

## Vegetarian Diets and Estrogen Metabolism in Korean Premenopausal Women

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### ABSTRACT

It has been suggested that dietary fat increases the risk of breast cancer by elevating serum estrogen concentrations. However, studies on a relationship between fat intake and breast cancer risk have shown contradictory results, possibly because the levels of fat intake in study populations were too high to observe differences. Also, the effect of other dietary factors may present. The present study was performed to investigate the relationship between diet and estrogen metabolism in premenopausal women whose usual fat intake is relatively low compared to their western counterparts. Twenty lacto-ovo vegetarians (LOV) and twenty omnivores participated in the study. Three day food records including a Saturday or a Sunday were used to estimate nutrient intake. Serum lipids, estradiol, sex-hormone binding globulin, and urinary estradiol were measured. Study results showed 24.8% and 20.9% of energy intake were provided from fat in omnivorous and LOV subjects, respectively. Serum and urinary levels of estradiol were two times higher in omnivores. Fat intake was not related to either serum estradiol nor urinary estradiol when the Spearman correlation coefficient analysis was performed. Carbohydrate, total dietary fiber and soluble dietary fiber intakes were negatively related to serum estradiol concentration. Legumes, vegetables and fruit consumption showed significantly negative relationships with both serum and urinary estradiol concentrations. These results indicate lower estrogen availability may be associated with plant food-based diets in premenopausal women whose usual diets contain less than 25% of energy as fat. (*J Community Nutrition* 7(1) : 29~35, 2005)

**KEY WORDS :** estrogen · breast cancer · vegetarians · phytoestrogens.

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### Introduction

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Breast cancer is one of the most common cancers in women. Incidences of breast cancer has been generally low in Asian countries, but the rates are increasing (The American Institute for Cancer Research 1997). The Japanese migrant study showed Japanese women living in Hawaii have four-times greater incidence of breast cancer compared to their counterparts living in Japan (Dunn 1975) supporting the hypothesis that environmental factors, especially diets, may be important in the occurrence of breast cancer.

Elevated serum concentrations of estrogens are suggested to be a major risk factor for breast cancer development in

premenopausal women (Bernstein, Ross 1993). Different types of diet affect the absorption, metabolism and excretion of estrogens (Goldin et al. 1986), and the western-type diets were suggested to increase the risk of breast cancer by elevating circulating concentrations of estrogens (Adlercreutz 1990). Although most of the studies have focused on the effects of fat intakes on estrogen metabolism and breast cancer risk, findings from pooled cohort studies and case-control studies revealed no association between fat intake and breast cancer risk (Hunter et al. 1996 ; Brandt et al. 1993). Protective roles of dietary fiber and complex carbohydrates in breast cancer development have been postulated in many case-control studies, however, the results are not consistent (Willett 2003 ; Stoll 1996 ; Willett et al. 1992 ; Baghurst, Rohan 1994 ; Goldin et al. 1994). Therefore, it is not clear whether total fat or total fiber intake is related to the development of breast cancer at this point.

Most of the cohort studies are conducted in countries where the population's average fat intake is relatively high.

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In these subjects, daily fat intake may be too high to observe differences between high-fat and low-fat consuming groups for circulating estrogens. Also, a recent study indicated that the types of fat, not the total fat, intake may have a relationship with breast cancer risk (Richter 2003). Fiber or fiber-associated components, especially phytoestrogens which possess weak estrogenic or antiestrogenic activities are suggested as possible modulators (Duncan 2004).

The objective of this study was to examine the relationship between diet and estrogen availability in a population whose average fat intake is lower compared to that of western populations. To achieve the study objective serum/urinary estradiol and serum sex hormone binding globulin (SHBG) concentrations of premenopausal omnivores and LOV were compared and relationships between diet and above biochemical markers of estrogen metabolism were analyzed.

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## Materials and Methods

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### 1. Subjects

Study subjects were premenopausal Korean female volunteers ( $n = 40$ ) aged between 18 and 27 years of age; all of them had regular menstrual cycles. Twenty subjects were Seventh Day Adventists (SDA) and twenty other subjects were age-matched omnivores. SDA subjects had consistently excluded animal foods except milk, eggs or fishes from their diets at least for five years and were considered as lacto-ovo vegetarians (LOV). Omnivores were university students who agreed to participate in the study. Subjects with family histories of cancer, heart disease, high blood pressure, diabetes, kidney disease and/or disease were excluded. Oral contraceptive users, antibiotic or corticosteroid users, and regular consumers of alcohol were not included in the study. Informed consent was obtained from each individual and anthropometric measurements were done. Also, menstrual history was obtained.

### 2. Dietary data

Participants were instructed to complete a three day dietary record including a Saturday or a Sunday. Portion sizes were estimated by measuring apparatus including bowls, spoons, cups and food models. Dietary records were coded and analyzed for nutrient intake by a computer-aided nutritional analysis program for professionals (CAN-PRO, APAC Intelligence, Seoul, Korea) except for soluble and insoluble dietary fiber

intake and fatty acids intake, which were calculated based on previously reported database (Hwang 1995; Lee et al. 1995).

### 3. Sample collection

Fasting venous blood samples were placed into a collecting tube on a day between the 21st and 23rd day of the menstrual cycle. Serum was separated by centrifugation and stored at  $-80^{\circ}\text{C}$  until analysis. Twenty-four hour urine samples were collected in a bottle containing 1mg of toluene on the same day blood samples were collected. After total urine volume was measured, aliquots of urine were stored at  $-20^{\circ}\text{C}$  until analysis.

### 4. Methods

#### 1) Blood lipids

Total cholesterol was measured based on the methods of Allain et al. (1974). Total HDL-cholesterol was determined by precipitation of LDL and VLDL-cholesterol from total cholesterol using dextran sulfate- $\text{Mg}^{2+}$  (Warnick et al. 1982). LDL-cholesterol was calculated based on Friedewald formula (Friedewald et al. 1972). Total triglyceride was analyzed by enzymatic procedure based on the method of Fletcher (1982).

#### 2) Serum estradiol, sex hormone binding globulin (SHBG) and urinary estradiol

Estradiol and SHBG concentrations were measured by a double-antibody  $^{125}\text{I}$  radio-immunoassay kit (ICN Pharmaceuticals, Inc., CA, USA). Serum SHBG was quantified using a monoclonal anti-SHBG  $^{125}\text{I}$  immunoassay kit (Diagnostic Products Co., CA, USA).

### 5. Statistical analysis

Values in the tables and figures were expressed as the mean  $\pm$  standard deviation of twenty subjects in each group. Significance of the differences between two groups was examined by Student's t-test. Relationships between dietary intakes and biochemical indices were analyzed by the Spearman correlation coefficient test. All statistical analysis were performed using the Statistical Analysis System (SAS/STAT version 6, SAS Institute Inc., Cary, NC, USA).

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## Results

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### 1. Demographic and anthropometric characteristics

Anthropometric indices and ages at menarch are shown in Table 1. Average age, height, weight and body fat content

**Table 1.** General characteristics of subjects

	Vegetarians	Omnivores
	Mean ± S.D. <sup>a</sup>	Mean ± S.D.
Height (cm)	159.7 ± 6.8	161.5 ± 5.4
Weight (kg)	51.7 ± 5.8	52.3 ± 6.7
xBody fat (%)	20.1 ± 2.6	20.8 ± 3.6
Systolic blood pressure	101.8 ± 7.0*	106.5 ± 6.5
Diastolic blood pressure	61.6 ± 8.4	66.2 ± 7.2
Menarch (years)	13.6 ± 1.4	12.9 ± 1.2

<sup>a</sup>Significantly different from the value of omnivores, \*p < 0.05, \*\*p < 0.01

**Table 2.** Average daily intake of selected nutrients

	Vegetarians	Omnivores
	Mean ± S.D. <sup>a</sup>	Mean ± S.D.
Energy (kcal)	1769.6 ± 199.5	1682.3 ± 165.6
Carbohydrate (g)	293.2 ± 45.2	236.2 ± 30.7
(% of energy)	(66.5)	(58.8)
Protein (g)	55.6 ± 8.3*	65.8 ± 19.1
(% of energy)	(12.6)	(16.4)
Animal protein (g)	6.5 ± 4.4***	31.5 ± 11.7
Fat (g)	43.7 ± 7.0*	49.8 ± 7.6
(% of energy)	(20.9)	(24.8)
Total fatty acids (g)	38.9 ± 6.4*	43.9 ± 7.0
Saturated fatty acids (g)	11.6 ± 3.3**	14.6 ± 3.1
Monounsaturated fatty acids (g)	14.0 ± 2.5**	16.8 ± 2.7
Polyunsaturated fatty acids (g)	14.9 ± 2.6	14.2 ± 1.9
Cholesterol (mg)	101.7 ± 52.2***	244.4 ± 115.6
Total dietary fiber (g)	31.3 ± 6.9***	21.1 ± 9.6
Insoluble dietary fiber (g)	15.6 ± 3.1***	11.3 ± 3.6
Soluble dietary fiber (g)	15.7 ± 4.3**	9.8 ± 6.4

<sup>a</sup>Significantly different from the value of the omnivores : \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

were not different between the two groups. Systolic blood pressure was significantly higher in omnivores than that of LOV (p < 0.05). The age at menarch was not significantly different between the two groups being 13.6 ± 1.4 years and 12.9 ± 1.2 years in vegetarians and omnivores, respectively.

## 2. Dietary intakes

Table 2 shows the average intakes of selected nutrients. Daily energy intakes of LOV and omnivores were not different from each other. LOV subjects had a significantly lower intake of protein (p < 0.05) and fat (p < 0.001). Energy supplied from fat was less than 25% of total energy supply in both groups. Omnivores had higher intake of total (p < 0.05), saturated and monounsaturated fatty acids (p < 0.01); however, polyunsaturated fatty acids intakes of omnivores were not significantly different from that of vegetarians. The average cholesterol intake by LOV was 59% less than that of

**Table 3.** Food intake from different food groups unit : g

	Vegetarians	Omnivores
	Mean ± S.D. <sup>a</sup>	Mean ± S.D.
Cereals	511.5 ± 122.3	487.7 ± 84.1
Refined	170.4 ± 112.3**	483.6 ± 81.8
Unrefined	347.8 ± 149.3***	0.7 ± 3.1
Potatoes	37.1 ± 25.8	23.6 ± 37.0
Sugar	1.7 ± 1.8**	5.0 ± 4.0
Legumes	150.3 ± 71.6***	34.4 ± 36.5
Nuts & seeds	9.0 ± 17.5	1.4 ± 3.4
Vegetables	298.9 ± 91.7**	169.9 ± 85.4
Mushrooms	13.9 ± 13.0*	5.4 ± 10.7
Fruits	195.0 ± 77.8*	126.1 ± 115.3
Eggs	13.2 ± 12.6	23.6 ± 24.5
Fish & shellfish	5.8 ± 9.1**	58.1 ± 62.8
Seaweeds	1.6 ± 1.4	1.5 ± 2.0
Milk	98.4 ± 73.9	138.2 ± 95.9

<sup>a</sup>Significantly different from the value of omnivores : \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

**Table 4.** Serum lipid levels

	Vegetarians	Omnivores
	Mean ± S.D. <sup>a</sup>	Mean ± S.D.
Total lipids (mg/dl)	580.4 ± 208.4	612.7 ± 215.3
Total cholesterol (mg/dl)	142.7 ± 24.4	149.7 ± 16.3
High density lipoprotein (mg/dl)	49.1 ± 11.5	48.3 ± 8.0
Low density lipoprotein (mg/dl)	75.1 ± 18.7*	87.9 ± 19.8
Triglyceride (mg/dl)	75.2 ± 36.0	68.1 ± 17.7

<sup>a</sup>Significantly different from the value of the omnivores : \*p < 0.05

omnivores, while total dietary fiber consumption of omnivores was 33% less than that of LOV.

Daily intake of foods from different food groups is shown in Table 3. Although there was no significant difference in cereal intake, LOV consumed two thirds of cereals from unrefined sources; however, omnivores consumed most of cereals from refined sources. Intakes of legumes including soy foods, vegetables, mushrooms and fruits were higher in LOV subjects, while omnivores had higher intake of refined sugar. One LOV subject consumed fish during dietary intake was measured.

## 3. Serum lipid levels

Serum lipids, total cholesterol, HDL-cholesterol, and triglyceride levels of LOV subjects were not significantly different from those of omnivorous subjects (Table 4). However, the average serum LDL-cholesterol level was 75.1 ± 18.7 mg/dl and that was significantly lower than 87.9 ± 19.8mg/

**Table 5.** Serum and urinary levels of estrogens and serum SHBG concentrations

	Vegetarians	Omnivores
	Mean $\pm$ S.D. <sup>a</sup>	Mean $\pm$ S.D.
Serum estradiol (pmol/l)	238.5 $\pm$ 100.1*	455.6 $\pm$ 93.7
Serum SHBG (nmol/l)	46.3 $\pm$ 14.1	54.0 $\pm$ 23.3
Urinary estradiol (nmol/day)	17.6 $\pm$ 5.2*	28.3 $\pm$ 10.7

<sup>a</sup>Significantly different from the value of omnivores : \*p < 0.001

**Table 6.** Correlation coefficients between nutrient intake and estradiol or SHBG

	Serum estradiol	Serum SHBG	Urinary estradiol
Energy		-0.35*	-0.34*
Protein			
Fat			
Carbohydrate	-0.49**		-0.45**
Cholesterol	0.44*		
Total fatty acids			
Saturated fatty acids	0.37*		
Monounsaturated fatty acids	0.38*		
Insoluble dietary fiber			
Total dietary fiber	-0.40*		
Insoluble dietary fiber			
Soluble dietary fiber	-0.43*		

\*p < 0.05, \*\*p < 0.01

**Table 7.** Correlation coefficient between food intake and estradiol or SHBG

	Serum estradiol	Serum SHBG	Urinary estradiol
Cereals			
Refined			
Unrefined	-0.38*		-0.42*
Potatoes			
Sugar	0.45*		
Legumes	-0.59**		-0.36*
Nuts & seeds			
Vegetables		-0.37*	-0.44*
Mushrooms			
Fruits	-0.44*	-0.37*	
Eggs		-0.35*	
Fish & shellfish			0.35*
Seaweeds			
Milk			

\*p < 0.05, \*\*p < 0.001

dl in omnivores (p < 0.05).

#### 4. Serum and urinary levels of estradiol and sex hormone binding globulin (SHBG)

Premenopausal LOV women had significantly lower levels

of both serum and urinary estradiol (p < 0.001) (Table 5). Serum estradiol concentrations were 238.5  $\pm$  100.1 pmol/L in LOV and 455.6  $\pm$  93.7 pmol/L in omnivores. Urinary estradiol levels were 17.6  $\pm$  5.2 nmol/day and 28.3  $\pm$  10.7 nmol/day, respectively. There was no statistically significant difference in serum SHBG concentration.

#### 5. Relationship between dietary intakes and estradiol or SHBG levels

The associations between selected nutrients and serum or urinary estradiol and SHBG were evaluated (Table 6). There was no significant correlation between intake of total fat or individual fatty acid and serum or urinary estradiol. However serum estradiol concentration was negatively related to the intake of carbohydrate (p < 0.05), total dietary fiber (p < 0.01) and soluble dietary fiber (p < 0.01). Urinary estradiol level showed negative relationships with energy and carbohydrate intake (p < 0.01). Energy and carbohydrate intakes were negatively related to SHBG concentration.

Among different food groups, legume and soy food intake showed the strongest negative relationship with serum estradiol concentration (Table 7, p < 0.01). Intakes of cereals, vegetables and fruits also had significant negative associations with serum estradiol concentration, while sugar intake was positively related. Urinary estradiol level was negatively correlated to the intake of cereals, legumes, nuts, vegetables and fruits. SHBG was inversely related to the intake of vegetables, fruits and eggs (p < 0.05).

## Discussion

The present study was performed to investigate the relationship between plant food-based diets and estrogen metabolism. Results from this study showed omnivores consumed significantly higher amounts of protein, total fat, and cholesterol compared to the LOV. However, total energy intake was not different and energy intake from carbohydrate was higher in LOV compared to those of omnivores.

Although nutritional studies of breast cancer development have focused on determination of the role of fat, more recent data suggest fat intake may not be related to the risk (Willett 2003). Possible explanations for this contradiction are : 1) these studies are executed in populations whose average fat consumption is too high to observe the differences in cancer risk ; 2) other food components such as phytoestrogens

and fibers are involved in the process of breast cancer development. The results from this study indicated fat intake is not related to serum nor urinary estradiol concentrations even in a population whose fat intake is 20 – 25% of energy consumption. Wu et al. (1999) conducted a meta-analysis of 13 dietary fat intervention studies that investigated serum estradiol concentrations. Results showed reduction in estradiol levels of –6.6% (95% CI = –10.4% to –2.7%) when dietary fat was reduced to 18 – 25% of total calories from 30 – 35% of total calories. In their review, the authors pointed out that previous reports may have failed to detect a positive relationship between dietary fat intake and breast cancer risk (Hunter, Willett 1993 ; Graham et al. 1982) because the number of people who consume fat less than 20% of their calorie intake was too small to observe the difference. However, in our study subjects whose average dietary fat intake was 20.9% of total energy, and we could not observe a relationship between fat intake and estradiol concentrations indicating a dramatic reduction in fat intake may be necessary to observe changes in estrogen concentration.

However, the present study showed total and soluble dietary fiber intakes possess significant negative associations with serum estradiol concentration. Several epidemiological studies have suggested total dietary fiber intake is negatively associated with the incidence of breast cancer (Duncan 2004 ; Baghurst, Rohan 1994). Diets containing approximately 40g/day of dietary fiber, significantly decreased serum estrogen concentrations in premenopausal women (Goldin et al. 1994). However, omnivorous and vegetarian women who daily consume 12g and 28g fiber, respectively, no significant difference in plasma estrogen concentration was observed although fecal excretion of estrogens was higher in vegetarian subjects (Goldin et al. 1982). In our study, insoluble fiber had no relationship, and this is contradictory to an intervention study by Rose et al. (1991) in which oat bran consumption did not affect the serum estradiol concentration while wheat bran lowered it. These results imply that the types of fiber and the existence of other compounds present in fiber-rich foods may need more careful examinations.

The correlation coefficient analysis of food consumption and estrogen concentrations in this study clearly showed that daily intakes of cereals, legumes, nuts, fruits, and vegetables are significantly related to lower serum and urinary estradiol concentrations. Whole-grain cereals and legumes are rich sources of phytoestrogens such as lignans and isoflavones. These

are a group of compounds structurally similar to estrogens possessing weak estrogenic activity. Isoflavones and lignans have been suggested to suppress the development of breast cancer (Limer, Speirs 2004). In populations consuming large amounts of whole-grain products, vegetables and soybeans, blood and urinary concentrations of these phytoestrogens are reported to be high (Adlercreutz 1990). Also, lower excretion of lignans has been observed in subjects with breast cancer compared to control subjects (Adlercreutz et al. 1982). Several epidemiological studies showed that total grain fiber intake was significantly different between breast cancer patients and control subjects (Adlercreutz et al. 1989), and this may be related to phytoestrogen in these grain products. A summary report based on several study results indicated that a 17% reduction in estrogen may significantly reduce the risk of breast cancer (Prentice et al. 1990).

Serum concentration of SHBG is believed to negatively modulate the biotransformation of sex hormones to target cells. Few studies observed the relationship between dietary components and serum SHBG concentration. A report by Longcope et al. (2000) showed protein intake was negatively correlated with SHBG concentration, but total caloric intake and intake of fat or carbohydrate were not significant. However, the result from our study indicated SHBG concentration had significantly negative correlations with total caloric intake and intake of carbohydrates. More studies on diet and sex hormone-binding globulin are necessary to understand the relationship between SHBG and diets.

Most of the studies so far have focused on the reduction in fat intake and dramatic increases in fiber consumption to reduce breast cancer risk by controlling available estrogens. However, no consistent cause-effect relationship has been observed. Also, these reductions or increases are too dramatic to introduce into the average diet. The present study indicates that vegetarian diets high in cereals and legumes with moderate fat content may be effective in regulating serum estradiol concentrations in premenopausal women. Also, development of complete analysis data for food phytoestrogen content will facilitate the understanding of the relationship between diet and estrogen metabolism.

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## Summary and Conclusion

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Results from the present study are summarized as follows :

- 1) Premenopausal LOV consumed less protein and fat

compared to omnivores, while dietary fiber consumption was higher in LOV.

2) While no significant difference was found in cereal intake, LOV consumed two thirds of cereals from unrefined sources. Also, intake of legumes, vegetable, mushrooms, and fruits were higher in LOV compared to those of omnivores.

3) Serum LDL-cholesterol level of LOV was significantly lower than that of the omnivores. Also, LOV had significantly lower level of serum and urinary estradiol while no difference was observed for serum SHBG level.

4) Serum estradiol concentration was related to the intake of carbohydrate, total dietary fiber, and soluble dietary fiber. Among food groups, intake of legume, cereals, vegetables and fruits showed negative relationships with serum estradiol concentration.

5) Urinary estradiol level showed negative relationships with energy and carbohydrate intake, while the intake of cereals, legumes, nuts, vegetables, and fruits were negatively related to urinary estradiol.

These results indicate that vegetarian diets high in plant-based foods with a moderate level of fat may reduce the risk of breast cancer through modulating circulating level of estrogen.

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