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# Soy isoflavone supplementation increases equol-producing capability in postmenopausal women with osteopenia

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## ABSTRACT

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**BACKGROUND**
Isoflavone containing soy protein has been associated with beneficial health effects. Equol, a gut bacterial metabolite of the isoflavone daidzein, has been hypothesized to be the cause of the effectiveness of isoflavones. The objective of this study was to evaluate the effect of 100 mg/day soy isoflavone supplementation for 3 months on the ability to produce equol in postmenopausal women with osteopenia.

**METHOD**
A pre-post experimental study was conducted to assess the effects of 100 mg/day soy isoflavone supplementation on equol production capability and the proportion of equol producers to non-equol producers in postmenopausal women with osteopenia. A total of 39 subjects received 1 supplement tablet containing 100 mg soy isoflavones (genistein, daidzein) for 3 months. Determination of serum genistein, daidzein, and equol concentrations was performed twice at baseline and at 3 months post-supplementation using high-performance liquid chromatography mass spectrophotometry (HPLC-MS). Equol producer status was determined by the detection of a serum equol concentration of 5 ì g/L.

**RESULTS**
Mean genistein and daidzein concentrations at baseline were 86.2 ± 68.4 ì g/L and 16.7 ± 18.6 ìg/L, respectively. The proportion of equol producers was 69.2%. After 3 months of soy isoflavone supplementation the serum concentrations of genistein and daidzein significantly increased to 161.0 ± 5.8 ìg/L (p=0.000) and 49.9 ± 40.4 ìg/L (p=0.000), respectively, and the proportion of equol producers also significantly increased (100.0%).

**CONCLUSION**
Soy isoflavone supplementation was capable of increasing the serum concentrations of isoflavones (genistein and daidzein) and the equol-producing capacity of postmenopausal women with osteopenia.

**Key words:** Soy isoflavone, equol, postmenopausal, osteopenia

# Suplementasi isoflavon kedelai meningkatkan produksi equol pada perempuan pascamenopause dengan osteopenia


## ABSTRAK

***LATAR BELAKANG***

*Protein kedelai yang mengandung isoflavon bermanfaat bagi kesehatan. Equol, yaitu metabolit bakteri usus dari isoflavon daidzein, diperkirakan merupakan penyebab efektivitas isoflavon. Penelitian ini bertujuan untuk menilai efek suplementasi isoflavon kedelai 100 mg/hari selama 3 bulan terhadap kemampuan memproduksi equol pada perempuan pascamenopause dengan osteopenia.*

***METODE***

*Penelitian ini menggunakan desain* pre-post experimental study *untuk menilai efek suplementasi isoflavon kedelai 100 mg/hari pada kadar isoflavon dalam serum, kadar equol, dan proporsi* equol producer *terhadap* non-equol producer *pada perempuan pascamenopause dengan osteopenia. Sebanyak 39 subjek setiap hari menerima 1 tablet suplemen yang berisi 100 mg isoflavon kedelai (genistein, daidzein) selama 3 bulan. Pengukuran kadar genistein, daidzein, dan equol dalam serum dilakukan 2 kali pada kadar awal dan pada 3 bulan pascasuplementasi menggunakan* high performance liquid chromatography mass spectro-photometry *(HPLC-MS). Status equol producer ditetapkan bilamana dapat terdeteksi kadar equol serum sebesar 5µg/L.*

***HASIL***

*Rerata asupan genistein dan daidzein masing-masing besarnya 149,0 ± 138,2 mg/hari dan 239,5 ± 175,8 mg/hari. Proporsi kelompok yang memproduksi equol besarnya 69,2%. Setelah suplementasi isoflavon kedelai selama 3 bulan, kadar genistein dan daidzein meningkat menjadi 161,0 ± 5,8 ì g/L (p=0,000) dan 49,9 ± 40,4 ì g/L (p=0,000) serta proporsi kelompok yang memproduksi equol naik menjadi 100,0%.*

***KESIMPULAN***

*Suplementasi isoflavon kedelai 100 mg/hari selama 3 bulan mampu meningkatkan kadar isoflavon dalam serum (genistein dan daidzein) dan kemampuan memproduksi equol pada perempuan pascamenopause dengan osteopenia.*


*Kata kunci: Isoflavon kedelai, equol, pasacamenopause, osteopenia*

## INTRODUCTION

Menopause is the most important risk factor for osteopenia and osteoporosis.[1] Postmenopausal women experience a permanently decreased secretion of the ovarian hormones estrogen and progesterone as a result of depletion of ovarian follicles.[1] The higher prevalence of osteopenia, osteoporosis, and cardiovascular disease in post-menopausal women is presumably due to decreased estrogen concentrations.[2,3] Although it has been demonstrated that the decrease in estrogen concentrations may actually reduce bone density, numerous studies are being conducted to clarify the latter process. Studies using tissue cultures have demonstrated direct effects of estrogen on bone cells via estrogen receptors.[4] Several of the most recent studies indicate that estrogen plays a role in regulation of bone homeostasis through regulatory effects on the immune system, oxidative stress, and direct effects on bone cells.[5] The direct effects of estrogen on bone cells comprise effects on

osteoblasts, osteocytes, and osteoclasts, mediated through estrogen receptors (ERs). ERs are of two types, ERá and ERâ, both of which are present on bone cells, although not uniformly distributed. ERá being predominantly in cortical bone, while ERâ is mainly found in trabecular bone.[4,6]

Accelerated postmenopausal osteoporosis requires effective management to prevent the occurrence of fractures, which are characteristic of osteoporosis due to reduced bone density. Hormonal replacement by administration of synthetic estrogens or selective estrogen receptor modulator (SERM) has been demonstrated to be an effective treatment option for postmenopausal osteoporosis.[7-10] The clinical trial conducted by the Women's Health Initiative (WHI) revealed that hormonal replacement reduced the risk of all fractures associated with osteoporosis, including femoral neck and vertebral fractures, although only in patients with a low risk of fractures.[8] However, hormonal replacement also shows an increased risk of cardiovascular disorders such as venous thromboembolism and breast and endometrial malignancies, such that the benefits to risks ratio should be considered.

In Japanese and Asian women with a high consumption of dietary products derived from isoflavone-containing soy beans, the incidence of osteoporosis was found to be lower than in Western women with a low consumption of isoflavones.[11,12] This reinforces the idea that isoflavones in soy beans serve to inhibit postmenopausal osteoporosis, because soy isoflavones have been identified to possess phytoestrogenic effects.[13,14] The major class of phytoestrogens attracting attention from the nutritional and medical viewpoint are the ligans and isoflavones. Genistein, daidzein, and glycitein, which are free forms of soy isoflavones, have a molecular structure similar to estradiol and display estrogenic effects (Figure 1).[15]

There are many pharmacokinetic studies on isoflavones in connection with equol, which is an active metabolite of daidzein. Equol enters the enterohepatic circulation and is actively secreted in the renal tubules, such that it is detectable in the blood and urine. Equol has a greater estrogenic effect than genistein or daidzein.[16-18] Various studies also demonstrate an association of equol concentrations with the variable biologic effects as well as the clinical benefits of isoflavone administration.[18,19]

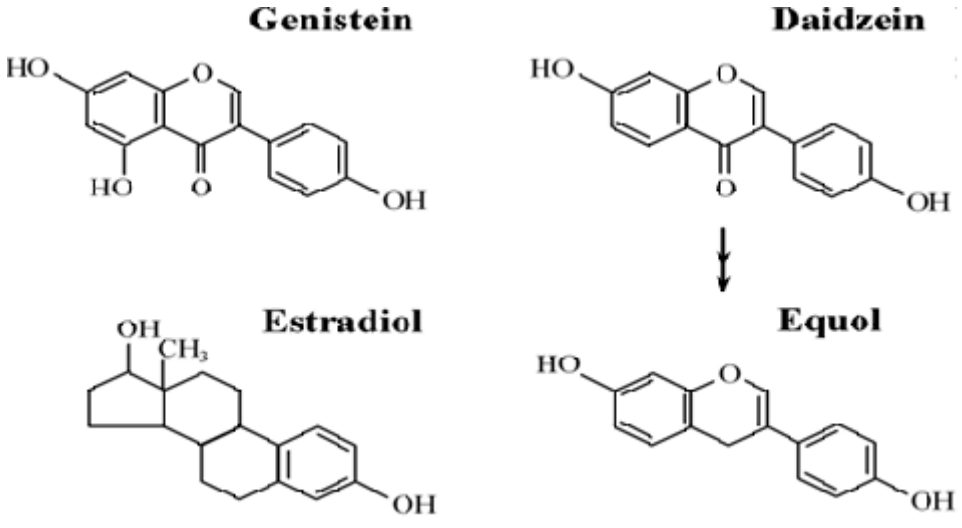


Figure 1. Molecular structure of isoflavone derivatives and estradiol.[15]

Individuals differ in their capacity to metabolize daidzein into equol, and therefore can be classified into equol producers and non-equol producers.[21,22] Numerous *in vitro* studies have found evidence that the characteristics of intestinal bacteria play a role in the metabolism of daidzein into equol, with daidzein being hydrolyzed into equol by bacterial endo-â-glycosidases.[20-22]

There is a wide variation in the capacity of individuals to produce equol, with a proportion of equol producers of 30-40% in Western countries,[23,24] while several studies have reported a higher proportion of equol producers in Asian as compared with Western populations.[20,25] Lampe et al.[26] reported that genetic variation may affect phytochemical metabolic pathways such as to potentially induce changes in their biological response. Vergne et al.[27] reported that isoflavone pharmacokinetics and bioavailability are influenced by ethnicity and dietary history of isoflavone intakes.

In our study, the criterion of osteopenia was used on the consideration that postmenopausal women with osteopenia comprise the largest group compared to normal bone mineral density (BMD) and osteoporosis groups.[28] There are few reports of studies on equol producers in Indonesia, although Indonesian communities consume a high proportion of soy-based foods, usually in the form of tofu, tempeh, soy sauce, and soy milk. The aim of the present study was to evaluate the effects of soy isoflavone supplementation on serum isoflavone concentrations and the potential to stimulate equol-producing capacity in postmenopausal women with osteopenia.

## METHODS

### Research design

This study was a study of pre-post test experimental design conducted between January and July 2010 to assess the effects of soy isoflavone supplementation on equol-producing capacity.

### Study subjects

The study subjects were 48-60 year old postmenopausal women with osteopenia, who were recruited by consecutive random sampling from the Mampang Parapatan District, South Jakarta. Screening was performed by means of questionnaires, physical examination, and measurement of BMD. The inclusion criteria were as follows: postmenopausal women 48-60 years of age, duration of menopause of more than one year but not exceeding 10 years, osteopenia (T score between –1 and –2.5), without a history of medications in the past 6 months, such as hormonal replacement, isoflavone-containing supplements, antidiabetics, antihyper-lipidemic agents and anticoagulants, agreeing to participate in the study and signing informed consent. Laboratory investigations: bilirubin $\leq$ 2.0 mg/dL, glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) within normal limits, creatinine $\leq$ 1.5 mg/dL, capable of walking unaided and capable of communication. Exclusion criteria were: women with a past history of hysterectomy or oophorectomy, irradiation, chemotherapy, malignant disease (such as mammary, cervical or endometrial carcinomas), and chronic disease (such as hepatic cirrhosis, renal failure, diabetes mellitus, cardiac disorders, hyperthyroidism, or stroke).

### Intervention

Soybean extract was imported from Huisong Pharmaceuticals, China. Each 100 mg of soy extract contained 40% soy isoflavones. The supplement tablets used in this study were prepared and packaged by PT Ikapharmindo Putramas. The supplements contained 250 mg soybean extract equivalent to 100 mg isoflavones (composed of genistein 56%, daidzein 41%, and glycitein 3%). All postmenopausal women took 1 tablet daily for 3 months. The supplementation was administered at daily home visits under direct observation by the cadre on duty and recorded in a check list.

**Measurements**

Subjects meeting the inclusion and exclusion criteria on the basis of interviews, were screened by physical examination, laboratory tests, and BMD measurement. Physical examination comprised determination of pulse rate, body weight, and height. Dual-energy X-ray absorptiometry (DXA) was used for BMD screening, with the subjects with T-scores between –1 and –2.5 being selected for participation in the study.[7] The BMD was determined at Budi Jaya Hospital, Jakarta, using the Lunar DPX Bravo Nomusa densitometer (GE Medical Systems). Laboratory screening tests were done on fasting subjects and comprised blood glucose, bilirubin, SGPT, SGOT, and creatinine.

**Soy intake measurement**

Intake of soybean-containing foods was assessed at baseline by means of food records and food recall. The subjects recorded their complete dietary intake per day for 3 days (2 work days and 1 holiday), to be confirmed by food recall. The dietary intake was calculated semi-quantitively using the soy food frequency questionnaire (FFQ) and the results of an analysis of the soy isoflavone content of foods frequently consumed by the subjects.

**Isoflavone analysis**

The serum concentrations of genistein, daidzein, and equol were determined at baseline and at 3 months postsupplementation. Measurement of isoflavone concentrations was performed by means of the high-performance liquid chromatography mass spectrophotometer (HPLC-MS) using the Waters 2690 separation module-Alliance-MS (Micromass Quattro LC Triple Quadropole). A C18 column with a photo diode array detector was used at 200-350 nm.[29] The detection limit was 0.5 ng/mL, with an individual being classified as equol producer if the serum equol concentration was ≥ 5 ìg/L.[23] The coefficients of variation of daidzein, genistein and equol were 1.51%, 1.53%, and 2.67%, respectively.

**Compliance measures**

Subject compliance was assessed by means of the supplementation checklist and by counting the remaining tablets in the bottle, and was monitored by the investigators in periodic meetings with the subjects. The subjects were categorized as dropouts if they failed to take the supplements for a total of more than 10% of the tablets (9 tablets) within 3 months of supplementation.

**Statistical analysis**

Subject characteristics such as age, body weight, height, body mass index (BMI), blood pressure, and laboratory screening test results were presented as descriptive statistics, with the values given as mean ± standard deviation (SD). The paired t-test and McNemar test were used to compare the differences in isoflavone concentration and equol producer status between baseline and post-supplementation, p-value of <0.05 being considered significant.

**Ethical clearance**

The study protocol was approved by the Committee of Medical Research Ethics of the Faculty of Medicine, University of Indonesia.

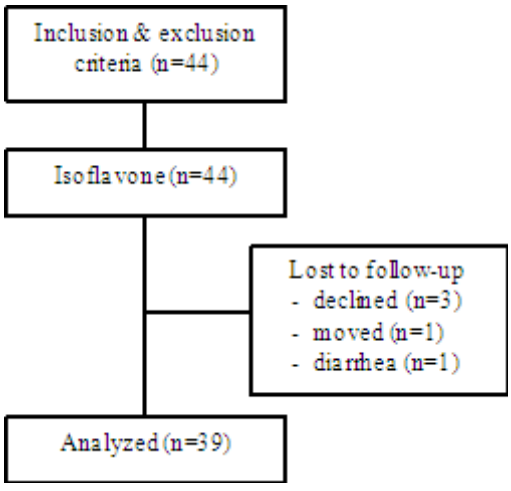


Figure 2. Flowchart of study subjects

Table 1. Subject characteristics at baseline (n=39)

| Characteristic | Mean ± SD |
|---|---|
| Age (years) [a)] | 53.0 ± 3.5 |
| Duration of menopause (years) [a)] | 4.4 ± 2.1 |
| Marital status [b)] | |
| Married (w/ living husband) | 27 (69.2) |
| Unmarried/widow | 12 (30.8) |
| Level of education [b)] | |
| Low (= primary school) | 25 (64.2) |
| Medium (junior high/senior high) | 7 (17.9) |
| High (= diploma 1) | 7 (17.9) |
| Employment [b)] | |
| Employed | 19 (48.7) |
| Unemployed | 20 (51.3) |
| Pulse rate (per minute) [a)] | 78.2 ± 5.6 |
| Height (cm) [a)] | 148.7 ± 4.9 |
| Weight (kg) [a)] | 61.8 ± 10.9 |
| Body mass index (kg/m$^2$) [a)] | 27.9 ± 4.4 |
| Estradiol concentration (pg/mL) [a)] | 7.3 ± 5.6 |
| Isoflavone intake [a)] | |
| Genistein (mg) | 149.0 ± 138.2 |
| Daidzein (mg) | 239.5 ± 175.8 |
| Serum isoflavone concentration [a)] | |
| Genistein (µg/L) | 86.2 ± 68.4 |
| Daidzein (µg/L) | 16.7 ± 18.6 |
| Equol (µg/L) | 7.5 ± 4.6 |
| Proportion of equol producers [b)] | |
| Equol producers | 27 (69.2) |
| Non-equol producers | 12 (30.8) |

[a)] Values are mean ± standard deviation; [b)] number of subjects (%)

## RESULTS

### Study subjects

In this study, 44 subjects fulfilled the inclusion and exclusion criteria. Five subjects dropped out for the following reasons: 3 subjects declined to continue participation, 1 subject had a change of residence, and 1 subject discontinued participation due to diarrhea in the third month. A total of 39 subjects participated until completion of the study, and were analyzed as per protocol (Figure 1).

Baseline characteristics of the study subjects are listed in Table 1. Age, years since menopause, body weight, height, BMI, isoflavone intake, and serum isoflavone concentration are presented as mean ± SD. The subjects were postmenopausal women aged 48-60 years with osteopenia. Among the 39 subjects who completed the study, the youngest was 48 years and the eldest 60 years of age, with mean age of 53.0 ± 3.5 years. The shortest duration of menopause was 1 year and the longest 9 years, with a mean duration of 4.4 ± 2.1 years.

Marital status was categorized as married with living husband 27 (69.2%) and unmarried (single) or widow 26 (30.8%). Most of the subjects (25 or 64.2%) had a low educational level (no education – primary school). Employment status was divided into employed (48.7%) and unemployed (51.3%).

Mean BMI was 27 ± 4.4 kg/m$^2$, and estradiol concentration was 7.3 ± 5.6 pg/mL. Isoflavone intake was assessed by means of the FFQ method for the isoflavone derivatives genistein and daidzein. Daily genistein intake was 149.0 ± 138.2 mg, while daidzein intake was higher at 239.5 ± 175.8 mg.

In this study, serum isoflavone concentrations, comprising genistein, daidzein, and equol concentrations, were determined twice, namely at baseline and at 3 months postsupplementation. Baseline isoflavone concentrations, presented in Table 1, showed mean genistein concentration of 86.2 ± 68.4 ìg/L, mean daidzein concentration of 16.7 ±18.6 ìg/L, and mean equol concentration of 7.5 ± 4.6 ìg/L, with 27 (69.2%) equol producers and 12 (30.8%) non-equol producers.

After 3 months of isoflavone supplementation there was a significant increase in the concentrations of genistein (86.2 ± 68.4 vs. 161 ± 5.8 ìg/L; p = 0.000), daidzein (16.7 ± 18.6 vs. 49.3 ± 40.4 ìg/L; p=0.000), and equol (7.5 ± 4.5 vs 13.0 ± 5.9; p =0.000) (Table 2).

As presented in Table 3, the results of McNemar's test showed a significant difference in equol-producer status at baseline and at 3 months post-supplementation (p=0.000).

Tabel 2. Mean isoflavone at baseline and at 3 months post-supplementation

| Isoflavone | Concentration | | p [b)] |
|---|---|---|---|
| | Baseline (n = 39) | 3 months (n = 39) | |
| Genistein (µg/L) [a)] | 86.2 ± 68.4 | 161.7 ± 64.4 | 0.000 |
| Daidzein (µg/L) [a)] | 16.7 ± 18.6 | 49.9 ± 40.4 | 0.000 |
| Equol (µg/L) [a)] | 7.5 ± 4.5 | 13.0 ± 5.9 | 0.000 |

[a)] Values are mean standard deviation; [b)] p value calculated by paired t-test

## DISCUSSION

The study subjects were postmenopausal women with osteopenia, which is a condition that without intervention is at risk of deteriorating to osteoporosis. The results of the present study indicate high genistein and daidzein daily intakes of 149.0 ± 138.2 mg and 239.5 ± 175.8 mg, respectively, which are higher than those of postmenopausal women in Texas (58.7 ± 21 mg/d for genistein and 53.4 ± 10.7 mg/d for daidzein).[30] A study in Japan[31] showed that older Japanese adults consume approximately 6-11 g of soy protein and 25-50 mg of isoflavones per day (expressed as aglycone equivalents), which are lower than the isoflavone intakes among postmenopausal women in our study. In Indonesia, soy beans are on the daily menu in processed form as tempeh, tofu, soy sauce, and soy milk, which are much consumed by the subjects of this study in particular and by Indonesian communities in general, in view of their low cost and ready availability in traditional markets. Dietary isoflavone intakes show considerable individual variation in many countries.

In our study, serum isoflavone concentrations were already high at baseline, before the administration of the isoflavone supplements, while post-supplementation serum isoflavone concentrations increased significantly (p=0.000). These data may be considered as objective evidence confirming subject compliance in taking the supplements and at the same time demonstrating that the administered supplementation was able to reach the systemic circulation.

Pharmacokinetics deals with the fate of drugs in the body and comprises 4 processes, i.e. absorption, distribution, metabolism, and excretion. Inter-individual variation in metabolizing capacity for a drug may influence the effectiveness of the drug. Modification of soy isoflavones by intestinal bacteria may produce metabolites that differ in biological activity from the parent compounds. Bacterial metabolism of soy isoflavone varies among individuals.[32] Usually, further degradation and transformation of aglycones produce less active compounds, but hydrolysis of daidzein glycosides results in the more active compound equol [7-hydroxy-3-(4'-hydroxyphenyl)-[chroman]]. Although the

Table 3. Comparison of equol producer status before and after administration of soy isoflavones for 3 months (n=39)

| Equol before | Equol after | | p [b)] |
|---|---|---|---|
| | Non-producers | Producers | |
| Non-producers [a)] | 0 | 12 | 0.000 |
| Producers [a)] | 0 | 27 | |

[a)] Number of subjects; [b)] p value calculated by Mc Nemars test

factors that influence equol-producing capacity are not clearly established, intestinal physiology, host genetics, and diet appear to contribute to interindividual differences in the conversion of daidzein to equol.[32] Other studies state that intake of fructo-oligosaccharidies (FOS)[21] or *Lactobacillus casei* with isoflavone may increase equol production.[17] A high carbohydrate diet may also increase the production of equol.[13] In contrast, administration of antibiotics has the opposite effect, modifying the numbers and characteristics of intestinal bacteria and thus inhibiting the metabolic conversion of daidzein into equol.

Assessment of equol may be done on serum or urinary samples. On the basis of their equol-producing capacity, individuals can be classified into equol producers and non-equol producers. There is as yet no standard criterion for this classification, as many studies use different criteria. A number of studies consider their subjects to be equol producers if their serum equol concentration is ≥5 ìg/L or their urinary equol is ≥20 ìg/L.[23] In the present study, a value of serum equol of ≥5 µg/L was used, while the limit of detection of the measuring instrument used for determining equol concentration was 0.5 ìg/L.[23]

The proportions of equol producers between countries show considerable variation, with the proportion of equol producers in Western countries being approximately 33%, while Japan and Korea, with their high dietary soy isoflavone consumption, show a proportion of approximately 55-60%.[33] In Indonesia there are not many study reports on the proportions of equol producers and non-equol producers. In the present study, serum isoflavone concentrations were determined twice, namely at baseline and at 3 months post-supplementation. At baseline, mean serum equol concentration was 7.5 ± 4.6 ìg/L and the proportion of equol producers was 27/39 (69.2%). Although no supplementation had yet been administered, the proportion of equol producers was already relatively high, because soy beans are much consumed by the subjects of this study in particular and by Indonesian communities in general. At 3 months post-supplementation the proportion of equol producers increased to 39/39 (100%). This indicates that mean equol concentration and the proportion of equol producers was already high since the start of the study, while the proportion increased at 3 months post-supplementation.

Differing results were found in the study by Vedrine et al.,[20] who reported that soy isoflavone supplementation for one month was unable to convert non-equol producers into equol producers. The modification of individual equol-producing status is as yet controversial and affected by a variety of factors. In contrast, Liu et al.[34] reported the existence of an association between age, isoflavone intake, and proportion of equol producers in ethnic Chinese. Supplementation of 41 mg genistein for 3 days was capable of raising the proportion of equol producers from 26.8% to 60.4%. Vergne et al.[27] reported that ethnic differences and dietary history play a role in the differing bioavailability of isoflavones in ethnic Asians and Caucasians. A one-time dose of isoflavone led to an increase in maximal isoflavone concentration ($C_{max}$) and a larger area under the plasma concentration time curve (AUC) in Asians as compared to Caucasians. On isoflavone administration for 10 days, $C_{max}$ and AUC were raised in Caucasians but not in Asians.[27] The factor of dietary background in Asian ethnic groups with a prior high consumption of isoflavones plays a role in inter-ethnic differences in bioavailability. Setchell [23] reported a proportion of equol producers of 33.3% in non-vegetarians and a higher proportion (51.7%) in vegetarians.

Lampe et al.[32] have explained inter-individual differences in metabolism of naturally occurring compounds. Intake of naturally occurring compounds does not always accurately reflect their tissue concentrations. Inter-individual differences may be due to genetic variability involving pharmacokinetic factors, such as absorption, distribution, and metabolism, thus resulting in different tissue

concentrations and modification of disease risk. Plant polyphenols including the isoflavones as phytoestrogens are extensively metabolized by intestinal bacteria. Inter-individual differences influencing the host bacteria are associated with isoflavone metabolism. Soy isoflavones contain flavonoids in the form of glycosides that are difficult to absorb. Under the influence of â-glycosidases from bacteria as well as the intestinal mucosa, these glycosides are hydrolyzed into readily absorbable aglycones, such that their absorption rate is influenced by the hydrolyzing capacity. Similarly to other naturally occurring compounds or xenobiotics, isoflavones also undergo biotransformation. Polymorphisms in biotransforming enzymes, such as glutathione S-transferase (GST), uridine 5'-diphosphate (UDP)-glucuronosyltransferase (UGT), sulfotransferase (SULT), cytochrome P-450, also play a role in the varying effects on natural compounds. Pharmacodynamic factors such as changes in target receptor sensitivity or mutations may affect the binding site of drug receptors, resulting in modification of drug effects.

The study conducted by Pusparini[35] on a population of Indonesian postmenopausal women irrespective of osteopenic status, showed a lower proportion of equol producers of 60.4%. In the present study, there exists the possibility that the specific subject characteristic of osteopenia in postmenopausal women also contributed to a more homogenous group of equol producers. It is apparent that there is a need for further extensive studies on the effects of dietary patterns on daidzein metabolizing capacity in various Indonesian communities, considering that equol producer status is highly dependent on the characteristics of intestinal bacteria, which are in turn affected by individual dietary patterns.

A history of high isoflavone intake in all study subjects since the start of the study has an important role in the equol-producing capacity of the subjects. High carbohydrate intake and a history of high longterm dietary isoflavone intakes in Indonesian communities in general, also constitute factors that affect isoflavone metabolism in the subjects of this study. There is a need for further studies to acquire a more comprehensive insight into the factors influencing equol-metablizing capacity.

This study has a number of limitations, the first being that the isoflavone intake levels of the study participants were probably underestimated, because the estimation was based on a questionnaire. Secondly, the isoflavone content of some modern processed protein-containing foods (e.g. white bread) could not be estimated because the ingredients and composition were not available. Finally, the effect of isoflavone supplementation may have been influenced by the fact that the dietary soy isoflavone intake of the study subjects could not be controlled, since they lived at their own homes.

## CONCLUSION

The increase in equol producer status following isoflavone supplementation in the present study demonstrates that administration of soy isoflavones 100 mg/day for 3 months resulted in increased equol-producing capacity.

## ACKNOWLEDGEMENTS

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