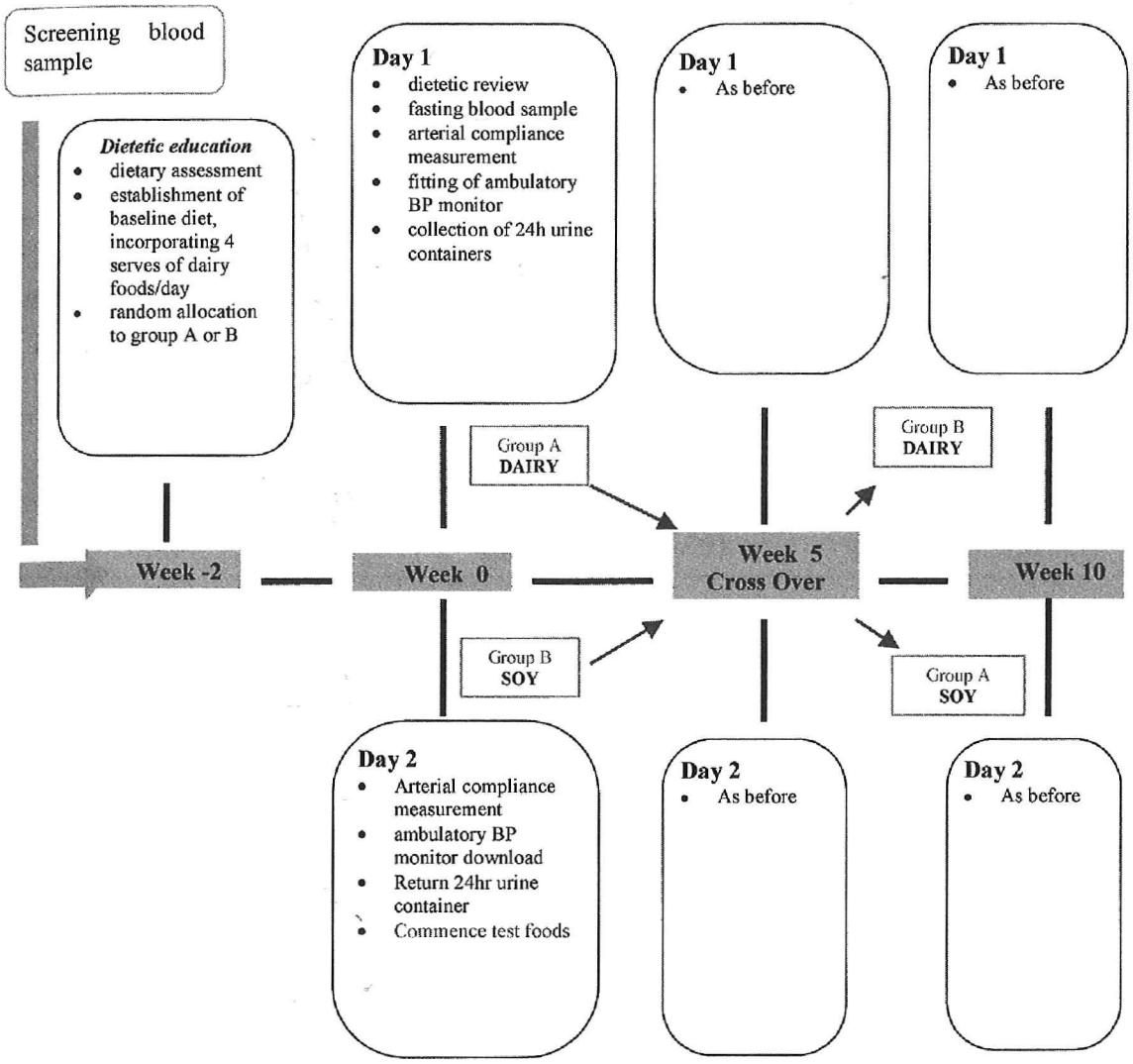
Therefore, the aim of the present study was to investigate the potential cardiovascular benefits of regularly consuming foods made with whole soy bean extracts containing soy protein, soy isoflavones and polyunsaturated fatty acids including α-linolenic acid (LNA) for optimal health in people with either mild hypercholesterolaemia or slightly elevated blood pressure. With consideration of the potential for equol to contribute to cholesterol lowering, a retrospective analysis was conducted to differentiate between equol positive and equol negative subjects with regard to the cardiovascular effects of whole soy consumption.

Methods

The study design was a randomised placebo controlled cross over intervention trial. Figure 1 illustrates that after a two week run-in phase, where 4 serves per day of dairy milk/yoghurt were incorporated into their diets and soy based foods were not consumed, the study participants were randomly assigned to either continue the dairy phase for 5 week or commence on the soy phase for 5 weeks. At 5 weeks of intervention, the study participants swapped to the other dietary phase, ie dairy to soy or soy to dairy for the remainder 5 weeks. Eligible subjects were men and post-menopausal women who had either a total plasma cholesterol level greater than 5.5 mmol/L, or mildly elevated blood pressure (>140/90 mmHg) and were not taking medication for either condition.

Clinic visits were conducted on two consecutive days at 0, 5 and 10 weeks. On day 1 of each clinic visit dietary assessments, fasting blood sample, arterial compliance, fitting of the 24 hour ambulatory blood pressure monitors, and subjects collected their urine containers. On day 2 of each clinic visit, a second fasted blood sample was taken, arterial compliance, downloading of the 24 hour ambulatory blood pressure assessment and subjects returned their 24 hour urine containers and the subjects commenced the test foods. These measurements were repeated at week 5 and 10 of intervention.

Figure 1 - Study design



The composition of the food products are shown in Table 1. Four serves per day (1000 ml or 1000 g) will provide at least 30 g of soy protein and 80 mg of isoflavones per day.

To assess the study participants diets, a diet history interview was conducted by a dietitian (Tapsell et al., 1999). Urinary and plasma isoflavones were assessed using high performance liquid chromatography with electrochemical detection based on the method by Gamache and Acworth (1998). Plasma fatty acids were measured using direct transesterification method of Lepage and Roy (1986). Plasma lipids were analysed using commercially available kits on an autoanalyser. HDL-cholesterol was measured using the method by Warnick et al (1982) and LDL-cholesterol was calculated using the Friedewald calculation (Friedewald et al 1972). Arterial compliance and supine blood pressure were assessed using the CR-2000 cardiovascular profiler instrument (HDI, Eagan, MN, USA). 24hour ambulatory blood pressure was measured using monitors from Spacelabs Medical Inc. A repeated measures

of ANOVA was used to test for effects of diet order. Paired t-tests were used to determine the effect of soy versus dairy on lipid levels.

Table 1 *- Composition of Soy milk and Soy yoghurt*

	Calci-forte Soy milk (100ml)	*So Natural Soy yoghurt (100g)*
Energy (kJ)	287	393
Soy Protein (g)	3.1	4.0
Carbohydrate (g)	7.5	15.0
Fat - total (g)	2.9	2.0
- saturated (g)	0.4	0.3
- monounsaturated (g)	1.3	0.7
- polyunsaturated (g)	1.2	1.0
- omega-3 as ALA (mg)	240	200
Isoflavones (mg)	8.8	9.0

Results

There were 13 male and 10 female subjects that completed the study. On average they were 54 years old, weighed 76 kg and had a body mass index of 26. The 23 study participants' baseline plasma total cholesterol was 6 mmol/L, LDL-cholesterol was 4.13 mmol/L, HDL-cholesterol was 1.28 mmol/L and plasma triglycerides were 1.30 mmol/L. Their clinic systolic and diastolic blood pressure was 132 and 77 mmHg, respectively.

There was good compliance to the test foods throughout the intervention as assessed by dietary intakes, plasma fatty acids and urinary and plasma isoflavones. The dietary intake data showed that after whole soy bean milk and yoghurt consumption, the polyunsaturated fatty acid intakes doubled from approximately 15% to 30% of total fat, with a concomitant decrease in saturated fat (from 44% to 37% of total fat) and monounsaturated fat (from 42% to 37% of total fat). There were no other significant changes in the diet as a result of soy consumption.

Plasma fatty acids reflected the dietary changes seen. There were significant increases in linoleic acid (from 25% to 29% of total fatty acids), LNA (from 0.6% to 0.9% of total fatty acids) as a result of soy supplementation with concomitant significant decreases in oleic acid (from 22% to 20% of total fatty acids) and palmitic

acid (from 27% to 24% of total fatty acids).

Urinary isoflavone analysis demonstrated a 5 to 8 fold increase in genistein and daidzein after soy consumption compared with either baseline or the dairy phase. This suggests good compliance to the test foods during the soy phase of the study. Equol was detected in the urine or plasma in eight out of the twenty-three study participants and these eight participants were defined as equol positive whilst the remaining fifteen study participants were classed as equol negative.

Table 2 - Plasma lipids at baseline and after the dairy and soy interventions

	Total cholesterol (mmol/L)	*LDL-cholesterol (mmol/L)*	*HDL-cholesterol (mmol/L)*	*LDL: HDL ratio*	*Plasma triglycerides (mmol/L)*
Whole Group Baseline (n = 23)	6.00 (0.25)	4.13 (0.24)	1.28 (0.09)	3.6 (0.3)	1.30 (0.14)
Whole Group Dairy (n = 23)	5.83 (0.22)	3.98 (0.21)	1.28 (0.07)	3.4 (0.3)	1.25 (0.13)
Whole group Soy (n = 23)	5.83 (0.21)	3.92 (0.21)	1.35 (0.07)	3.2 (0.3)	1.22 (0.13)
Equol Positive Baseline (n = 8)	6.26 (0.37)	4.49 (0.93)	1.19 (0.13)	4.0 (0.5)	1.27 (0.11)
Equol Positive Dairy (n = 8)	6.07 (0.37)	4.20 (0.36)	1.19 (0.10)	3.7 (0.4)	1.49 (0.16)
Equol Positive Soy (n = 8)	5.53* (0.28)	3.78* (0.28)	1.24 (0.10)	3.2* (0.4)	1.12* (0.09)
Equol Negative Baseline (n = 15)	5.86 (0.33)	3.93 (0.32)	1.33 (0.11)	3.3 (0.4)	1.32 (0.21)
Equol Negative Dairy (n = 15)	5.70 (0.27)	3.85 (0.27)	1.33 (0.09)	3.2 (0.4)	1.12 (0.18)
Equol Negative Soy (n = 15)	5.99 (0.29)	4.00 (0.29)	1.40 (0.10)	3.2 (0.5)	1.28 (0.20)

Values are Mean (SEM).

* The Soy minus Dairy is significantly different from zero ($p < 0.05$).

Table 2 shows the plasma lipids as a result of the intervention. When assessing the differences in total cholesterol, LDL cholesterol, HDL cholesterol, LDL:HDL cholesterol ratio and plasma triglycerides in the whole study population (n = 23), there were no significant changes. The retrospective analysis according to equol status however, revealed significant reductions in total cholesterol (8.5%), LDL cholesterol (10%), LDL:HDL cholesterol ratio (13.5%) and plasma triglyceride levels (21%; Table 2).

There were no significant differences in blood pressure or arterial compliance after soy supplementation in the whole study population, or in subgroups of equol-positive and equol-negative, although diastolic blood pressure tended to be lower after the soy diet. The ambulatory blood pressure assessment suggests this tendency may have predominated at night.

Discussion

A few different indicators, namely dietary assessments, plasma fatty acids, plasma and urinary isoflavones, suggest the study participants' compliance to the soy foods was high. The soy diet supplied at least 25 g soy protein and 80 mg of isoflavones per day, but despite good compliance, there were no cholesterol lowering benefits in the whole study group. However, a meta-analysis of 38 clinical trials (Anderson et al 1995) found significant reductions in total cholesterol (9.3%), LDL cholesterol (12.9%) and plasma triglycerides (10.5%) as a result of soy supplementation. As the extent of cholesterol reduction correlates with the baseline cholesterol levels (Anderson et al 1995), the lack of cholesterol lowering benefit in our study could be explained by starting plasma cholesterol of 6.0 mmol/L. However, according to Anderson et al (1995) study participants with starting cholesterol levels of approximately 6 mmol/L, a 4% reduction in plasma cholesterol is expected. Since the meta-analysis (Anderson et al 1995), negative studies in terms of the effect of soy on cholesterol levels are emerging. For example, Potter et al (1998) found that daily consumption of 40 g of soy protein with either 56 mg or 90 mg isoflavones failed to reduce either total cholesterol or LDL cholesterol in mildly hypercholesterolaemic postmenopausal women.

Our study results add to the controversy in the literature. It is still unclear what is the active component of soy that is responsible for the cholesterol lowering effect, with soy protein and isoflavones being the primary contenders. In a study by Crouse et al (1999), soy protein lowered plasma cholesterol levels and the addition of isoflavones resulted in further reductions of cholesterol. This suggests that isoflavones, in addition to soy protein, may be responsible for the cholesterol reduction. However in studies that assessed the effect of isoflavones without soy protein, there were no lipid lowering effects (Greaves 1999, Nestel 1997, 1999).

Equol, referred to as the forgotten isoflavone by Setchell at the Soy and Health con-

ference, is a metabolite of daidzein produced primarily in the gut by bacterial digestion (Lampe et al 1998). There are emerging health benefits associated with equol. Premenopausal women who are equol excretors have been shown to have plasma hormone profiles that are associated with lowered risk of breast cancer (Duncan et al 2000). At this Soy and Health conference, the results of a 2 year study on the effect of soy on bone loss in postmenopausal women was presented by Lyderking-Olsen et al (2002). This study showed that women who were equol producers (defined as plasma equol levels of 10ng/ml or greater) showed increases of bone mineral density significantly more than the non-equol producers (Lyderking-Olsen et al 2002). Hence we decided to conduct a post-hoc analysis on equol status and lipid lowering benefits in our study.

In our study eight subjects (35%) were identified as being equol-positive (having equol in any of their urine or plasma samples), a similar proportion as found in other studies (Lampe et al 1998). Comparison of the eight equol-positive subjects to the remaining equol-negative subjects (n = 15) revealed that the equol-positive group had significant improvements in their lipid profiles (Table 2). Equol-positive subjects had highly significant reductions in total cholesterol (8.5%), LDL cholesterol (10%), LDL:HDL ratio (13.5%) and plasma triglyceride levels (21%). Hence, our post-hoc analysis suggests that the hypocholesterolaemic effect of soy consumption may be mediated through the production of equol.

In conclusion, consumption of 25 g soy protein and 80 mg isoflavones per day in a group of mildly hypercholesterolaemic subjects resulted in increased levels of plasma and urinary isoflavones, dietary and plasma polyunsaturated fatty acids but did not result in an overall improvement in cardiovascular risk. The retrospective analysis shows that the beneficial effects of soy may be limited to equol positive subjects.

Acknowledgements

We would like to acknowledge the cooperation of the study volunteers and the research team for their contributions to the study: Suzanne Johnson (clinic assessments and processing of blood samples), Sharyn Denmeade and Gina Martin (dietetic assessments), Liz Grigonis-Deane, Leisa Ridges and Beata Peter-Przyborowska (plasma lipid and fatty acid assessments), A/Prof Will Price (phytoestrogen analysis) and A/Prof Ken Russell for assistance with the statistical analysis. The financial assistance as well as the supply of dairy and soy milks and yoghurts by So Natural Foods is gratefully acknowledged.

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