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Dietary intake of soybean protein and menstrual cycle length in pre-menopausal Singapore Chinese women

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Abstract

Background: Intake of soybean protein was associated with a reduced risk of breast cancer in a case–control study. It has also been demonstrated to increase menstrual cycle length in an experimental setting.
Objective: To ascertain whether the association of soybean protein intakes with menstrual cycle length persists in an uncontrolled community setting.
Design: Cross-sectional food frequency dietary survey, menstrual cycle survey and prospective collection of menstrual cycle data.
Setting: A hospital clinic and a nursing college.
Subjects: Two hundred menstruating women.
Results: An association ($P = 0.034$) of higher intakes of soybean protein with increased menstrual cycle length, as recorded by self report and by prospectively recording three consecutive cycles, was observed. The risk of menstrual cycle length being greater than the median, when comparing the upper quartile (8.7–35.2 g day^{-1}) of soybean intake and the lowest quartile (0.1–3.3 g day^{-1}) was double, and this approached statistical significance (OR = 2.02, 95% CI = 0.88–4.64 and OR = 1.93, 95% CI = 0.82–4.56 for self-reported cycle length and cycle length as recorded by diary, respectively). In terms of the absolute association with cycle length, subjects in the upper quartile of soybean intake demonstrated a cycle length 1–2 days longer than did subjects in the lowest quartile.
Conclusions: It is likely that the association between dietary intake of soybean protein and length of menstrual cycle prevails in the community setting. This is shown using both self-reported cycle length and cycle length as recorded in a prospective diary.

Keywords
Phyto-oestrogens
Soybean protein
Menstrual cycle length
Breast cancer risk

Dietary intakes of different forms of protein, i.e. animal and plant, have been shown to affect breast cancer risk in retrospective and prospective studies[1–5]. The proportions of intake, as well as the total intake, of the different forms of protein, may have an effect on the overall risk of breast cancer. A high proportion of protein intake from soybean sources has been found to be associated with a significantly lower risk of breast cancer in a case–control study in Singapore[1]. In addition, a prospective study in Japan found a non-significant reduction in risk with daily consumption of soybean paste soup[3]. More recently, both positive[6] and negative[7,8] findings in relation to soybean intakes have resulted from epidemiological studies.

One proposed mechanism whereby the risk might be affected by soybean products is the replacement of relatively active endogenous oestrogens by inactive plant oestrogens, in which soybean protein is relatively rich[9]. Thus, such foods may have an oestrogen suppressive effect. High intakes of soybean products have been shown to reduce oestrogen receptor concentrations in animal experiments[9]. Other animal studies have reported a higher incidence of mammary tumours in the presence of isoflavones[10]. In controlled experiments on human subjects, high soybean protein diets have been shown to increase menstrual cycle length and to bring about various hormonal changes[11]. A study conducted in

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Japan suggests intake of soybean milk can lower serum oestrogen levels[13].

The effect of soybean protein in reducing breast cancer risk, with its possible hormonal mechanism, is of particular interest as it suggests a potential to unite the well established reproductive epidemiology with the often postulated, but still unclear, dietary aetiology. Here we report a study to assess whether the hormonal and ovulatory effects of variation in soybean protein intakes, observed in controlled experiments, are also observed in response to the variation in voluntary diet in the community at large. The motivation for this research is that the protective effect of soybean protein has the potential to explain, at least in part, the dramatic international variation in breast cancer incidence, with very high rates in western Europe and North America, and low rates in eastern Asia[14]. If it is part of the hormonal aetiology of the disease, it should be active in the community as well as in controlled dietary experiments.

The pre-menopausal female population of Singapore, which has relatively high average intakes of soybean protein and reasonable variation around this average, was considered suitable for this study[2]. In this study we assessed soybean protein intakes in a survey sample of Singaporean women, and related these to menstrual cycle length and blood hormone concentrations.

Methods

Subjects and eligibility

The subjects were recruited as volunteers from two sources: a referral clinic at a local hospital ($n = 85$) and from a group studying for a nursing diploma at a polytechnic ($n = 115$). The subjects were attending the hospital clinic for a variety of reasons including general check-ups, pap-smears and fibroids. All subjects were screened before invitation to the study to confirm that they were menstruating regularly and did not have any condition that might affect their menstrual cycle or the level of sex hormones. Thirty of the eligible subjects approached from the hospital clinic refused consent, mainly for fear of pain from giving blood. Exclusions before invitation included current pregnancy, post-natal (3 months) or breast feeding, current oral contraceptive use, use of an intra-uterine device, hysterectomy, having significant medical or endocrine illness including pituitary, adrenal or thyroid disease. Following explanation of the study aims, signed consent was obtained.

It was intended to recruit the subjects from a common source, but the slow rate of accrual in the clinic setting prompted us to find an alternative source.

Investigations

Each subject responded to an interviewer-administered questionnaire that elicited intakes of soybean products, self-reported length and regularity of the menstrual cycle, age at menarche, number of pregnancies and births, use of exogenous hormonal drugs and time in days since last menstrual period. A semi-quantitative food-frequency questionnaire, previously validated for intakes of broad food groups and macronutrients, but not for soybean protein in particular, was used to assess dietary intake of soybean products[1,2].

A blood sample was taken and analysed for concentrations of the following hormones: oestrone (E1), oestradiol (E2), oestriol (E3), luteinizing hormone (LH) and follicle stimulating hormone (FSH). E2 levels were measured on the Immulite analyser using the Immulite Estradiol kit. Intra-assay precision at a mean of 480 (SD 6.9) s.i. units has a CV of 6.3%; inter-assay precision at a mean of 482 (SD 31) s.i. units has a CV of 6.4%. E2 and E3 concentrations were measured using kits developed at the National University Hospital, Singapore. FSH and LH were measured on the Abbott's AxSYM using Abbott's diagnostic kits. For LH, intra-assay precision at a mean of 36.16 (SD 1.21) s.i. units has a CV of 3.3%; inter-assay precision at a mean of 38.36 (SD 2.63) s.i. units has a CV of 6.7%. For FSH, intra-assay precision at a mean of 27.73 (SD 0.99) s.i. units has a CV of 3.6%; inter-assay precision at a mean of 25.59 (SD 1.41) s.i. units has a CV of 5.7%.

Diary charts were completed for three consecutive menstrual cycles following the interview, and returned to the investigators by post. Whether the subject was in follicular phase or luteal phase on the day of blood sample collection was calculated approximately, assuming ovulation was 14 days prior to the date of the start of the first cycle reported in the diary chart.

Statistical considerations

The results of Cassidy suggest that the mean difference between cycle length for a high intake of isoflavonic phyto-estrogens and that for a low intake would be 1.5 days, with a population standard deviation of around 2.2[12]. On this basis, for 80% power for a comparison of two such extreme groups, we would require 33 per group. We envisaged the consumption of soybean products between low and high intake groups in the Singapore population to be around 75% of that in the experimental situation, which would suggest a sample size of 64 per group, a total of 128 women[2]. The actual regression analysis is likely to be more powerful than a dichotomization followed by a *t*-test, although noise introduced by dietary measurement is likely to counteract this. Further, previous studies using dietary instruments have experienced difficulties in compliance; it was estimated that as many as 25% of subjects would be unable to complete the interview. We therefore proposed a sample size of 200 women.

The association between the intake of soybean protein and cycle length was estimated first by simple correlation coefficients, then proportional odds modelling was used to take account of menstrual cycle length being ordered

Table 1 Study group characteristics stratified by source of subjects

	Hospital clinic ($n = 69$)		Nursing college ($n = 115$)	
	Mean	SD	Mean	SD
Age (years)	40.3	6.8	26.8	11.0
Age at menarche (years)	13	1.5	12	1.2
Length of cycle (days)				
Self-reported	28	2.6	29	7.8
Diary	29.2	4.8	30.6	4.5
Soybean protein (g day^{-1})	5.4	4.5	7.8	5.4
% Parous	81		18	
FSH (s.i. units)	10.8	11.9	6.2	6.6
LH (s.i. units)	9.4	11.0	7.5	9.9
Oestrone (s.i. units)	428	216	550	314
Oestradiol (s.i. units)	321	292	309	341
Oestrone (s.i. units)	0.63	0.17	0.71	0.23

but discrete (measured to the nearest day), adjusting for age and subject source[15]. The potential association between soybean protein intakes and measured hormone levels was estimated using correlation coefficients, stratified by whether the blood sample was taken during the follicular or luteal phase of the menstrual cycle.

Results

Following completion of interviews nine subjects were excluded as a result of a clinical diagnosis that would affect a normal menstrual cycle (sub-fertility treatment, mid-cycle bleeding, dysmenorrhoea (four subjects), dysfunctional uterine bleeding, prolonged menses, and menorrhagia). Other exclusions included five subjects who were subsequently found to be receiving drug treatments that would affect levels of hormones (mercilon, norethisterone, danazol, oestriol and nordette), one schizophrenic subject, and one subject who had a hysterectomy soon after her interview. The remaining 184 subjects (69 from the hospital clinic and 115 from the nursing college) were included in all analyses; however, 12 subjects failed to return a complete menstrual diary chart and so could not be used in analyses where diary chart data were used as an endpoint.

Table 1 shows mean values and standard deviations for covariates separately for subjects from the hospital clinic and nursing college. The nursing college subjects are considerably younger than the clinic subjects and this is reflected in differences in cycle length and parity. The overall average soybean protein intake was 6.9 (SD 5.2) g day^{-1} with absolute range 0.1–35.2 g day^{-1} (lower quartile 3.3, median 5.7, and upper quartile 8.7). Mean levels of the measured hormones are also described in Table 1.

Table 2 shows the unadjusted effects of covariates on cycle length. A total soybean protein intake above the median (5.7 g day^{-1}) was significantly associated with a longer menstrual cycle length as measured in the diaries ($P = 0.034$). The test for trend with continuous values of intake was not significant ($P = 0.16$). For self-reported cycle length, soybean protein intakes above the median were associated with an increased risk of longer cycle length and this approached significance ($P = 0.056$). The trend in risk with continuous soybean protein intakes showed a similar result ($P = 0.052$).

The effect of soybean protein intake on cycle length as recorded in the diaries was slightly attenuated when adjusted for age (OR = 1.81, $P = 0.07$), subject source (OR = 1.70, $P = 0.09$) and parity (OR = 1.73, $P = 0.08$).

Table 2 Unadjusted effects on risk of having menstrual cycle length greater than the median, as estimated by odds ratios from logistic regression models

		Self-reported cycle length		Average cycle length from diary	
		n	OR (95% CI)	n	OR (95% CI)
Soybean protein (g day^{-1})	<5.7	92	1	88	1
	≥5.7	92	1.77 (0.99–3.18)	84	1.93 (1.05–3.53)†
Subject source	Hospital clinic	69	1	64	1
	Nursing college	115	1.70 (0.93–3.11)	108	2.31 (1.22–4.37)†
Age at menarche (years)	<13	98	1	90	1
	≥13	86	0.72 (0.40–1.30)	82	0.62 (0.34–1.14)
Age (years)*		184	0.95 (0.93–0.98)†	172	0.94 (0.91–0.96)†
Parous	No	107	1	102	1
	Yes	77	0.56 (0.31–1.02)	70	0.37 (0.19–0.69)†

* Age fitted as a continuous variable.
† $P < 0.05$.

Table 3 Odds ratios for length of cycle associated with quartiles of intakes of soybean protein

	Self-reported cycle length			Average cycle length from diary		
Intake of soybean protein (quartiles) (g day^{-1})	*n*	Mean days	OR (95% CI)*	*n*	Mean days	OR (95% CI)*
<3.3	46	28.2	1	44	29.7	1
3.3–5.6	46	27.3	1.09 (0.48–2.52)	44	29.2	0.82 (0.35–1.94)
5.7–8.6	46	29.1	1.70 (0.74–3.88)	42	30.7	1.59 (0.68–3.73)
≥8.7	46	30.8	2.02 (0.88–4.64)	42	30.9	1.93 (0.82–4.56)
Linear test for trend			$P = 0.052$			$P = 0.16$

* Odds ratios for menstrual cycle length greater than the median, by logistic regression.

Adjusting for age in 5-year age categories[16] and parity simultaneously, gave an OR for the effect of soybean protein intake of 1.84 (95% CI = 0.97–3.50, $P = 0.06$).

Similar results were observed using self-reported cycle length. Odds ratios, for length of cycle greater than the median, associated with quartiles of soybean intake showed a trend in the anticipated direction, but these did not reach statistical significance (Table 3). Again, adjusted for age in 5-year age groups and parity simultaneously produced an OR of 1.73 (95% CI = 0.88–3.37, $P = 0.1$).

Table 4 shows correlation coefficients between hormone levels, cycle length, number of pregnancies and soybean protein intakes stratified by phase. There was a significant positive correlation between oestrone in the luteal phase and soybean protein intakes. Two subjects provided an insufficient volume of blood for a full hormone profile so do not feature in this matrix.

Correlation coefficients for non-hormonal outcomes are described in Table 5 and include all eligible subjects who returned the menstrual diary. A statistically significant correlation between soybean protein intake and self-reported length of cycle was found. Self-reported cycle length also correlated well with cycle length as collected by the diaries.

Discussion

Soybean protein intakes have been observed to reduce the incidence of mammary tumours in animals[9]. Soybean protein is rich in isoflavonic phyto-oestrogens[12], and has been observed to increase menstrual cycle length in controlled human experiments[11]. In addition, high soybean protein intakes were associated with reduced risk of human breast cancer in a case–control study in Singapore[1].

Two caveats should be borne in mind with respect to the results. First, the effects on menstrual cycle of soybean protein intakes are not invariably and unequivocally significant. They should therefore be regarded as strongly suggestive rather than definitive. Secondly, the absolute size of the effect is modest. The results do, however, at

Table 4 Correlation matrix for measured hormones, cycle length, pregnancies and soybean protein intake, stratified by phase of cycle

	E1	E2	E3	FSH	LH	Cycle*	Cycle†	Pregnancy	Soybean
Luteal ($n = 80$)									
Oestrone (E1)	1								
Oestradiol (E2)	0.5346‡	1							
Oestriol (E3)	0.4449‡	0.1575	1						
FSH	0.1755	0.0147	−0.0213	1					
LH	0.3979‡	0.5081‡	0.2305‡	0.4586‡	1				
Cycle length*	−0.0466	−0.0419	0.0387	−0.1654	0.0563	1			
Cycle length†	−0.0480	−0.2041	−0.0190	0.0856	0.0983	0.4772‡	1		
No. pregnancies	0.2485‡	0.1356	0.1936	0.2978‡	0.0853	−0.0935	−0.1866	1	
Soybean protein	0.2221‡	−0.0326	0.0667	0.0526	−0.0262	0.1848	0.0521	−0.0074	1
Follicular ($n = 90$)									
Oestrone (E1)	1								
Oestradiol (E2)	0.6796‡	1							
Oestriol (E3)	0.2437‡	0.0149	1						
FSH	−0.0975	−0.1379	−0.1069	1					
LH	−0.0103	0.0329	−0.1730	0.6500‡	1				
Cycle length*	−0.1786	−0.1519	−0.0436	−0.1711	−0.1267	1			
Cycle length†	0.0254	−0.0840	−0.1819	0.0738	0.0431	0.6155‡	1		
No. pregnancies	−0.0794	0.0245	0.1280	0.1249	0.1186	−0.0125	−0.1588	1	
Soybean protein	0.0853	−0.0403	0.1985	0.0143	0.0422	0.1653	0.1437	−0.1730	1

FSH, follicle stimulating hormone; LH, luteinizing hormone.
* Self-reported cycle length.
† Cycle length from diary cards.
‡ $P < 0.05$.

Table 5 Correlation matrix for cycle length, pregnancies and soybean protein intake ($n = 172$)

	Cycle length*	Cycle length†	No. pregnancies	Soybean protein
Cycle length*	1			
Cycle length†	0.5613‡	1		
No. pregnancies	−0.0461	−0.1706‡	1	
Soybean protein	0.1728‡	0.0949	−0.0941	1

* Self-reported cycle length.
† Cycle length from diary cards.
‡ $P < 0.05$.

least support, complementary to the experimental evidence, that high intakes of soybean protein can influence the menstrual cycle at community level.

The phyto-oestrogens in soybean protein are weak oestrogens, and their effect in animals is likely to be anti-oestrogenic[17]. There is also evidence, approaching statistical significance, that soybean milk lowers serum oestrogen levels in human subjects[13], although this was not observed in our study. This is consistent with increased menstrual cycle length as a result of high intakes and this increase has been demonstrated in experiments on human subjects[11]. Our results lend support to the evidence that variation in soybean protein intakes at a community level correlates with menstrual cycle length and with serum oestrone levels.

Although the reporting of results stratified by phase of cycle was not a primary endpoint, it should be acknowledged that using time to next cycle to determine whether the blood sample was taken in the follicular or luteal phase is a very approximate method. Also, some of the women may have been sampled during anovulatory cycles. With a more exact method of calculating phase, our secondary results, correlations with hormone levels might have been more statistically significant, particularly with respect to the luteal phase. The study population comprised subjects drawn from two sources. However, the major difference was that of age and adjustment for age had little effect on the results.

If we translate a modest increase in cycle length of 1.2 days between the lower and upper quartiles of soybean intake as shown in these data, using cycle length recorded by diary, the effect over a lifetime is approximately 20 fewer cycles. At a mean cycle length of 30.9 days this equates to 2 fewer years of menstruating life for those in the upper quartile of soybean intake.

A reduction in lifetime ovarian activity has been related to reduced breast cancer risk[18]. Other studies have demonstrated a reduced risk of breast cancer with high soybean protein intakes[1]. Our results suggest that dietary intake of soybean protein lengthens menstrual cycle length, possibly through a biological mechanism that modifies ovarian activity. This may have important implications for the modification of breast cancer risk through dietary interventions.

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