ORIGINAL ARTICLE

Relationship between Lifestyle, Body Mass Index, and Dietary Factors with the Equol Production

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Abstract

Aim: This study was aimed to find the relationship between the lifestyle factor (smoking, physical activity), the body mass index, and the dietary component with the equol-producing phenotype. Our work can be useful as basic data to modify lifestyle and nutrition to improve the metabolism of equol production, which is needed for women's health.

Materials and methods: Data were collected using the interview and the semi-quantitative food frequency questionnaire. Equol was measured in urine and was collected after a 3-day soy challenge to determine equol-producing phenotypes.

Results: The correlation analysis among lifestyle factors, the dietary component, with the equol-producing phenotype was performed. Data show that equol producers accounted in 60.7% of the participants. Smoking was significantly correlated to the equol-producing phenotype ($p = 0.030$; $r = 0.224$). Carbohydrate and dietary fiber were positively correlated with equol-producing phenotypes ($p = 0.011$; $r = 0.202$) and ($p = 0.004$; $r = 0.218$). No significant correlation was found between physical activity, BMI, dietary protein, and fat intake with equol-producing phenotypes ($p = 0.677$; $r = 0.035$), although we observed a lower dietary fat intake in equol producer compared to non-equol producer.

Conclusion: These findings suggest that smoking habit, carbohydrate, and dietary fiber significantly influence equol-producing phenotypes.

Clinical significance: Our study may be useful as basic data to modify lifestyle and nutrition to improve the metabolism of equol production, which is needed for women's health.

Keywords: Body mass index, Dietary factors, Equol production, Nutrition, Physical activity, Smoking.

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Introduction

Equol is a daidzein isoflavones metabolite with the help of intestinal microflora, has a similarity in chemical structure with 17β-estradiol so that it has an estrogenic activity, and is able to stimulate gene transcription via estrogen receptors.[1] There are several factors that are known affecting the metabolism of daidzein into equol, including the specific strain of intestinal bacteria, which is able to metabolize daidzein into equol.[2,3] Lifestyle factors such as smoking, physical activity, and obesity are also known to influence the composition of the microflora in the gastrointestinal tract, which reduce the diversity of the intestinal microflora and reduce the population of bacteria that are able to convert daidzein into equol.[4,5] Dietary components like carbohydrates, fats, protein, and fiber also can determine the bacterial population that can grow predominantly in the gastrointestinal tract. Equol production has correlation with the intake of carbohydrates and high fiber. Carbohydrates and fiber, which are not digested by the small intestine, will undergo a fermentation process that selectively will stimulate the growth and the activity of beneficial intestinal bacteria after entering the colon. Protein and fat intake is known to inhibit the production of equol. They can produce harmful metabolites that are toxic, carcinogenic, and inhibit the growth of beneficial bacteria.[6] There are two phenotypes in the human population, namely the equol-producing and non-equol-producing. In this study, soy milk was given for 3 days, for the substrate additional isoflavones in daily diet, as a challenge test to distinguish the two phenotypes of the subjects. Equol levels can be detected in plasma, feces, and urine. However, equol levels in urine are lower than the levels of equol in plasma; equol detection by using urine is easier and less invasive.

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Materials and Methods

This study was an observational study with a cross-sectional method, analyzing the correlation of lifestyle factors (smoking habits, physical activity), the body mass index, and dietary components with the equol-producing phenotype. Research samples were obtained from the study subjects who met the inclusion criteria, which were reproductive, perimenopausal, and postmenopausal women who were healthy and were voluntary to be the subject on the study. The exclusion criteria were those subjects who did not follow the whole steps of the study, including those who did not drink soy milk >1 day prior to the examination of urinary equol concentration and those who were known based on their history had allergic to soy milk.

The method that was used to differentiate the equol-producing and the non-equol-producing status of the subjects was by giving the subjects 2 cups (250 mL) of soy milk daily in morning and evening in addition to their daily dietary consumption for 3 consecutive days; then on the 3rd day, 24-hour urine samples were collected for urinary equol concentration examination. Urinary equol concentration is stable at room temperature up to 14 days. Equol examination was conducted by using electrospray ionization mass spectrometry (ESI-MS). Equol producing groups were determined if the urinary equol concentration was >20 ng/mL.

Dietary intake of a specific compound was obtained by using a semiquantitative food frequency questionnaire method, so their daily intake of carbohydrates, protein, fat, and fiber could be determined; then the result was analyzed by using Food processor II version 3.

Ethics Approval and Consent to Participate

This study was ethically conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Clearance Committee Review Board, Dr Hasan Sadikin General Hospital No: LB.04.01/A05/EC/120/IV/2015.

Results

This research was conducted from January to June 2015. During the period, 140 subjects met the inclusion criteria. The subjects were reproductive-age, perimenopausal and postmenopausal healthy women. Some were RSHS staffs and group of women from Posbindu Teratai Komplek Bukit Cimindi Raya, Pasirkaliki, Posyandu and Posbindu in Cileunyi area.

Most of the subjects in both groups were in the perimenopausal age (45–55 years). The previous report says that age and the menopausal status have no influence on the ability to produce equol. Age only became a substantial factor among newborns in the first month of life, compared to infants or adults, due to the absence of intestinal flora in newborn infants (Table 1).

From Table 2, only the smoking habit factor had a significant correlation with the equol-producing and non-equol-producing groups. An increase in the number of cigarettes consumed equals lower equol production. Physical activity did not have a significant correlation with both equol-producing and non-equol-producing groups. The BMI variable tended to give effect that higher the BMI of a person, the person's ability to produce equol will be lower, although it was not statistically significant.

Table 3 shows that higher intakes of carbohydrate and fiber were obtained in the equol-producing group compared to the non-equol-producing group. These variables had positive correlation and were statistically significant ($p < 0.05$), although the strength of correlation was weak ($r < 0.4$).

Table 1: Characteristics of subjects

Variable	*Equol-producing phenotype*		*p value*
	Non-equol-producing (n = 55)	*Equol-producing (n = 85)*	
Age (years old)			
20–39	10	11	0.089
40–59	42	62	
>60	3	12	
Menopause status			
Premenopause	18	16	0.107
Perimenopause	23	45	
Postmenopause	14	24	
Education level			
Elementary school	16	42	0.074
Junior high school	2	16	
Senior high school	28	14	
Diploma degree	7	8	
Bachelor degree	2	5	
Occupation			
Housewives	36	50	0.569
Entrepreneur	6	24	
Civil servant	9	9	
Teacher/lecturer	4	2	

Table 2: Relationship of smoking habit, physical activity, and body mass index with equol-producing phenotype

Variable	*Equol-producing phenotype*		*p value*	*Phi correlation*
	Non-equol-producing (n = 55)	*Equol-producing (n = 85)*		
Smoking habits			0.030*	0.224
Do not smoke	42	75		
5–10 cigarettes/day	7	9		
>10 cigarettes/day	6	1		
Physical activity			0.677	0.035
Irregular	35	57		
Regular	20	28		
BMI			0.209	0.180
Underweight	1	3		
Normal	30	55		
Overweight	14	21		
Obesity	10	6		

*Based on Chi-squared test

Discussion

Analysis of Lifestyle Factors (Smoking Habit, Physical Activity) and BMI

From two lifestyle factors and BMI variables, only smoking habit had significant correlation to the equol-producing phenotype. Higher number of cigarettes smoked gave a lower percentage of equol-producing phenotypes. This finding was similar to Liu et al., which showed the ratio between smokers in the non-equol-producing group was statistically higher than equol-producing groups.[7] The effect of smoking in inhibiting the conversion of daidzein into equol was by reducing Bifidobacterium in composition of intestinal microflora.[8] Besides, smoking has been reported to affect mucus production in the colon and affects the immune system in the gastrointestinal mucosa. The differentiation

Table 3: The relationship between the intake of carbohydrates, protein, fat, and fiber with the equol-producing phenotype

	Equol-producing phenotype			
Variable	*Non-equol-producing (n = 55)*	*Equol-producing (n = 85)*	*p value*	*Biserial correlation point*
Carbohydrate (g/day)			0.011*	0.202
Mean (SD)	190.1 (72.5)	222.2 (78.9)		
Median	180.3	219.9		
Range	63–363	63–419		
Protein (g/day)			0.736	−0.051
Mean (SD)	48.7 (18.60)	46.9 (16.8)		
Median	45.6	46.2		
Range	15–99	15–112		
Fat (g/day)			0.109	−0.161
Mean (SD)	66.41 (34.86)	55.8 (30.1)		
Median	57.9	52.5		
Range	10–157	5–137		
Fiber (g/day)			0.004*	0.218
Mean (SD)	9.76 (4.06)	11.4 (3.35)		
Median	9.4	11.0		
Range	2.8–21.4	3–18.7		

*Statistically significant ($p < 0.05$)

of the environment within the lumen of the intestine allows different types of bacteria to grow, including bacteria that are able to produce equol from daidzein.[3]

In this study, physical activity showed no significant correlation to the equol-producing phenotype. This finding was contradictory to a study conducted by Wu et al., which showed that isoflavonoid excretion of urine was correlated with regular physical activity.[9] Another study by Rybak et al. showed physical activity was significantly correlated with enterolactone concentration in urine, which was consistent with a healthy lifestyle.[4] The mechanism of how physical activity correlated with the ability of converting daidzein into equol was predicted to be similar to the smoking habit, which indicated that lack of physical activity affects the composition of intestinal microflora, which were able to convert daidzein into equol. In addition, lack of physical activity can cause the composition of intestinal microflora to resemble the obesity condition.

The lack of significant correlation between physical activity and the equol-producing phenotype could be caused by lack of accuracy during data collection. The criteria of physical activity habits on the subject were less strict. Besides, data collection was only done through interviews. The observation method would be better to be used for collecting the subjects' physical activity habits. The research regarding correlation of physical activity to the equol-producing phenotype between groups of athletes with none athletes can be conducted to obtain better descriptions.

The BMI variable also gave no significant correlation. This was similar to Frankenfeld et al., who stated that obesity was not correlated with the equol-producing phenotype. Yet in this study there was a tendency that if a person was more obese, their ability to produce equol was getting lower, even though it was not statistically significant.[5] One study found that the concentration of several isoflavones, such as enterodiol and enterolactone, was weakly and inversely correlated with BMI.[9]

The mechanism of how obesity affects the metabolism ability of daidzein into equol was predicted relate to the change of intestinal microflora composition in obese individuals. Previous research described that human with obesity had more Firmicutes species, which is a type of bacteria that are known to be able to metabolize daidzein into equol.[10]

Effect of Dietary Component Analysis (Intake of Carbohydrates, Protein, Fat, and Fiber)

Carbohydrate Intake

Another factor that can affect the production of equol is the differences of dietary intake. Many studies reported correlation between the equol-producing group with components of the dietary intake including carbohydrates, proteins, fats, PUFAs, dairy products, lactose, green tea, seaweed, and soy intake, but definitive conclusions were not yet ascertain. Long-term soy consumption was also not a factor that affects the formation of equol, but the intake of food containing daidzein substrates, such as soy, and the presence of bacteria in the intestine are essential.

In this study, the analysis of nutrient intake was taken by using the interviews reports with the semiquantitative food frequency questionnaire method; the analysis was divided based on the equol-producing group and the non-equol-producing group. The data obtained showed that there was a positive correlation between the intake of carbohydrates and fiber with the equol-producing phenotype. The data signified that there was a higher carbohydrate intake on the equol-producing group and it was statistically significant, although there was a weak strength of correlation.

High dietary intake of carbohydrates was known to stimulate the production of equol in individuals who had equol-producing bacteria species in their intestine. Previous research by Setchell et al. showed a differentiation of carbohydrate intake between the equol-producing and non-equol-producing equol.[6] The observational study using fecal flora culture in an environment of high polysaccharides stimulated the fermentation of bacteria that can change daidzein into equol faster. Unfortunately, in the conditions that are similar to the low carbohydrate intake, equol was not formed. Yuan et al. stated that equol production was stimulated by the amount of hydrogen gas that might act as an electron donor in the biotransformation reaction of daidzein into equol. This finding indicated that hydrogen has an important role in the production mechanism of equol.[11] Slavin showed that inulin, oligofructose, lactulose, and resistant starch or other fiber types and prebiotic carbohydrates were able to stimulate the growth of Bifidobacterium, which was a type of bacteria that is capable to convert daidzein into equol.[12]

Fiber Intake

The analysis showed that fiber intake has a significant correlation with the equol-producing phenotype. This finding was similar to the previous study by Lampe, which stated that women who excreted equol consumed more fiber and vegetation protein than those who did not excrete equol. This finding assumed that in those women, fibers or other high-fiber components increased the growth and/or the activity of bacteria that play a role in the production of equol in the intestine. In addition, the equol-producing groups who were vegetarian produced 4.25 times higher S-equol compared to nonvegetarian groups.[12]

Fiber is composed of carbohydrates that are resistant to the digestion and absorption process in the small intestine. Dietary fiber forms are including resistant starch, nonstarch polysaccharides (cellulose, hemicellulose, pectin, and gums), oligosaccharides, lignin, and other substances. Fiber can undergo fermentation in the colon. The mechanism of action of fiber as a prebiotic, undergo fermentation in the colon and alter the composition of intestinal microflora. The process of fermentation in the colon stimulates the growth of beneficial bacteria colonies such as Bifidobacterium type and inhibits pathogen bacteria.[13] The bacteria have the same characteristics as the bacteria that are capable to convert daidzein into equol.

Protein Intake

There was no significant correlation between protein and fat components with the equol-producing phenotype found in this study. Previous research conducted by Rowland mentioned that the consumption of meat and high fat could inhibit the intestinal flora, which was needed for the production of equol. High-protein diet also increased the proteolytic fermentation process that can produce useful substances, but the decay process can also produce substances that are toxic to the body. Those toxic substances were including ammonia, amines, phenols, and sulfides, and they also have a role in colorectal cancer. Previous researches described that a high-protein diet could increase Bacteroides type of bacteria.[14,15]

In this study, the intake of protein did not affect significantly the ability of converting daidzein into equol and can be caused by a high intake of protein cannot increase the specific bacterial strain, which was able to convert daidzein into equol. In this case, further research was needed to detect the specific strain that was affected by the presence of high levels of protein in the digestive tract. In addition, the effect of protein intake on the equol-producing phenotype in our study was affected by its confounding factors, including other macronutrient components. Further research should be done to minimize the effect of other macronutrient components in the subject's intake.

Fat Intake

In this study, we used the total fat intake as a dietary intake of fat. The analysis of fat intake showed that there was no significant correlation between fat intakes with the equol-producing phenotype. Data showed that fat intake on the equol-producing group was lower than the non-equol-producing group, although it was not statistically significant. Rowland's research conducted on healthy adults had similar findings, which stated that a good equol-producing group consumed less fat than the non-equol-producing group.[16] High-fat diet, approximately 49.5%, may alter the composition of intestinal microflora and eliminate *Bifidobacterium* spp., *Clostridium eubacterium* rectale-coccoides, and bacteroides. The reduction of the beneficial bacteria strain will also affect the person's ability to convert daidzein into equol. Furthermore, a saturated fat diet can increase pro-inflammatory intestinal microbes by stimulating the formation of taurine-conjugated bile that enhances the growth of pathogenic bacteria.[17,18]

Conclusion

The smoking habit and the intake of carbohydrates and fiber in dietary consumption had an effect on the equol-producing phenotype.

Clinical Significance

Our study may be useful as basic data to modify lifestyle and nutrition to improve the metabolism of equol production, which is needed for women's health.

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