Original Article

Plasma isoflavones in Malaysian men according to vegetarianism and by age

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Epidemiological studies indicate lower prevalences of breast and prostate cancers and cardiovascular disease in Southeast Asia where vegetarianism is popular and diets are traditionally high in phytoestrogens. This study assessed plasma isoflavones in vegetarian and non-vegetarian Malaysian men according to age. Daidzein, genistein, equol (a daidzein metabolite), formononetin, biochanin A, estrone, estradiol and testosterone were measured by validated liquid chromatography tandem mass spectrometry (LCMSMS). Plasma isoflavone and sex hormone concentrations were measured in 225 subjects according to age (18-34, 35-44 and 45-67 years old). In all age groups, vegetarians had a higher concentration of circulating isoflavones compared with non-vegetarians especially in the 45-67 year age group where all isoflavones except equol, were significantly higher in vegetarians compared with omnivores. By contrast, the 18-34 year group had a significantly higher concentration of daidzein in vegetarians and significantly higher testosterone and estrone concentrations compared with non-vegetarians. In this age group there were weak correlations between estrone, estradiol and testosterone with some of the isoflavones. This human study provides the first Malaysian data for the phytoestrogen status of vegetarian and non-vegetarian men.

Key Words: isoflavones, vegetarian, sex-hormones, LCMSMS, Malaysians

INTRODUCTION

Epidemiological studies have shown that vegetarians have a lower incidence of several diseases including heart disease,¹ prostate and breast cancer.² This is the case for vegetarians living in western countries and those consuming plant based diets in developing countries.³

Although vegetarians are missing nutrients that are more available from meat and fish, they have a higher dietary exposure to some bioactive substances that are present in vegetables including phytochemicals. Among these phytochemicals are phytoestrogens - a group of plant-derived compounds that can mimic estrogens.

Phytoestrogens have been reported to be involved in the prevention of the above-mentioned diseases⁴⁻⁶ therefore it seems that the beneficial effect of a vegetarian diet is partly related to the presence of these phytoestrogens. There are two principal classes of phytoestrogens: the isoflavonoids and the mammalian lignans.⁷

Isoflavones are present in great amount only in soy and soy products. In fact soybeans contain glycoside conjugates of the isoflavonoids genistein and daidzein which can be metabolized by gut bacteria to produce their respective aglycones. Daidzein can be further metabolized to the estrogenic isoflavan, equol. Equol is an interesting metabolite because, in humans, only one third of the population is able to produce it⁸ and because in vitro, it has shown to have the highest estrogenic capacity.⁹

Phytoestrogens have the ability to affect sex hormone

levels by binding the estrogen receptor in cells, and blocking the action of estrogens. They can also manipulate sex hormones metabolism via other mechanisms. In fact phytoestrogens can directly increase hepatic sex hormone binding globulin (SHBG) synthesis.¹⁰ They can also inhibit enzymes related to testosterone metabolism such as 17 beta-hydroxysteroid dehydrogenase¹¹ and 5 alpha-reductase.¹² Although the mechanism of action of isoflavones is known in animals and in *in vitro* studies; little is known about their effect on sex hormone concentrations in human populations.

The main source of isoflavones is soy beans. On average, Asians consume 20 to 80 g of soy food daily consisting mainly of tofu, miso and tempeh.¹³ Malaysians consume soy products in their daily dietary intake, since the food consumption statistics for Malaysia¹⁴ indicated that intakes of tempeh and tofu were 6.9 g/daily and 19.4 g/daily, respectively. Haron et al¹⁵ described selected local soy products commonly consumed by Malaysians as:

Corresponding Author: Dr Wided Kouidhi, Department of Pharmacology Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. Tel: +603-7967 6619; Fax: +603-7967 5726 Email: wided.kouidhi@gmail.com Manuscript received 23 September 2014. Initial review completed 27 October 2014. Revision accepted 01 January 2015. doi: 10.6133/apjcn.2016.25.1.02 soy bean drinks, egg tofu, soft tofu, tofufah (soft tofu served in syrup normally taken as dessert by Malaysians) and fujook (tofu skin made by boiling soy milk in pan and then dried).

Because of the popularity of vegetarian diets in Southeast Asia, there is an important public health need to understand the relationship between a vegetarian soy isoflavones rich diet in men and sex hormone profiles. Therefore the purpose of the present cross-sectional study was to investigate the isoflavone and sex hormonal profiles in vegetarians and compare with the profiles in omnivores Malaysian men according to their age.

METHODS

Chemicals

Daidzein, genistein, formononetin, equol, biochanin A, estrone and estradiol were purchased from Sigma Aldrich, USA. Testosterone was purchased from Ridel-de-Haen, Germany and 4-hydroxybenzophenone (4-HBPH) used as the internal standard was from Merck, Germany. Crude solution of β glucuronidase of Type H-2, from *Helix* pomatia extract (Sigma Aldrich, United Kingdom) was used to hydrolyse glycoside conjugates of isoflavones. HPLC grade Methanol was purchased from Mallinckrodt, USA. Acetone was from JT Baker, USA. Sodium acetate anhydrous 99% (Alfa Aesar, Lancaster) was used to prepare buffer solution. All solvents were filtered using a vacuum filtration system with a 0.45μ membrane filter. Ammonium hydroxide was from Fisher Scientific. All solvent and water used for Liquid Chromatography Tandem Mass Spectrometry (LCMSMS) were filtered with Millipore membrane filter of 0.45 μ pore size for solvent and 0.2 µ for water. Purified water was from PureLab Option-Q system (18.2 MQ/cm). All samples were filtered with a 4 mm diameter syringe filter of regenerated cellulose by Phenomenex (0.2 μ pore size).

Stock standard solutions (1 mg/ mL) were prepared and stored at -20^oC. Working standard solutions of different concentrations were prepared daily by mixing aliquots of each individual stock solution and diluting with methanol.

Study population

This cross-sectional study included 225 healthy men, aged between 18 and 67 years, who practice vegetarian diets; they were recruited from various temples, religious associations and public advertisements in Malaysia. Controls (non-vegetarian males) were randomly selected from the public through a blood donation venue, after matching for age and race. The exclusion criteria were -any medical illness or any form of antibiotics medication. All subjects gave written consent to participate in the study. They were briefly interviewed for basic background information, and blood sample was taken. Blood samples were analysed for phytoestrogens: daidzein, genistein, formononetin, biochanin A, and equol, for endogenoussex-hormones: estrone, estradiol and testosterone. Ethical approval was granted by the Ethics Committee of the University Malaya Medical Centre (Ethics No. 607.6).

Plasma samples

About 6-10 mL of blood was collected from each subject.

Blood samples were then centrifuged at 4000 rpm for 10 minutes at room temperature. Separated plasma was stored in several aliquots in -80°C freezer until extraction. The sample extraction method was adapted from Chan et al¹⁶ An aliquot of 0.5 mL plasma with 0.5 ng internal standard was added to 3 mL of 50 mM sodium acetate buffer (pH 5) and mixed. Deproteinisation was done by adding 4 mL of acetone to the mixture and vortexed for 30 seconds. The sample was then transferred into a tube after centrifugation at 4000 rpm for 10 mins. The tube was placed into 40° C water bath and acetone was evaporated from the sample under a steady flow of nitrogen stream. Finally, 25 µL of β-glucuronidase enzyme was added and shaken gently. Sample was incubated in a water bath at 45° C for an hour.

LCMSMS

The solid phase extraction cartridges used were Strata-X 33 μ with polymeric reversed phase (60 mg/3 mL), supplied by Phenomenex, USA. The cartridges were fitted onto an IST Vacmaster manifold. The apparatus was connected to a vacuum pump. The cartridges were conditioned with 3 mL of 100% methanol followed by 3 mL of water. Sample was then loaded onto the SPE column, washed with water (5 mL) and eluted with methanol (4 mL). Using nitrogen stream, sample was dried and reconstituted with 250 μ L of 30% methanol, vortexed and filtered with 0.2 μ syringe filter before injection into LCMSMS.

LCMSMS was performed using a Shimadzu LC system consisting of a binary pump (LC-20AD), an autosampler SIL-20AC (set at 4°C), a column oven (set at 40°C) and a system controller (CBM-20A). All phytoestrogens and sex estrogens were separated in one single gradient run in negative mode while testosterone was run separately under a mix mode program. LCMSMS was carried out on an API-3200 QTrap (Applied Biosystems/MDS Sciex). Analyte was detected by Multiple Reaction Monitoring (MRM) mode and each analyte was monitored for two MRM transitions. Mass spectral data were analysed using Analyst 1.4.2. Software. A seven point calibration curve was included with each assay using calibrator concentrations from 1, 3, 10, 15, 30, 60 and 75 ng/mL. Limits of detection were between 0.20-0.5 ng/mL. Accuracy, precision and recovery range were within 85-115%, 2.7-15% and 58-202%, respectively.

Separation was performed on reversed phase C-18 Zorbax Extend column, 100 mm X 4.6 mm with 3.5 upacking coupled with a guard cartridge from Phenomenex. Gradient system with constant flow rate of 0.5 mL/min was used to separate the analytes. The mobile phase consisted of two eluents, solvent A (water/methanol at 98:2 by volume) and solvent B (100% methanol), both containing 0.01% ammonium hydroxide. Sample injection volume and total run time was 15 µL and 15 mins for negative mode and 10 µL and 8.51 mins for mix mode, respectively. In the negative mode, initial conditions were set at 30% solvent B, increased to 60% in 2 mins, and held for another 3 mins. This was further increased to 95% over the next 1.5 mins and held for another 3 mins. Gradient was immediately returned to initial condition of 30% solvent B and maintained for the final 6 mins of each run. Gradient for the mix mode was started at 65% of B and maintained for half a minute. This was increased to 95% over 2 mins and maintained for another 2 mins. Immediately, it was returned to the initial proportion of 65% of solvent B for the final 4 mins.

Statistics

Statistical analyses were performed by the non-parametric Mann-Whitney test as data were not normally distributed. Most of the data were skewed to the right. The data remained skewed even after log transformations were attempted. Spearman correlations were performed to examine the relationship between the phytoestrogens and the natural hormones among the studied population and 95% confidence internal (CI) were calculated. Spearman correlations were not in a normal bivariate distribution. All the statistical analysis was performed using SPSS 15 (SPSS, Inc., Chicago, IL, USA). Significance was accepted at p<0.05.

RESULTS

Table 1 shows the characteristics of the study population. The duration of being vegetarian ranged from three months to more than 55 years, with a mean of 16.4 years. In response to the reason for being vegetarian, the majority cited religious belief (71.7%) while others stated personal health (12.1%) and environmental and ecological concerns (10.1%) as the answer. Since there are variations among equal producers in the population, some researchers use the ratio of urinary concentration of equoldaidzein to categories equol producers¹⁷ while others take the limit of quantification (LOQ) as the cut-off point. We defined equol producers as those with equol plasma concentration exceeding LOQ (0.5 ng/mL).¹⁸ Setchell and Cole however in 2006 have proposed another method to define equol producers in vegetarians but their method requires equol concentrations in urine which were not measured in our current study.¹⁷

There were more equal producers among vegetarians (41.7%) than non-vegetarians (24.6%) in this study. The majority (90.2%) of the vegetarians were found to have two or more types of isoflavone detected while only 66.4% of the non-vegetarians have more than 2 isoflavone types.

Ethnicity is considered as a confounding factor, in this study. Therefore, we divided the vegetarians into four sub-groups; Lacto-ovovegetarian (consume dairy and egg products), Lactovegetarian (consume dairy products but exclude eggs), Ovovegetarian (consume egg products but exclude dairy products) and Vegan (exclude any kind of animal products) according to their ethnicity (Table 2).

Table 3 indicates that the majority of the young generation 18-34 years were Lacto-ovovegetarians as well as the 35-44 years group, while in the 45-67 years group the majority were vegans.

Table 4 compares the median analyte concentration between vegetarians and non-vegetarians for the age groups 18-34, 35-44 and 45-59 years old, respectively.

Vegetarians show higher median plasma concentration for the majority of the analytes for all age groups. In the young group, 18-34 years only daidzein concentrations were significantly higher in vegetarians compared to omnivores, while in the 35-44 years old group daidzein and genistein were found to be significantly higher in vegetarians. Vegetarians of 45-67 years group had higher concentrations of all isoflavones except equol compared to the other age groups. In this age group; daidzein, genistein, formononetin, biochanin A were found to be significantly higher in vegetarians than non-vegetarians. Testosterone and estrone were found to be significantly higher only in vegetarians in the age group 18-34 years.

We then investigated the relationship between the analyzed isoflavones and sex hormones among the studied population. Correlations were performed in each age

Table 1. Parameters an	d data c	observed	from the	ne study
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Variable	Male veget	arian (n=103)	Male non-vegetarian (n=122)		
variable	Mean (SD)	Frequency (%)	Mean (SD)	Frequency (%)	
Ethnicity					
Chinese		30 (29.1)		33 (27)	
Indian		63 (61.2)		69 (56.6)	
Others		10 (9.7)		20 (16.4)	
Age	39.0 (10.7)		38.1 (9.92)		
Smoking					
Yes		7 (6.8)		35 (28.7)	
No		96 (93.2)		87 (71.3)	
Drinks coffee					
Yes		79 (76.7)		89 (73)	
No		24 (23.3)		33 (27)	
Drinks alcohol					
Yes		14 (13.6)		40 (32.8)	
No		89 (86.4)		82 (67.2)	
Equol producer		43 (41.7)		30 (24.6)	
Number of types of isoflavone detected					
0		6 (5.8)		26 (21.3)	
1		4 (3.9)		15 (12.3)	
2		17 (16.5)		16 (13.1)	
3		17 (16.5)		16 (13.1)	
4		40 (38.8)		26 (21.3)	
5		19 (18.4)		23 (18.9)	

	Ethnicity						
Vegetarian status	Chinese		Indian		Others		Total
c	n	%	n	%	n	%	•
Non-vegetarian	33	27.0	69	56.6	20	16.4	122
-		52.4		52.3		60.0	
Lacto-ovovegetarian	12	30.0	22	55.0	6	15.0	40
c		19.0		16.7		24.0	
Lactovegetarian	8	22.2	27	75.0	1	2.8	36
c		12.7		20.5		4.0	
Vegan	10	41.7	12	50.0	2	8.3	24
-		15.9		9.1		8.0	
Ovovegetarian	0	0.0	2	66.7	1	33.3	3
2		0.0		1.5		4.0	
Total	63		132		30		225

Table 2. Frequency and percentage of vegetarian subgroup status according to ethnicity

Table 3. Frequency and percentage of vegetarian subgroup status according to age group

	Age (years)						
Vegetarian status	18-34		35-44		45-67		Total
	n	%	n	%	n	%	
Non-vegetarian	44	36.1	44	36.1	34	27.8	122
-		52.4		60.3		50.0	
Lacto-ovovegetarian	19	47.5	13	32.5	8	20.0	40
-		22.6		17.8		11.8	
Lactovegetarian	13	36.1	11	30.6	12	33.3	36
		15.5		15.1		17.6	
Vegan	6	25.0	4	16.7	14	58.3	24
		7.1		5.5		20.6	
Ovovegetarian	2	66.7	1	33.3	0	0	3
-		2.4		1.3		0	
Total	84		73		68		225

Table 4. Median plasma concentrations (ng/mL) of analytes in vegetarian and non-vegetarian groups aged between 18-34, 35-44 and 45-67 years old

	Median plasma o	concentration (IQR)			
Analyte	Vegetarian	Non-vegetarian	z statistics [†]	p value [†]	
	(ng/mL)	(ng/mL)			
18-34 years old (n)	40	44			
Daidzein	9.9 (25.5)	2.6 (8.12)	-2.06	0.039	
Genistein	20.9 (55.1)	8.9 (32.8)	-0.83	0.407	
Equol	0.0 (5.35)	0.0 (0.82)	-1.48	0.139	
Formononetin	2.6 (14.0)	1.4 (11.1)	-1.16	0.247	
Biochanin A	1.7 (10.7)	1.9 (10.5)	-0.18	0.860	
Estrone	0.7 (1.26)	0.0 (0.07)	-4.59	< 0.001	
Estradiol	0.0 (0)	0.0 (0)	-0.43	0.666	
Testosterone	2.4 (2.45)	1.7 (1.77)	-2.64	0.008	
35-44 years old (n)	29	44			
Daidzein	7.3 (43.0)	0.0 (4.06)	-3.79	< 0.001	
Genistein	15.2 (91.9)	4.9 (34.3)	-2.27	0.023	
Equol	0.0 (1.08)	0.0 (0.11)	-1.38	0.169	
Formononetin	1.7 (13.0)	0.0 (10.9)	-0.89	0.371	
Biochanin A	1.3 (7.99)	0.1 (8.32)	-0.48	0.633	
Estrone	0.0 (0.58)	0.0 (0.11)	-0.97	0.331	
Estradiol	0.0 (0)	0.0 (0)	-0.13	0.895	
Testosterone	1.4 (1.41)	1.2 (1.37)	-0.07	0.946	
45-67 years old (n)	34	34			
Daidzein	19.8 (37.8)	0.1 (6.37)	-4.92	< 0.001	
Genistein	50.8 (97.1)	3.1 (15.8)	-4.35	< 0.001	
Equol	0.0 (4.82)	0 (1.06)	-1.14	0.255	
Formononetin	6.6 (23.8)	0 (12.9)	-2.34	0.019	
Biochanin A	4.5 (20.9)	0.5 (8.57)	-2.05	0.040	
Estrone	0.0 (1.08)	0.0 (0)	-2.38	0.170	
Estradiol	0.0(0)	0.0 (0)	-0.04	0.965	
Testosterone	1.8 (6.76)	1.4 (1.51)	-0.46	0.646	

[†]Mann-Whitney test.

Variable	Estrone	p value	Estradiol	p value	Testosterone	p value
18-34 years old (n=84)		-		-		
Daidzein	0.03*	0.028	0.32^{*}	0.003	0.27^{*}	0.014
Genistein	0.09	0.095	0.25^{*}	0.020	0.28^{*}	0.009
Equol	0.17	0.166	0.22^{*}	0.049	-0.11	-0.338
Formononetin	0.35^{*}	0.001	0.25^{*}	0.021	0.13	0.229
Biochanin A	0.15	0.147	0.31*	0.004	0.06	0.563
35-44 years old (n=73)						
Daidzein	0.18	0.131	0.06	0.594	0.06	0.598
Genistein	0.20	0.085	-0.04	0.747	0.14	0.223
Equol	0.01	0.966	-0.04	0.765	0.22	0.060
Formononetin	0.35^{*}	0.002	0.20	0.088	0.00	0.997
Biochanin A	0.11	0.334	0.23^{*}	0.048	-0.03	0.786
45-67 years old, n=68						
Daidzein	0.35^{*}	0.004	0.19	0.125	0.35^{*}	0.004
Genistein	0.26^{*}	0.036	0.11	0.392	0.32^{*}	0.007
Equol	0.07	0.586	0.03	0.821	-0.03	0.811
Formononetin	0.40^{*}	0.001	0.22	0.073	0.38^{*}	0.002
Biochanin A	0.22	0.070	0.13	0.288	0.29^{*}	0.016

Table 5. Correlation coefficients between the analytes by each age group

*Spearman correlation is significant at the 0.05 level (2-tailed).

group (refer Table 5) to investigate the associations between isoflavone and sex-hormone concentrations. Based on Munro (2000), when the rho value=0.0-0.25 (very low or no correlation) and 0.26-0.49 (low correlation). All significant correlations shown in table 5 are positive correlations. In the group 18-34 years old, estrone was found to have significantly low correlation with formononetin while estradiol had significantly but low correlation with all the isoflavones. Testosterone has a significant low correlation with only daidzein and genistein. For the age group of 35-44 years old, none of the isoflavones were found to have any significant correlations with testosterone, and only weak correlations were found between estrone and formononetin and between estradiol and biochanin A. Concerning the age group of 45-67, estrone was significantly but weakly correlated to daidzein, genistein and formononetin. Testosterone was found to have significantly low correlation with all isoflavones except with equol.

DISCUSSION

Our present study provides comparative data on plasma concentrations of selected phytoestrogens, such as daidzein, genistein, formononetin, biochanin A and equol (a metabolite of daidzein) and sex steroid hormones (estrone, estradiol and testosterone), among vegetarian and nonvegetarian Malaysian men.

The median plasma concentration of analytes revealed interesting results regarding age groups. In the young generation (18-34 years), only a slight increase of daidzein in the vegetarian group was observed compared with the omnivores and surprisingly significant greater levels of the estrone and testosterone concentrations in vegetarians were shown. These results are in contradiction to the another study of Howie and Shultz (1985) who reported lower plasma testosterone and estradiol concentrations in male vegetarians than in omnivores,¹⁹ but the majority of the studies that have compared plasma androgens and estrogens in vegetarians and meat eaters do detect any differences.²⁰⁻²² In these studies the age of the subjects

were up to 40 years; in fact these results are more consistent with the sex hormones concentrations found in our study, in the middle age group (35-44 years) and in the group (45-67 years). Moreover, when compared with the reference ranges of normal sex-hormone concentrations our results seem to correspond to the normal levels: the testosterone concentration evaluated in our study corresponded to the bioavailable testosterone which vary in normal healthy men, aged between 20-39 years, from 0.8 to 2.3 ng/mL, and in men aged between 40-59 years from 0.5 ng/mL to 1.9 ng/mL²³, our results showed in the group (18-34 years) a value of 1.7 ng/mL in nonvegetarians and 2.4 ng/mL in vegetarians which is slightly higher than the normal values. In the groups (35-44 years) and (45-67 years) the concentration of testosterone varied from 1.2 to 1.8 ng/mL between vegetarians and non-vegetarians, which is in the reference ranges. Concerning the concentration of estrone, the normal ranges in healthy adults men are between 0.001 and 0.06 ng/mL, and our results are in this range except for the vegetarians of the group (18-34 years) where the estrone level was much higher (0.7 ng/mL). The reason for significantly higher concentration of estrone despite the lack of change in estradiol concentrations in the vegetarian young group compared to the omnivore is not clear. This is especially so since that we have shown that the estrone concentration is weakly correlated with the presence of isoflavones and there is no other study that has examined the estrone concentration in vegetarians. Therefore this variability of sex hormones seems to be related to another mechanism. In men, circulating estradiol is derived partly from direct testicular secretion and partly from peripheral aromatization of testosterone, whereas circulating estrone is derived predominantly from peripheral conversion of delta 4androstenedione. Since we did not measure serum and rostenedione it is difficult to conclude. Moreover inflammatory markers such as interleukin-6 and interleukin-11 and cytokine have a role in the regulation of aromatization as described previously and cannot be excluded.²⁴

In contrast, vegetarians aged between 45 and 67 years

have different isoflavone and hormone profiles, with all the isoflavones except equal being higher in vegetarians compared with omnivores, and no difference in the level of sex hormones was observed. This generation gap in the isoflavone concentrations might be explained by the change in dietary habits of the young generation of Malaysians who prefer fast food.²⁵ Young vegetarians these days can have access to more fried food, soda, macaroni and cheese. The majority of the vegetarians in this age group are lacto-ovovegetarians, therefore they can have a high intake of highly processed foods which are rich in carbohydrates, which could explain in part the higher significant testosterone concentration in the young vegetarian generation since a high carbohydrate diet can increase testosterone concentration.²⁶ The older generation on the other hand are more vegan, who traditionally prefer the intake of soybean products, which include tempeh, tofu and soymilk which contain 31-35 mg/100 g,¹⁵ 16.2- $31.2 \text{ mg}/100 \text{ g}^{27}$ 7.6-19.9 mg/100 g²⁷ of isoflavone, respectively. This could explain the higher isoflavone concentrations compared with the non-vegetarians and compared with the young generation.

Over the past decade, researchers have obtained evidence suggesting that soy isoflavones and their metabolites may be beneficial for the prevention or treatment of certain diseases. Some population-based studies have shown that a vegetarian diet is associated with a reduced risk of prostate cancer, cardiovascular and diabetes type 2.²⁸⁻³⁰ But our results in the young vegetarians (18-34 years) are in contrast with the majority of the studies on vegetarians which have shown health benefits, for the reason that a high estrone concentration is associated with an increased incidence of diabetes type 2 in men³¹ and a high level of testosterone is associated with a high risk for prostate cancer.³²

The present study has several limitations. First, this cross-sectional study lacks detail concerning potential confounding factors such as body composition and dietary intake. The differences in hormone concentrations between vegetarians and non-vegetarians in the young generation could be attributed to factors such as Body Mass Index (BMI) because BMI in men can influence the concentration of testosterone.³³ Again, exercise (training and sport) can raise the level of testosterone³⁴ and vitamin D as can.³⁵ Nonetheless, the present study indicates that the new generation of vegetarians in Malaysia may not have as favourable an isoflavone profile as previous generations. Further work is needed to assess the health risk related to diet in the young generation of Malaysian men and presumably, women.

To our knowledge, this study is the first assessment of phytoestrogen distribution among vegetarians and non-vegetarians in Malaysia. It establishes the baseline concentrations of selected isoflavones in male vegetarian and non-vegetarians according to age by using a sensitive analytical method with liquid tandem mass spectrometry. It adds to existing databases for maternal and cord blood,^{36,37} on compounds such as plant oestrogens and potential endocrine disruptors, in Malaysia.

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AUTHOR DISCLOSURES

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Original Article

Plasma isoflavones in Malaysian men according to vegetarianism and by age

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马来西亚男性血浆异黄酮浓度因素食和年龄而不同

流行病学研究表明东南亚乳腺癌、前列腺癌和心血管疾病的发病率较低,素食 在这些地区备受欢迎,传统饮食中富含植物雌激素。本研究根据年龄评估了马 来西亚素食和非素食男性血浆中异黄酮的含量。通过高效液相色谱-串联质谱 法(LCMSMS)测定血浆中大豆异黄酮、染料木素、雌马酚(一种大豆异黄 酮的代谢产物)、芒柄花素、鹰嘴豆芽素A、雌酮、雌二醇和睾酮的浓度。按 照年龄(18-34岁、35-44岁和45-67岁)测定了225名研究对象的血浆异黄酮 和性激素的浓度。在所有年龄组,尤其是45-67岁组,素食者循环异黄酮浓度 高于非素食者,在45-67岁组,除雌马酚外的所有异黄酮,素食者均显著高于 杂食者。相反,在18-34岁组,素食者的大豆异黄酮、睾酮和雌酮的浓度显著 高于非素食者。在这个年龄组中,雌酮、雌二醇和睾酮与一些异黄酮之间有弱 的相关性。本研究提供了马来西亚素食和非素食男性植物雌激素状态的第一手 资料。

关键词:大豆异黄酮、素食者、性激素、LCMSMS、马来西亚人