

RESEARCH ARTICLE

Effects of Tofu Consumption on Menopause Symptoms and Equol Level [7-Hydroxy-3-(4-Hydroxyphenyl) Chroman]

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ABSTRACT

Background: Improvements in both health and welfare in Indonesia have increased the life expectancy; thus, the number of women who experience menopause period would be increasing. Menopause symptoms resulting from estrogen deficiency often cause problems, such as menstrual cycle changes, hot flushes, dyspareunia, urethritis, incontinence, urgency, frequent micturition, insomnia, or sexual dysfunction. This symptom happened since perimenopause age. Phytoestrogens and their metabolites, equol, binds to estrogen receptors and induce biological effects resembling estrogen. The most important content of phytoestrogens is isoflavones. Isoflavones could be found in foods or drinks that contain soya (soybeans), such as tofu. Equol, a bacterial metabolite, and the only hormonally active daidzein metabolite is a good predictor to determine the degree of menopause symptoms. This was an experimental study with pre and post design.

Aim: The aim of this study was to determine the effect of regular tofu consumption for three months on the symptoms of menopause and excreted human urinary equol level. Subjects were perimenopause women (n = 25) that were interviewed using questionnaire guidance of menopause rating scale (MRS). Urine samples were analyzed using high-performance liquid chromatography (HPLC). Results The result showed that regular tofu consumption significantly affected menopause symptoms and equol level (p value <0.05).

Method: This was an experimental study with pre- and post-design. Subjects were perimenopause women (n = 25) that were interviewed using questionnaire guidance of menopause rating scale (MRS). Urine samples were analyzed using high performance liquid chromatography (HPLC).

Conclusion: Research concluded there was a decrease in the degree of menopause symptoms and increased equol level in perimenopause women after consuming tofu regularly for 3 months.

Clinical significance: Results can be an alternative choice besides hormonal drugs that we used regularly to decrease menopause symptoms.

Keywords: Equol, Isoflavones, Menopause symptoms, Phytoestrogens, Tofu.

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BACKGROUND

Improvements in both health and welfare in Indonesia have increased the life expectancy; thus the number of women who experience menopause period would be increasing.¹ Menopause symptoms resulting from estrogen deficiency often cause problems, such as menstrual cycle changes, hot flushes, dyspareunia, urethritis, incontinence, urgency, frequent micturition, insomnia, or sexual dysfunction.^{2,3}

Phytoestrogens and their metabolites, equol, binds to estrogen receptors and induce biological effects resembling estrogen. The most important content of phytoestrogens is isoflavones. Isoflavones could be found in foods or drinks that contain soya (soybeans), such as tofu. Consumption of tofu containing 50–100 mg of isoflavones daily for 3 months is believed to reduce the symptoms of menopause.⁴

Equol [7-Hydroxy-3-(4-Hydroxyphenyl) Chroman] is the result of isoflavone daidzein metabolism by the intestinal microflora. The absorption of equol is influenced by dietary carbohydrate and fiber. Equol had a chemical structure that was similar to 17- β estradiol. Thus, it could have the estrogenic effect to stimulate gene transcription via. estrogen receptors. Equol levels could be detected in plasma, feces, and urine. Detection of equol in urine was easier and the least invasive.^{5,6}

Some studies suggest that higher level of equol was associated with lower menopause symptoms.⁵ Therefore, the aim of this study was to determine the effect of giving tofu regularly for 3 months on the symptoms of menopause and excreted human equol level in urine.

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METHODS

Tofu

We made the tofu based on the traditional book. The tofu was homemade from 500 gram of soybean, soaked in 3–4 L of previously boiled water for one night. The next day, water was drained out, sediment was obtained then blended for 1–2 minutes until becoming soybean milk. Then filter the milk until the sediment obtained, then boiled at 160°C until forming a solid phase. This solid phase was left to cool down and form tofu. The tofu was divided each 120 g (containing about 75 g of soybean). We served the tofu fried by coconut oil as this method is preferred.

Study Design

This was a categoric-analytic experimental study with pre-post test design from June 2016 to August 2016 in Gynecology-Endocrinology outpatient installation at Dr. Hasan Sadikin Hospital Bandung, regional public health community group at Cileunyi Bandung, Indonesia and Pharmacokinetic Laboratorium, Health Research Unit, Faculty of Medicine, Universitas Padjadjaran Bandung.

Subject Selection and Sample Size

This study used convenience sampling method for the inclusion of the subjects. Inclusion criteria were has stopped menstruating <1 year, not consuming foods containing isoflavones except tofu, accepting the tofu diet programme, and has normal body mass index (BMI) calculated using the metric system from Center of Disease Control according to the Asia Pacific region guidelines. Exclusion criteria are not compliance to tofu diet, has an allergic reaction to tofu and foods-containing tofu, has used antibiotic drugs or hormonal therapy in the past 3 months, vegetarian diet, or alcohol consumption, have chronic diseases in the digestive tract or have a certain dietary restriction such as diabetes mellitus. This study determine the confidence level as 95% ($Z_a = 1,96$) and power test 80% ($Z_b = 0,84$). Placebo control subject was not available due to difficulties searching perimenopause subject and limited fund.

Study Variable and Operational Definition

Independent variable of this study were three pieces of tofu whereas the dependent variable is menopause symptom and level of equol concentration. Other food-containing tofu such as tempeh, soy milk, soy flour, flax seed bread, yogurt could become confounding variable.

Procedure and Data Collection

History taking and urine measurement were conducted at the time of admission and after intervention (3 months later). The questionnaire was collected using menopause rating scale (MRS) and a urine sample was collected and processed by using high-performance liquid chromatography (HPLC). This method is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition. It is a highly improved form of column liquid chromatograph so it much faster, but the same basic principle like other chromatography, separation of a sample into its constituent parts because of the differences in relative affinities of different molecules for the mobile phase and the stationary phase used in the separation. The basic part of the tool is a solvent reservoir, pump, sample injector, column that often referred as microbore column, detector, and data collecting devices so it can extract equol from the urine sample. The first urine sample was taken 1 day before regular tofu consumption (24 hours urine sample collection) and then directly we take the sample to the laboratorium for equol level measurement. The second urine sample was taken 1 day after regular tofu consumption (24 hours urine sample collection) and take the sample to the laboratorium again with the same procedure.

Tofu is administered regularly 1 piece three times a day (morning, afternoon, evening) for 3 months and the subject was instructed to fill the table of diet every day. The weight of each tofu is mentioned above. Before this study conducted, we used semi-quantitative food frequency questionnaire method which then converted into household size and the list of food composition to make sure the subjects had almost similar diet characteristic. On the semi-quantitative food frequency questionnaire, we only ask some questions about the subject food habit weekly, monthly, or yearly. We estimate it by using number spoon or plates that subject eat and then we converted into how many grams subject eat their food based on carbohydrate, protein, fat, or vitamin composition. We did not count it and the only estimate to make sure that there were no far differences food habit between each subject. It was a recall method which the subject might be forgotten some of their food habit.^{7,8}

Data Analysis

Bivariable analysis was intended to discover the relationship between tofu administration on perimenopause symptoms and equol concentration in urine. Normality of numerical data was assessed using Shapiro–Wilks and analyzed by unpaired statistical t-test for normal

distribution and Wilcoxon statistical test for the abnormal distribution. Chi-square statistical test used for categorical data with exact Fisher and Kolmogorov–Smirnov test as the alternative. The result will be considered significant if *p* value ≤0.05. The data calculated by using SPSS version 21.0 for Windows software.

RESULTS

The final subjects of this study were 25 persons since two subjects resigned during the procedure and two subjects did not adhere to a tofu diet. Characteristics of the study subjects are presented in Table 1.

Table 2 revealed the examination result of various variables. The mean of menopause symptoms score before the study was 19.2 (severe symptoms) and mean of menopause symptoms score after the study was 14.3 (moderate symptoms). The mean of equol concentration in blood was 114.9 nmol/L and mean of equol concentration after the study was 262.7 nmol/L.

Table 1: Characteristics of study subjects (n = 25)

Characteristic	Total
<i>Age (years)</i>	
Mean (SD)	48.4 (2.6)
Median	48
Range	44–55
<i>Education</i>	
Primary school	15
Junior high school	6
Senior high school	2
Academic/college	2
<i>Work</i>	
Working	5
Not working	20
<i>Last menstruation (month)</i>	
Mean (SD)	7.7 (4.3)
Median	6
Range	3–12
<i>BMI (Kg/m²)</i>	
Mean	22.1 (0.8)
Median	22.2
Range	20–23.3

Table 3: Comparison analysis of menopause symptoms (MRS score) and equol concentration before and after tofu administration

Variable	Measurement		<i>p</i> value
	Pre	Post	
<i>Equol concentration (nmol/L):</i>			
Median	71.5	259.6	<0.001*
range	8.25–419.74	29.04–639.91	
<i>MRS score:</i>			
Median	20	15	<0.001*
range	8–28	8–24	
Correlation coefficient level of equol concentration with MRS score (<i>r</i>)	-0.821	-0.837	<0.001**

* Wilcoxon test; ** *r* = Spearman rank correlation coefficient

Comparison of measured variables before (pre) and after (post) study is presented in Table 3. A significant change occurred in the degree of menopause symptoms (MRS score) and level of equol concentration between before and after the study. Menopause rating scale has 11 parameters that divided into three big categories: somatic (hot flushes, heart discomfort, sleeping problems, joint and muscular discomfort), psychological (depressive mood, irritability, anxiety, physical and mental exhaustion), and urogenital (sexual problems, bladder problems, dryness of vagina). On this research, we give each parameter a score. Score 0 for no complain, score 1 for mild symptoms, score 2 for moderate symptoms, score 3 for severe symptoms, and 4 for very severe symptoms. After all, we sum all the 11 parameter scores. If the total score range was 0–4 it is no symptom, if the total score range is 5–8 it is mild symptoms, score range 9–16 is moderate symptoms, and score range more than 16 is severe symptoms.⁹ After that, we use statistical analysis to count how significant was the change of the total 11 menopause symptoms parameter based on the menopause rating scale, so we did not do statistical analysis for each parameter. Correlation between the MRS score and level of equol concentration was also detailed in Table 3 which showed a significant correlation between the degree of menopause symptoms (MRS score) with the level of equol concentration. The higher levels of equol, the lower the degree of menopause symptoms.

The effect of routine tofu administration on the degree of menopause symptoms is presented in Table 4. There was a significant association between regular tofu con-

Table 2: The result of examined variable

Variable	Statistical parameters		
	Mean (SD)	Median	Range
Pre-equol concentration (nmol/L)	114.9 (105.2)	71.5	8.25–419.74
Pre-MRS score		20	8–28
Post-Equol concentration (nmol/L)	19.2 (4.4) 262.7 (196.9)	259.6	29.04–639.91
Post-MRS score		15	8–24
		14.3 (14.8)	

Table 4: Effect of daily tofu administration for three months on the degree of menopause symptoms

Degree of menopausal symptoms	Post			Number	<i>p</i> value
	Mild	Moderate	Severe		
Pre					
Mild	1	0	0	1 (4%)	0.003*
Moderate	3	4	0	7 (28%)	
Severe	3	8	6	17 (68%)	
Total	7 (28%)	12 (48%)	6 (24%)	25	

*Chi-square test Mc.Nemar-Bowker

sumption with a lower degree of menopause symptoms. This table show changes on the menopause symptoms based on severity. Before the regular tofu consumption, there were 17 subjects had severe menopause symptoms, 7 subjects had moderate menopause symptoms, and only one subject had mild menopause symptoms. After three months consuming tofu regularly three times a day, there were significant changes for menopause symptoms, 6 subjects with severe menopause symptoms, 12 subjects with moderate menopause symptoms, and 7 subjects with mild menopause symptoms.

DISCUSSION

Based on Table 1, the average of perimenopause women's age was 48.4 years old with a standard deviation of 2.6 years. The median age was 48-year-old, the youngest was 44-year-old and the oldest was 55 years old. Overall, it appears that the average age of perimenopause women without considering the variety of menopause symptoms was almost the same as the previous studies, which was around the age of 49–51 years. The average age of Asian women experiencing menopause was 51-year-old.^{2,10}

Most of the subjects in this study were elementary school graduates (60%). There were 20 subjects who were unemployed or housewives and only 5 subjects has jobs. Education level and occupation factor were independent factors that could affect menopause age among women.¹⁰ Socioeconomic status and higher education level have allegedly delayed the age of menopause.¹⁰ However, in other studies, these factors were inconclusive in affecting the age of menopause.¹¹

Diet was an important component in the study related to its effects on the menopause symptoms and equol level in perimenopause women. Previous studies often correlate equol production with high fiber diet, high carbohydrate diet, regular consumption of soy or low-fat diet.^{12,13} Soy-rich (isoflavones) diet was believed to have effects on equol level which was higher in Asian women.^{5,14} This study did not distinguish between the equol-producing and non-equol-producing diet, but there was an increasing trend in the subjects' equol level in this study.

Previous research was done at Dr Hasan Sadikin General Hospital stated that higher equol level was associated with lower MRS score which showed a milder degree of the symptoms.¹⁵ There was correlation between smoking and dietary intake, especially carbohydrates, fiber with equol-producing groups using soya consumption test.¹⁵

In this study, as listed in Table 3 which was based on the Wilcoxon test, showed that the consumption of 3 pieces of tofu every day on a regular basis for three months could significantly reduce the degree of

menopause symptoms which was shown by changes in median MRS score of 20 to 15. Tofu consumption on a regular basis could also significantly increase the median equol level of 71.5 nmol/L to 259.6 nmol/L. Table 3 also showed the negative correlation between the degree of menopause symptoms and equol level indicated by the significant changes of correlation coefficient value of -0.837 before treatment to -0.821 after treatment. Table 4 showed the degree of menopause symptoms changes in 25 study subjects. The number of subjects which had severe menopause symptoms decreased from 17 subjects (68%) to 6 subjects (24%) subjects, the number of subjects which had moderate menopause symptoms increased from 7 subjects (28%) to 12 subjects (48%), the number of subjects which had mild menopause symptoms increased from 1 subjects (4%) to 7 subjects (28%). It shows a decrease in the severity of menopause symptoms on the subjects because, after the study, there were more subjects with mild and moderate menopause symptoms and fewer subjects with severe menopause symptoms.

The limitations of this study were the direct observation of dietary intake was not performed. The researcher was unable to conduct a direct observation every day because of the time constraints. Diet-based research should be isolated, with a large number of subjects, and in a strict diet, setting to avoid bias from the other dietary components. The researcher was unable to isolate the subjects because of the limited funds to organize the subjects' dietary habit and social conditions.

CONCLUSION

Regular consumption of tofu for 3 months in perimenopause women resulted in increased equol level reduced the menopause symptoms and. Furthermore, there were fewer subjects with severe menopause symptoms after this same regular consumption of tofu. Further studies in menopause women about the effect of certain dietary patterns on the equol production and menopause symptoms with strict supervision should be conducted. It is also necessary to conduct research on the bacterial isolation for synthetic equol formation.

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Ethical Aspects

Urine sample collection does not cause any pain and discomfort. Study obliged to all ethical principles, autonomy, beneficence, nonmaleficence, and justice. Subjects could resign from the study at any time. The study was approved by the Health Research Ethical Committee Faculty of Medicine Padjadjaran University/Dr. Hasan Sadikin Bandung, Indonesia (No. LB 04.01/A05/EC/123/IV/2016).

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