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行政院國家科學委員會補助專題研究計畫成果報告

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- ☐赴國外出差或研習心得報告一份
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中 華 民 國 91 年 8 月 20 日

行政院國家科學委員會專題研究計畫成果報告

臺灣烏腳病盛行地區高血壓、糖尿病與砷誘發之活性氧物種與抗氧化防禦系統之相關性研究

The Relationship between Arsenic Induced Reactive Oxygen Species, Antioxidant Defense System and Hypertension and Diabetes in Blackfoot Disease Hyperendemic Area in Taiwan

計畫編號: NSC 90-2320-B-038-021
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主持人:薛玉梅教授 台北醫學院醫學系公共衛生學科

一、中文摘要

針對烏腳病盛行地區探討含錳超氧歧化酵素（MnSOD）基因多形性和高血壓之間的關係。以30歲以上並且每週居住在研究地區至少五天的居民作為研究對象，並利用78年收集的資料以及78–86年間所採集的生物檢體。兩位公共衛生護士以結構式問卷訪視每位研究對象，問卷內容包括社會人口學資料、抽菸與喝酒習慣、職業史、居住史、飲水史與家族疾病史等項目。血球萃取出之DNA利用聚合酵素連鎖反應及限制片段長度多形性分析含錳超氧歧化酵素粒線體目標序列Val/Ala變異。利用高效率液相層析儀分析血清中微量營養元素包括維生素A、維生素E、蕃茄紅素、β–胡蘿蔔素。調整年齡、性別、身體質量指數、累積砷暴露等重要危險子後，含錳超氧歧化酵素基因型為Val/Ala和Ala/Ala者不論三酸甘油脂及維生素E濃度為何，危險對比值有上升趨勢。調整含錳超氧歧化酵素基因型等其他重要危險因子之後，不論內皮一氧化氮合成酵素基因型是Glu/Glu或Glu/Asp和Asp/Asp者，隨著累積砷暴露或身體質量指數的增加，高血壓危險性顯著增加。調整了年齡和性別後，帶有Val/Val基因型且累積砷暴露越低、身體質量指數正常、三酸甘油脂濃度越低者，高血壓的相對危險性有下降的趨勢。因此本研究推論含錳超氧歧化酵素基因多形性可能是高血壓基因上的一個易感受性因子，基因型的不同可能修飾個體罹患高血壓的危險性，而後天環境的暴露、生活及飲食習慣更是影響高血壓相當重要的危險因子。

關鍵詞：含錳超氧歧化酵素、無機砷、高血壓、微量營養元素

Abstract

The relationship between hypertension and manganese superoxide dismutase (MnSOD) gene polymorphism has been studied in the blackfoot disease endemic area. The study subjects who were over 30 years old and lived at least 5 days a week in the three villages were recruited. The data of study subjects were collected in 1989 and biological samples were obtained from 1989 to 1997. Two well-trained public health nurses performed a standardized personal interview based on a structured questionnaire. Information obtained from the interview included demographic characteristics, alcohol drinking and cigarette smoking, working and residential history, history of well water consumption as well as family

history of hypertension. Moreover, DNA was extracted from buffy coat to analyze the Val/Ala variant in the mitochondrial targeting sequence in *MnSOD* gene utilizing polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Serum was measured for all trans-retinol, α-tocopherol, lycopene, β-carotene by high performance liquid chromatography (HPLC). After adjustment for age, sex, body mass index (BMI) and cumulative arsenic exposure, *MnSOD* Val/Ala and Ala/Ala genotype people had higher relative risk than *MnSOD* Val/Val genotype people in any concentration of triglyceride and vitamin E. After adjustment for *MnSOD* genotype and other important risk factors, The relative risk of hypertension was significantly increased with cumulative arsenic exposure or BMI increasing whether *eNOS* Glu/Glu genotype or Glu/Asp and Asp/Asp genotype. The relative risk of hypertension for *MnSOD* Val/Val genotype people in lower cumulative arsenic exposure, normal BMI and lower triglyceride level was decreased after age and sex were adjusted. The results of this study suggested that *MnSOD* gene polymorphism may be a genetic susceptible factor of hypertension and *MnSOD* genotype may modify individual hypertension risk. Acquired environment exposure, life style and dietary habit were also very important risk factors for hypertension.

Keywords：MnSOD, Inorganic arsenic, Hypertension, Micronutrients

二、緣由與目的

砷是地殼中廣布於各種化合物的元素，在環境中主要由水運輸傳佈，一般族群經由醫藥、環境和職業暴露暴露於無機砷。烏腳病為台灣西南沿海地區的地方性疾病，當地居民飲用高砷井水已達五十年之久。砷已經被公認為烏腳病主要危險因子之一，有研究指出烏腳病患罹患皮膚癌、肺癌、肝癌、膀胱癌、腎臟癌和缺血性心臟疾病等的危險性較非烏腳病患偏高（1）。

台灣流行病學研究指出烏腳病盛行地區居民高血壓盛行率比台灣其他鄉鎮顯著偏高（2，3），1995年的研究也發現調整年齡、性別後，烏腳病盛行地區居民高血壓盛行率比非烏腳病地區居民顯著偏高，且飲用水中的平均砷濃度、累積砷暴露和高血壓盛行率之間均呈劑量效應關係（1）。所以無機砷暴露會導致高血壓的發生，至於無機砷引起高血壓機制仍有待進一步的研究。

近幾年研究指出無機砷可以誘發超氧化自由基（$O_2^{\cdot -}$）生成，造成過氧化氫（H_2O_2）累積，進而生成氫氧根自由基（$OH^{\cdot}$）（4-6）。此外，細胞正常代謝過程中或是酵素系統包括粒線體電子傳遞鏈、黃嘌呤氧化酵素（xanthine oxidase）、環氧化酵素（cyclooxygenase）、脂質氧化酵素（lipoxygenase）、一氧化氮合成酵素（NO synthase）、血基質氧化酵素（heme oxygenases）、過氧化酵素（peroxidases）和NADH氧化酵素（NADH oxidases）等，也會生成氧自由基。這些氧自由基活性大，可能會改變轉錄因子的活性、調節細胞的訊號路徑、攻擊脂質或是造成血壓升高、改變血管的反應性，而引起血管發炎反應或是血管平滑肌細胞的增生、肥大，造成血管傷害形成（7）。

生物體內具有抗氧化防禦系統，包括抗氧化酵素及抗氧化物來移除活性氧及自由基。抗氧化酵素包括超氧歧化酵素（superoxide dismutase, SOD）、觸酶（catalase）以及麩氨基硫過氧化酵素（glutathione peroxidase, GPX）（8）；抗氧化物如維生素E、維生素C、β－胡蘿蔔素、麩氨基硫化合物（glutathione, GSH）（9）和蕃茄紅素（10）等。超氧歧化酵素已知有三種形式：（一）粒線體中的含錳超氧歧化酵素（MnSOD）；（二）細胞質中的銅鋅超氧歧化酵素（CuZnSOD）；（三）細胞外的銅鋅超氧歧化酵素（EC-SOD）（8）。

細胞粒線體中消耗約90%的氧氣，所

以在電子傳遞系統中生成的超氧化自由基，可由含錳超氧歧化酵素清除（11）。內皮細胞除了正常代謝過程生成的超氧化自由基之外，左旋精氨酸（L-arginine）轉換成左旋瓜氨酸（L-citrulline）過程中經內皮一氧化氮合成酵素（eNOS）催化會產生一氧化氮（NO），一氧化氮會進入鄰近的平滑肌細胞，促進GTP轉變成cGMP，此可使平滑肌鬆弛，降低血壓（12），但如果內皮細胞中有過多的超氧化自由基就可能和一氧化氮發生反應，生成過氧亞硝酸鹽（peroxynitrite, $ONOO^-$），因而造成含錳超氧歧化酵素蛋白質殘基產生硝酸化的情形，促使含錳超氧歧化酵素不活化（13）。所以是否因為含錳超氧歧化酵素未適當清除超氧化自由基，使得超氧化自由基與一氧化氮發生反應，降低了一氧化氮的含量，引起平滑肌收縮、血壓上升，進而造成高血壓，仍有待進一步研究。

有研究指出含錳超氧歧化酵素粒線體目標序列基因多形性和罹患帕金森氏病與乳癌的危險性有關，特別是有丙氨酸對偶基因（alanine allele）者有較高的危險性（8，14，15）。此外，內皮一氧化氮合成酵素麩氨酸298天門冬氨酸錯義變異（Glu298Asp missence variant）與人類本態性高血壓有顯著相關（16）；Glu298Asp變異在英國研究也發現與冠狀痙攣性心絞痛、心肌梗塞有相關（17）。除了抗氧化酵素的研究之外，最近許多研究也發現血清中微量營養元素和心血管疾病之間的關係，台灣研究指出砷誘發的缺血性心臟疾病和血清中的α－，β－胡蘿蔔素量之間有逆向的劑量效應關係存在（18），突尼西亞的研究則指出維生素E較低和提高低密度脂蛋白（LDL）氧化作用有關，所以維生素E可能可以預防致動脈粥狀硬化（19）。

無機砷引起高血壓，可能是由無機砷誘發反應性氧物種（ROS）所造成，而反應性氧物種的清除與含錳超氧歧化酵素以及血清中微量營養元素有關。內皮一氧化氮合成酵素所衍生的一氧化氮可能會與超氧化自由基進一步生成過氧亞硝酸鹽，降低內皮細胞中一氧化氮的含量，使血壓升高而引起高血壓的發生。由無機砷引起的高血壓和含錳超氧歧化酵素、血清中的微量營養元素以及內皮一氧化氮合成酵素基因多形性之間的關係，值得進一步研究。因此本研究選取烏腳病盛行地區高血壓患者和健康民眾為研究對象，探討含錳超氧歧化酵素粒線體目標序列基因多形性和高血壓之間的關係，並且評估高血壓傳統危險因子、慢性砷暴露、血清中微量營養元素以及內皮一氧化氮合成酵素基因多形性是否會影響兩者之間的關係。

三、結果與討論

表1指出高血壓患者有97人，其中以女性居多。高血壓患者的平均年齡約為54歲比血壓正常者的46歲顯著偏高，而且年齡每增加一歲罹患高血壓的危險性也隨之增加。另外，在調整年齡、性別後，抽菸、喝酒、教育程度和高血壓盛行率之間並無相關性存在。身體質量指數增加時，罹患高血壓的危險性也隨之增加，當身體質量指數為過重的情況，高血壓的危險性為2.0倍，如果到達肥胖的程度，危險性則為2.4倍，顯著偏高。

表1 血壓正常與高血壓者社會人口學特性及危險對比值分析

變項	血壓正常 人數（%）	高血壓 人數（%）	危險對比值[a] （95%信賴區間）
性別[b]			
男性	94（40.9）	40（41.2）	1.0
女性	136（59.1）	57（58.8）	1.1（0.7-1.9）
年齡（歲）[c]	46.6±0.6	54.4±0.7[d,*]	1.1（1.1-1.2）
抽菸			
沒有	189（82.2）	81（83.5）	1.0
有	41（17.8）	16（16.5）	0.6（0.3-1.4）
喝酒			
沒有	204（88.7）	88（90.7）	1.0
有	26（11.3）	9（9.3）	0.7（0.3-1.6）
教育程度			
不識字	66（28.7）	32（33.0）	1.0
小學程度	105（45.7）	52（53.6）	1.4（0.8-2.6）
國中程度以上	59（25.6）	13（13.4）	1.0（0.4-2.4）
身體質量指數（kg/m^2）			
<24	105（45.7）	23（23.7）	1.0[#,*]
24-26	64（27.8）	34（35.1）	2.0（1.0-3.8）
≧27	61（26.5）	40（41.2）	2.4（1.2-4.5）

[a]調整年齡、性別 [b]只有調整年齡 [c]只有調整性別 [d]T檢定 [#]趨勢檢定 [*]P<0.05

年齡增加使人類逐漸步入老化，隨之而來就是慢性疾病的發生，包括高血壓、動脈粥狀硬化和心血管疾病等，所以年齡是很重要的危險因子，此外，身體質量指數是衡量人類體態的一個指標，當指數值越高，即過重或肥胖的程度，高血壓或心血管疾病的危險性就會增加。

分析前依血壓正常者的慢性砷暴露指標先以三分位分層。表2指出調整年齡與性別後，隨著烏腳病地區居住年數、飲用深井水年數和累積砷暴露的增加，高血壓的危險對比值也隨之增加。如果居住年數越久、深井水飲用年數越長，高血壓的危險對比值大約是1.5倍到3.0倍之間，但累積砷暴露為4.8 ppm×年以上時，高血壓的危險性則達5倍以上，顯著偏高，因此慢性砷暴露指標對高血壓的影響是不容忽視的。

表 2 研究對象高血壓盛行率與慢性砷暴露指標危險對比值分析

變項	血壓正常 人數（%）	高血壓 人數（%）	危險對比值[a] （95%信賴區間）
烏腳病地區居住年數			
<32	77（33.5）	10（10.3）	1.0[#,*]
32-44	74（32.2）	31（32.0）	2.5（1.1-5.8）
≧45	79（34.3）	56（57.7）	2.9（1.3-6.4）
飲用深井水年數			
<3	71（30.8）	12（12.4）	1.0[#,*]
3-16	80（34.8）	26（26.8）	1.5（0.7-3.4）
≧17	79（34.4）	59（60.8）	2.3（1.1-5.0）
累積砷暴露[b]（ppm×年）			
<4.8	58（32.6）	4（5.0）	1.0[#,*]
4.8-14.7	60（33.7）	27（33.8）	5.6（1.8-17.6）
≧14.8	60（33.7）	49（61.2）	5.8（1.9-18.1）

[a]調整年齡、性別 [b]69人無資料 [#]趨勢檢定 [*]P<0.05

表3中族群含錳超氧歧化酵素Ala/Ala基因型頻率約1.7%。在調整年齡、性別後，含錳超氧歧化酵素Val/Ala和Ala/Ala基因型者，高血壓的危險對比值是Val/Val基因型者的1.8倍，有顯著邊緣性。高血壓病患含錳超氧歧化酵素Ala對偶基因頻率為22.8%比血壓正常者的17.3%高，但未達統計上的差異。另外，內皮一氧化氮合成酵素基因多形性和高血壓的盛行率之間並未看到相關性，而高血壓病患內皮一氧化氮合成酵素Asp對偶基因頻率為8.2%比血壓正常者的10.2%低，但沒有顯著差異。

表3 研究對象高血壓盛行率與含錳超氧歧化酵素、內皮一氧化氮合成酵素基因多形性的相關性分析

變項	血壓正常 人數（%）	高血壓 人數（%）	危險對比值[a] （95%信賴區間）
含錳超氧歧化酵素基因型[b]			
Val/Val	141（66.8）	45（57.0）	1.0
Val/Ala	67（31.8）	32（40.5）	1.8（1.0-3.2）[*]
Ala/Ala	3（1.4）	2（2.5）	
內皮一氧化氮合成酵素基因型[c]			
Glu/Glu	171（81.1）	66（83.5）	1.0
Glu/Asp	37（17.5）	13（16.5）	0.9（0.4-1.9）
Asp/Asp	3（1.4）	0（0.0）	
含錳超氧歧化酵素對偶基因[d]	血壓正常（211人）	高血壓（79人）	
Ala	17.3 %	22.8 %	P>0.05[#]
Val	82.7 %	77.2 %	
內皮一氧化氮合成酵素對偶基因	血壓正常（211人）	高血壓（79人）	
Asp	10.2 %	8.2 %	P>0.05[#]
Glu	89.8 %	91.8 %	

[a]調整年齡、性別 [b]37人無資料 [c]37人無資料 [*]P=0.05 [#]Z檢定
[d]對偶基因頻率：$Ala_{高血壓}$ = （32+2×2）/（79×2）×100% = 22.8%
$Ala_{血壓正常}$ = （67+3×2）/（211×2）×100% = 17.3%

表 4 中含錳超氧歧化酵素基因型為 Val/Val 者，三酸甘油脂濃度小於 111 mg/dl 與維生素 E 濃度在 4.20 μg/ml 以上為基準，危險對比值為 1.0。調整重要的危險因子之後，基因型為 Val/Val 者三酸甘油脂偏高或維生素 E 濃度偏低者，高血壓相對危險性偏高。基因型為 Val/Ala 和 Ala/Ala 者不管三酸甘油脂濃度及維生素 E 濃度為何，危險對比值有上升的趨勢。模式一中含錳超氧歧化酵素基因型為 Val/Ala 和 Ala/Ala 且三酸甘油脂濃度在 111 mg/dl 以上，危險對比值為 3.3。模式二中含錳超氧歧化酵素基因型為 Val/Ala 和 Ala/Ala 時且維生素 E 濃度小於 4.20 μg/ml 時，危險對比值為 3.2，呈統計顯著性，所以或許可推論維生素 E 對高血壓是具有保護作用的。另外，不管模式一或二在調整含錳超氧歧化酵素基因多形性與三酸甘油脂及維生素 E 組合後，累積砷暴露和身體質量指數對高血壓的影響依然存在，所以累積砷暴露和身體質量指數的重要性是不容忽視的。

表4 研究對象高血壓盛行率與含錳超氧歧化酵素基因多形性和三酸甘油脂、維生素E分層的多變項分析

變項		人數 正常/高血壓	模式一 危險對比值[a] (95%CI)[c]	模式二 危險對比值[b] (95%CI)
含錳超氧歧化酵素基因型[d]	三酸甘油脂[e] (mg/dl)			
Val/Val	<111	70/19	1.0[f,*]	
Val/Val	≧111	69/26	1.4 (0.6-3.3)	
Val/Ala和Ala/Ala	<111	37/11	1.6 (0.6-4.5)	
Val/Ala和Ala/Ala	≧111	33/23	3.3 (1.2-9.0)	
含錳超氧歧化酵素基因型	維生素E (μg/ml)			
Val/Val	≧4.20	69/20		1.0[f,*]
Val/Val	<4.20	72/25		1.5 (0.7-3.6)
Val/Ala和Ala/Ala	≧4.20	36/17		1.9 (0.7-5.0)
Val/Ala和Ala/Ala	<4.20	34/17		3.2 (1.2-8.5)
累積砷暴露[g] (ppm×年)				
<10.2		86/16	1.0	1.0
≧10.2		92/64	3.4 (1.5-8.0)	3.5 (1.5-8.3)
身體質量指數 (kg/m^2)				
<24		105/23	1.0	1.0
≧24		125/74	2.2 (1.0-4.5)	2.4 (1.1-4.9)

[a]調整年齡、性別 [b]調整年齡、性別及三酸甘油脂 [c]95% CI：95%信賴區間 [d]37人無資料 [e]3人無資料 [g]69人無資料 [f]趨勢檢定 [*]P<0.05

表5 高血壓盛行率與內皮一氧化氮合成酵素基因多形性和累積砷暴露、身體質量指數分層的多變項分析

變項		人數 正常/高血壓	模式一 危險對比值[a] (95%CI)[b]	模式二 危險對比值[f] (95%CI)
內皮一氧化氮合成酵素基因型[c]	累積砷暴露[d] (ppm×年)			
Glu/Glu	<10.2	65/8	1.0[f,*]	
Glu/Asp和Asp/Asp	<10.2	14/1	0.4 (0.0-4.3)	
Glu/Glu	≧10.2	70/44	2.7 (1.1-6.9)	
Glu/Asp和Asp/Asp	≧10.2	13/10	4.7 (1.4-15.9)	
內皮一氧化氮合成酵素基因型	身體質量指數 (kg/m^2)			
Glu/Glu	<24	78/14		1.0[f,*]
Glu/Asp和Asp/Asp	<24	21/6		1.5 (0.4-6.2)
Glu/Glu	≧24	93/52		2.5 (1.1-5.6)
Glu/Asp和Asp/Asp	≧24	19/7		2.9 (0.9-10.2)
含錳超氧歧化酵素基因型[e]				
Val/Val		141/45	1.0	1.0
Val/Ala和Ala/Ala		70/34	2.0 (1.0-4.0)	2.0 (1.0-3.9)
累積砷暴露 (ppm×年)				
<10.2		86/16		1.0
≧10.2		92/64		3.5 (1.5-8.1)
身體質量指數 (kg/m^2)				
<24		105/23	1.0	
≧24		125/74	2.5 (1.2-5.1)	

[a]調整年齡、性別及三酸甘油脂 [b]95% CI：95%信賴區間 [c]37人無資料 [d]69人無資料 [e]37人無資料 [f]趨勢檢定 [*]P<0.05

表5模式一中調整了含錳超氧歧化酵素基因型和高血壓重要危險因子之後，內皮一氧化氮合成酵素基因型為Glu/Glu且累積砷暴露小於10.2 ppm×年者為基準，基因型為Glu/Asp和Asp/Asp且累積砷暴露小於10.2 ppm×年者危險性偏低。不論內皮一氧化氮合成酵素基因型是Glu/Glu或Glu/Asp和Asp/Asp者，隨著累積砷暴露的增加，高血壓危險性顯著增加，危險對比值分別為2.7及4.7。在調整內皮一氧化氮合成酵素基因型與累積砷暴露的組合後，含錳超氧歧化酵素為Val/Ala和Ala/Ala基因型者罹患高血壓的危險性是Val/Val基因型者的2.0倍，表示此酵素的基因多形性對高血壓的生成或許扮演一個重要的角色，此外，身體質量指數仍然是高血壓重要的危險因子。模式二的結果和模式一相似，內皮一氧化氮合成酵素基因多形性和身體質量指數分組的組合中，內皮一氧化氮合成酵素基因型為Glu/Asp和Asp/Asp且身體質量指數小於24 kg/m^{2}者危險對比值為1.5；基因型為Glu/Glu且身體質量指數過重者，危險對比值為2.5，有統計上顯著性；基因型為Glu/Asp和Asp/Asp且身體質量指數過重者，危險對比值為2.9，但未達統計上的顯著性。另外含錳超氧歧化酵素基因多形性和累積砷暴露對高血壓的影響仍然很重要。

表6指出含錳超氧歧化酵素基因型為Val/Ala和Ala/Ala、累積砷暴露在10.2 ppm×年以上、身體質量指數過重且三酸甘油脂濃度在111 mg/dl以上者為基準，危險對比值定為1.0。四個變項中有任意三個變項為中位值以上或是基因型為Val/Ala和Ala/Ala者定為第二組，四個變項中有任意二個變項為中位值以上或是基因型為Val/Ala和Ala/Ala者為第三組，四個變項中任意有一個變項為中位值以上或是基因型為Val/Ala和Ala/Ala者及基因型為Val/Val而累積砷暴露小於10.2 ppm×年且身體質量指數正常且三酸甘油脂濃度小於111 mg/dl者定為第四組。調整了年齡和性別後，帶有Val/Val基因型且累積砷暴露越低、身體質量指數正常、三酸甘油脂濃度越低者，高血壓的危險對比值有下降的趨勢，第三組的危險對比值為0.3，第四組的危險對比值為0.1顯示有明顯的保護作用，所以先天基因的不同再加上後天環境影響以及生活、飲食習慣的差異，使得個人對高血壓有不同的易感受性。

表6 研究對象高血壓盛行率與含錳超氧歧化酵素基因多形性、累積砷暴露、身體質量指數以及三酸甘油脂分層的多變項分析

變項				人數 正常/高血壓	危險對比值[a] (95%CI)[b]
含錳超氧歧化酵素基因型[c]	累積砷暴露 (ppm×年)[d]	身體質量指數[e]	三酸甘油脂 (mg/dl)[f]		
Val/Ala和Ala/Ala	≧10.2	≧24	≧111	10/12	1.0[g]
Val/Ala和Ala/Ala	≧10.2	<24	≧111	27/24	0.7 (0.3-2.0)
Val/Val	≧10.2	≧24	≧111		
Val/Ala和Ala/Ala	<10.2	≧24	≧111		
Val/Ala和Ala/Ala	≧10.2	≧24	<111		
Val/Val	≧10.2	<24	≧111	64/22	0.3 (0.1-0.8)
Val/Val	<10.2	≧24	≧111		
Val/Val	≧10.2	≧24	<111		
Val/Ala和Ala/Ala	<10.2	<24	≧111		
Val/Ala和Ala/Ala	<10.2	≧24	<111		
Val/Ala和Ala/Ala	≧10.2	<24	<111		
Val/Ala和Ala/Ala	<10.2	<24	<111	59/5	0.1 (0.0-0.4)
Val/Val	<10.2	<24	≧111		
Val/Val	≧10.2	<24	<111		
Val/Val	<10.2	≧24	<111		
Val/Val	<10.2	<24	<111		

[a]調整年齡、性別 [b]95% CI：95%信賴區間 [c]37人無資料 [d]69人無資料
[e]身體質量指數單位：kg/m^2 [f]3人無資料 [g]趨勢檢定 [h]P<0.05

四、計畫成果自評

含錳超氧歧化酵素基因型為Val/Ala和Ala/Ala者且維生素E及β-胡蘿蔔素濃度越低，高血壓的危險對比值均有上升的趨勢。調整含錳超氧歧化酵素基因型等其他重要危險因子之後，不論內皮一氧化氮合成酵素基因型是Glu/Glu或Glu/Asp和Asp/Asp者，隨著累積砷暴露或身體質量指數的增加，高血壓危險性顯著增加。調整了年齡和性別後，帶有Val/Val基因型且累積砷暴露越低、身體質量指數正常、三酸甘油脂濃度越低者，高血壓的相對危險性有下降的趨勢。因此本研究推論含錳超氧歧化酵素基因多形性可能是高血壓基因上的一個易感受性因子，基因型的不同可能修飾個體罹患高血壓的危險性，而後天環境的暴露、生活及飲食習慣更是影響高血壓相當重要的危險因子。

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行政院國家科學委員會補助國內專家學者出席國際學術會議報告

90 年 9 月 11 日

附件三

報告人姓名	薛玉梅	服務機構及職稱	臺北醫學大學 醫學系公共衛生學科
時間 會議地點	8.27.2001-8.31.2001 Vienna, Austria	本會核定補助文號	NSC-90-2314-B038-021
會議名稱	(中文)第十七屆國際營養學會議 (英文)The 17th International Congresses of Nutrition		
發表論文題目	(中文)停經婦女服用 Soy Isoflavone 補充劑後微量營養素與骨質密度增加 (英文) Micronutrients and Bone Density Increment after Soy Isoflavone Supplementation in Postmenopausal Women		

報告內容應包括下列各項：

一、參加會議經過

第十七屆國際營養學會議於民國九十年八月二十七日至八月三十一日在奧地利維也納的 convention center 舉行。此會議是每四年一次的國際營養學大會，由營養科學國際聯盟(The International Union of Nutritional Sciences)贊助，奧地利營養學會(The Austrian Nutrition Society)主辦。參加會議人士來自美國、瑞典、荷蘭、日本、泰國及各地醫師、專家、學者，共計約 3100 人與會。

本人與本校公共衛生學院謝明哲院長率同一行四十餘人於八月二十二日搭乘長榮班機，經曼谷，八月二十三日抵達維也納機場，一行人直奔莎茲堡、捷克布拉格觀光後，八月二十六日返回維也納。八月二十七日在維也納會議中心報到後，隨即領了會議議程日程表、會議摘要論文集後由會議主席主持開幕儀式及欣賞維也納少年合唱團演唱後隨即展開一序列研討活動。本次會議內容針對食物成份、營養建議量與政策、營養與健康與疾病、營養研究的趨勢與發展、食品製造與加工的發展、特定族群之營養與食品安全等領域分別進行討論。

本人於八月二十九日上午九點以海報發表論文，題目為" Micronutrients and Bone Density Increment after Soy Isoflavone Supplementation in Postmenopausal Women" 論文張貼時，與會多位學者極表興趣，並與本人進行廣泛討論與交換意見。此外，八月二十九日晚間大會舉辦晚宴感謝與會學者，由於主辦單位為將三千餘人送往餐廳場面相當混亂，我們一行人只好作罷，大夥感到非常遺憾。由於九月一日班機客滿，本人只好提前於八月三十日下午搭乘長榮飛機返國，於次日早上十一半左右安全返抵國門。

二、與會心得

第一次參加營養國際會議，使筆者有機會與世界各國從事食品與營養研究的知名學者專家討論及交換意見，對未來研究工作有極大的助益。此次會議中以食品安全問題、生物科技是否能解決全球營養問題、健康生活型態與健康促進最受重視。在此會議中本人對營養與健康與疾病領域較有興趣，尤其特別關心營養與癌症、飲食中抗氧化物與與健康效應之流行病學的關係和氧化傷害與生物體關係等領域的發展成果。本人最近研究計畫探討砷誘發之高血壓的危險因子，故對探討高半胱胺酸(homocysteine)與血小板功能及血栓形成與缺血性心臟病的關係有濃厚興趣。在高半胱胺酸代謝過程中，參與的酵素有 5,10 Methylenetetrahydrofolate reductase (MTHFR), cystathionine synthase (CBS), methionine synthase 等。CBS 需要維生素 B_6，methionine synthase 需要維生素 B_{12}，MTHFR 需要葉酸。許多報告指出當葉酸，維生素 B_6，維生素 B_{12} 缺乏或 MTHFR 受損則高半胱胺酸分解有問題，影響血小板功能產生栓塞引起缺血性心臟病。高半胱胺酸分解抑制導致氧化性壓力(oxidative stress)造成之各種活化氧(reactive oxygen species; ROS)及自由基，如 $H^{\bullet}$、$O_2^{\bullet-}$、$OH^{\bullet}$、$RO^{\bullet}$、$RO_2^{\bullet}$、$NO^{\bullet}$、$NO_2^{\bullet}$等，使血管內皮細胞受到傷害產生栓塞，引發心血管疾病。此外，本人與研究 soy isoflavone 補充劑與骨質疏鬆關係的國外學者交換研究心得，似乎 soy isoflavone 結構式與 estrogen 相似也許可作為 photoestrogen 對骨質形成有刺激作用且對破骨細胞(osteoclastic)骨質再吸收有抑制作用，其作用機轉有待未來進一步探討。

三、考察參觀活動(無是項活動者省略)

此次參加第 17 屆國際營養大會之外於八月二十三至二十六日至奧地利莎茲堡與捷克布拉格參觀。奧地利莎茲堡為當年真善美影片拍攝地點，滿山片野小白花風景如畫，親身體驗感受片中之情景，瀝瀝在目。捷克布拉格是一個建築非常有格調且未曾經戰火洗禮的城市，哥德式、巴洛克與洛可可式建築為其特色，令人嘆為觀止。

四、建議

台灣營養學研究結果一向發表在國際水準的期刊上。尤其近年來對國民營養調查費盡心力，此對流行病學研究有相當重要性。以公共衛生立場而言，希望國內營養學界學者研究成果多與公共衛生學界交流，以便利用本土營養資訊應用在教導民眾如何養成健康飲食習慣去預防疾病的發生。

五、攜回資料名稱及內容

1.會議議程日程表

2.會議摘要論文集

六、其他

Micronutrients and Bone Density Increment after Soy Isoflavone Supplementation in Postmenopausal Women

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Running title: isoflavone supplementation and bone density

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Abstract

This study was designed to investigate isoflavone supplementation on plasma micronutrients and bone mineral density in postmenopausal women. Thirty-seven postmenopausal women were given 150 mg/d of isoflavone supplements twice daily for 6 months. Peripheral blood was sampled from the subjects before and after the isoflavone supplementation at 6 months. Subjects' concentrations of plasma lycopene, β-carotene and estradiol after 6-month supplementation were significantly different as compared with those of the baseline. After having adjusted other risk factors the increment of bone mineral density was significantly positively associated with the increment of plasma estradiol after receiving isoflavone supplements for 6 months. The effect of soy isoflavones in normal postmenopausal women may increase the plasma estradiol concentration and prevent bone mineral density loss.

Keywords: postmenopausal women; isoflavones; micronutrients; bone mineral density

Introduction

Postmenopausal women are at risk for estrogen-deficiency related health problems such as cardiovascular diseases and osteoporosis [1]. According to the literature the prevalence of coronary heart diseases is lower in Japan than in western countries, and the incidence of climacteric symptoms is lower in Japanese women as compared to Canadian women [2]. These findings are thought to be due to the high intake of soyfoods and high levels of phytoestrogens in the Japanese [3,4]. Soy is a rich source of nonestradiol estrogens [5], which are structurally similar to the mammalian estradiol and can bind to estrogen receptors [6]. Thus, the isoflavones are thought to regulate several biological processes including lipid and bone metabolism. Furthermore, soybean isoflavones and the metabolites of daidzein have been shown to inhibit lipoprotein oxidation in vitro [1,7], and the isoflavones isolated from the licorice root have been demonstrated to prevent β-carotene consumption and low-density lipoprotein (LDL) oxidation [8].

The literature shows that micronutrients from cereals are also important to maintain healthy bone structure [9]. For example, vitamin A deficiency slows down bone growth [10]. Selenium and vitamin E deficiencies cause bone abnormalities and weaken the mechanical strength of rabbits bone [11]. In the absence of vitamin C or E,

10 nM of 17 β-estradiol did not protect LDL samples against oxidation [12]. Besides, estrogenic effects of phytoestrogens are thought to prevent bone reabsorption and to increase bone density [13]. Dietary soy protein, which contains isoflavones, has also been shown to prevent bone loss caused by ovarian hormone deficiency in ovariectomized rats with osteoporosis [14]. Thus soy protein was used previously to evaluate the effect of isoflavones on plasma lipids and bone density [14].

The reports on isoflavone have focused only on examining their effects, but rarely on assessing plasma micronutrients and bone density in postmenopausal women. Isoflavone supplementation may have antioxidative and micronutrient-sparing effects. The purpose of current study was to examine the effects of isoflavone supplementation on plasma micronutrients and bone density in postmenopausal women. In addition, plasma estradiol concentrations were measured to determine whether isoflavone supplementation can influence plasma estradiol level.

Methods

Study Subjects

This 6-month study was conducted from December 1998 to December 1999 and enrolled 61 healthy postmenopausal women at Taipei Medical University-Municipal Wan-Fang Hospital. The TMU Ethical Committee approved the protocol, and all subjects gave written informed consent prior to their participation. All women had

taken no medication for over 6 months before being enrolled.

The subjects were told to return to the Clinic of Department of Obstetrics and Gynecology once a month. Being supplied with the isoflavone supplement at each clinic visit to last until their next visit, they were instructed to consume a package the isoflavone supplement each in the morning and in the evening with a glass of water and a meal. These directions were stressed verbally at each clinic visit. Subjects were instructed to eat as they normally would. The subjects brought in any unused packages to the Clinic at each visit for package count. The study recruited 37 subjects who consumed 90% packages and their blood were collected.

Study Materials

The product of the isoflavone supplement contained 25-mg isoflavones per gram, and one isoflavone package had 3 grams. Each subject consumed daily isoflavone 150 mg (i.e., 75mg twice/day). Blood samples were taken from each subject on Day 0 and at the end of 6 after having received isoflavone.

The blood was drawn after fasting the subject for 12 hours. The samples were stored in tubes containing ethylenediaminetetraacetic acid (EDTA) and were immediately centrifuged at 750 g for 10 minutes to separate the plasma. High-density lipoprotein (HDL) was precipitated from plasma by a modification of the dextan-sulphate method [15]. The reagent with phosphotungstic acid and magnesium chloride was used to precipitate apoprotein B-containing lipoproteins. Total cholesterol, triglyceride (TG) and HDL-cholesterol (HDL-C) was determined by colorimetric methods after enzymatic reaction with peroxide (Randox Co., Antrim, Ireland). LDL-cholesterol (LDL-C) was estimated by the Friedewald formula, which is reliable when TG levels are less than 400 mg/dl [16].

The packed erythrocytes were washed twice with isotonic saline, and stored at -70°C till the analysis of superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities (Randox Co., Antrim, Ireland). The enzyme concentrations were expressed in U/mg of hemoglobin (Hb), which was determined by cyanomethemoglobin assay (Sysmex F-500, San Tung Instrument Co., Taipei, Taiwan).

Since calcaneus contains high content of trabeculas, the bone density of calcanus was measured by dual-energy X-ray absorptiometry using OsteoAnalyzer (Dove Medical System, Inc., Newbeury Park, California, USA). Plasma estradiol concentration was determined by a competitive immunoassay using direct chemiluminescent technology (ASC-180 SE, Automatic Chemiluminescence System, Chiron Dianostic, Walpole City, Massetussets, USA). The coefficient of variance for plasma estradiol was 5.52 ± 2.85%. All laboratory personnel were blind from the information of subject's status.

Levels of β-carotene, lycopene, α-tocopherol and retinol of plasma samples were measured by HPLC according to the procedure described elsewhere [17]. Using reversed-phase HPLC (Hitachi) and multi-wavelength monitoring carried out analysis. The eluency expressed for mobile phase (methanol: acetonitrite: chloroform) was 47: 48: 5. Retinol was detected at 325 nm, α-tocopherol at 280 nm, and lycopene and β-carotene at 466 nm. The baseline and 6-month plasma samples of the same subjects were thawed from -70℃ refrigerator in the dim light at room temperature and assayed on the same day to ensure that temporal variability in the laboratory assays. All laboratory personnel were blind from the information of subject's status.

Recovery rates for β-carotene, lycopene, α-tocopherol and retinol were 87.6 ± 7.0%, 84.2 ± 7.1 %, 92.6 ± 8.0%, and 78.9 ± 4.6%, respectively. We also used internal

control α-tocopherol acetate to reduce the systematic error, and the coefficient of variance for α-tocopherol acetate was 4.5 ± 1.6%.

Statistics

All continuous variables were presented as means ± SE. The paired t test was used to analyze the difference of micronutrinets, estradiol and bone density between the baseline and 6-month after the isoflavone supplementation. Correlation analysis was used to examine estradiol, lipid profile, micronutrients and antioxidant enzyme, between baseline and 6 months after the isoflavone supplement. In addition, we also used multiple regression analysis to evaluate the associations of differences of bone density and estradiol between baseline and 6 month after the isoflavone supplement when lipid profile or lipid protein and nitric oxide variables were adjusted.

A p value < 0.05 was considered statistically significant.

Results

The characteristics of the subjects in this study were age 51.4 ± 4.3 years (with the ranges of 40-57 years), height 158.7 ± 5.8 cm, weight 53.0 ± 7.4 kg, age of onset of menopause 47.9 ± 4.6 years (with the ranges of 38-54 years), duration from menopause 3.1 ± 2.3 years (with the ranges 1-8 years), and parity 2.2 ± 1.0. Body weights (data not shown) were monitored during the 6-month study period, and did not change significantly.

There were no significant differences in the plasma retinol, α-tocopherol and bon density but significant differences in plasma lycopene, β-carotene and estradiol between the baseline and after the isoflavones supplementation 6-month (Table 1). The concentrations of plasma micronutrient were all in the normal range.

As shown in Table 2, the bone density was significantly related to α-tocopehrol

and triglyceride on baseline among postmenopausal subjects. But there were no association among bone density, micronutrients, lipid profile, and antioxidant enzymes after the 6 month isoflavone supplementation among postmenopausal subjects.

In Table 3 was the correlation coefficient of the difference of plasma lipid, lipoprotein concentrations, micronutrients, SOD, GSHPx and nitric oxide (NO) before and after the isoflavones supplementation for 6 months in postmenopausal subjects. The difference of estradiol had no linear relationship to the micronutrients, lipid profile and lipoprotein, so we can adjust with these variables in the same multiple regression model, but the difference of estradiol was significantly related with the difference of nitric oxide. The difference of LDL-C was significantly correlated with the difference of total cholesterol and HDL-C; therefore they were adjusted in the different model respectively.

Multiple regression analysis for the association between bone density and estradiol when other risk factors (lipid profile, lipoprotein and nitric acid) were adjusted, shown in Table 4. The older the age was the higher the difference of bone density when other risk factors adjusted. The higher the difference of the total cholesterol and LDL were the higher the difference of bone density when other risk factors adjusted. The difference of bone density was significantly positively associated with the difference of estradiol after adjusting the age, difference of α-tocopherol, nitric oxide, HDL-C, LDL-C or total cholesterol and triglyceride before and after isoflavone supplementation. It means that the difference of bone density was significantly increased with the difference of serum estridiol increment when the same level of age, and the same level of the difference of α-tocopherol, nitric oxide, HDL-C, LDL-C or total cholesterol and triglyceride before and after isoflavone

supplementation.

Discussion

Osteoporosis is characterized by low skeletal bone mass and fragility, which cause hip, radius, and vertebral compression fractures [18]. Osteoporosis is a major public health problem, particular for postmenopausal women. Menopausal loss of ovarian estrogen is known to be associated with a rapid decrease in bone mineral density, leading eventually to increased fracture risk [19].

The average per capita consumption of soy protein in Taiwan is 35 g/day [20]. Genistein and daidzein, both isoflavones and phytoestrogens, are found in relatively high concentration in soy-based foodstuffs [21, 22]. Genistein and daidzein, along with their β-glucoside conjugates, are present in soybeans in amounts of up to 3 mg/g. Hence; the per capita isoflavone intake among the Taiwanese may reach at 100 mg/day. In this study, we showed further significant increment of serum estradiol subjects receiving the soy isoflavones supplement for six months.

Isoflavones are nature plant hormones with the oestrogen-like structure. Hypothetically, dietary intake of isoflavones has a bone-sparing effect. Isoflavones mainly from soybeans, have also been suggested to give benefit of bone preservation. The exact mechanism has not yet demonstrated because the supporting data from laboratory and clinical studies are lacking. However, there are several reports showing positive correlation of the intake of soy protein and the bone density. Arjmandi et al. reported that bone loss in ovariectomized rats was prevented with a soy-protein diet, but not with a casein diet [23]. Erdman et al. also reported a significant increase in bone mineral content and bone mineral density of the lumbar spine in postmenopausal women having received a daily dose of 40 g soy protein containing 2.25 mg

isoflavones per gram protein for 6 months, as compared with a control group having received protein from casein/nonfat dry milk [24].

Limited vitamin A has anticarcinogenic properties through its abilities to stimulate the immune system and regulate cellular differentiation [25]. In this study, we found that the concentrations of serum lycopene and β-carotene, but note retinol, significantly increased after the soy isoflavone supplement for six months. Several studies demonstraed that β-carotene is safe and free form having teratogenicity for human [26]. Animal studies have shown the important role of vitamin A in the bone remodeling process [10]. Whereas vitamin A deficiency results in retarded bone growth [10], hypervitaminosis A accelerates bone resorption, bone fragility, and the risk for spontaneous fracture [27]. Retinoic acid inhibits osteoblast activity [28], stimulates osteoclast formation [29], and induces bone resorption [27,30]. However, carotenoids do not cause hypervitaminosis A even when ingested in large amount [31]. The response in persons with normal retinol concentrations indicates that absorption of carotenoids and their conversion to retinol may be regulated by the body's needs of vitamin A [32].

As indicated in Table 2, we analyzed plasma nitric oxide concentrations, as well as erythrocyte SOD and $GSHP_x$ activities, which protect tissues from the effects of free radical and lipidperoxides [33]. Nitric oxide, an intermediate metabolite generated during the biochemical transformation of arginine to citrulline [34], increases under oxidative stress [35]. The results in Table 2, showed no significant differences in erythrocyte SOD, $GSHP_x$ and plasma nitric oxide or α-tocopherol concentrations before and after the isoflavone supplement. Whether the higher plasma β-carotene and lycopene levels after the isoflavone supplement observed in this study

indicates that isoflavone have antioxidative micronutrient-sparing effect requires further investigation.

Besides binding metal ions [37], flavonoids have been shown to react with and to scavenge superoxide anions [38], hydroxyl radicals [39], and lipid peroxyl radicals [39]. As indicated in Table 1, serum lycopene and β-carotene significantly increased after receiving soy isoflavones supplements for six months, but the amount of isoflavone consumed had little effect on blood lipid variables in our previous study [36]. Further study is needed to clarify this discrepancy. The mechanisms by which flavonoids inhibit LDL oxidation are not clear. As show in Table 2, same plasma cholesterol and triglyceride level, or the same LDL and HDL level, plasma estradiol concentration were factors in bone density. As indicated in Table 1, α-tocopherol did not increase after the soy isoflavones supplement for six months in those postmenopausal women. These findings may suggest that soy isoflavones do not protect LDL against oxidation. The reason for these findings may be that the dietary concentration of isoflavones needed to affect lipid metabolism is different from that needed to influence bone metabolism [40].

The possibility also exits that the cholesterol-lowering component of soy is not or is only partially related to isoflavones [40]. We found that under the same lipid profile, α-tocopherol and defense enzyme level, the increment of serum estradiol was significantly related with the increment of bone density after receiving the soy isoflavones supplements for six months in those postmenopausal women. The reason may be related to the increased exposure to endogenous estrogen and increased protection from calcium loss, which in turn results in increased bone mineral density [41,42], or increased endogenous estrogen levels result in improved blood flow to muscles and bones. This in turn results in decreased bone turnover and increased bone mineral density [41,42], These interesting findings need clarification in further investigation.

Conclusion

The data from our study showed that after lipid profile, nitric oxide and α-tocopherol adjustments, the bone density increment was significantly related with the increment in plasma estradiol after receiving the isoflavone supplement for 6 months in postmenopausal women. Since lipid metabolism was not altered after isoflavone supplementation. These results may suggest that the requirement of isoflavone for bone metabolism is different from the requirement for lipid metabolism.

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Table 1. Plasma micronutrient, estradiol and bone density before and after the isoflavone supplementation for 6 months among 37 postmenopausal women

	Baseline	6 month
Variables	Mean ± SE	Mean ± SE
Retinol (μg/mL)	0.73 ± 0.03	0.74 ± 0.04
α-tocopherol (μg/mL)	9.36 ± 0.58	9.01 ± 0.53
Lycopene (μg/dL)	0.06 ± 0.01*	0.08 ± 0.01*
β-carotene (μg/dL)	0.34 ± 0.04*	0.44 ± 0.06*
Selenium (μg/L)	162.17 ± 8.14	170.11 ± 7.65
Estradiol (pg/ml)	9.22 ± 1.58**	15.27 ± 1.55**
Bone density (mg/cm^2)	352.88 ± 10.70+	358.12 ± 11.66+

SE, standard error

*Significantly different, $P<0.05$, tested by paired t test

**Significantly different, $P<0.01$, tested by paired t test

Table 2. The relationship of bone density for micronutrient, lipid profile, lipid protein and antioxidant enzymes before and after isoflavone supplementation for 6 months among 37 postmenopausal subjects

	Bone density			
	Baseline		6 month	
Variables	β	SE	β	SE
Retinol (μg/mL)	66.37	61.49	-10.90	7.88
α-tocopherol (μg/mL)	8.35*	3.54	3.75	4.12
Lycopene (μg/dL)	246.20	224.94	282.85	209.15
β-carotene (μg/dL)	-84.27	49.11	-51.92	33.60
Estradiol (pg/ml)	-1.02	1.31	-1.72	1.52
Triglyceride (mg/dL)	0.58*	0.21	-0.02	0.28
Total cholestrtol (mg/dL)	0.21	0.27	0.28	0.32
HDL-C (mg/dL)	-1.16	0.65	0.64	0.71
LDL-C (mg/dL)	0.27	0.28	0.23	0.38
SOD (U/mg Hb)	11.88	19.45	63.79*	24.90
GSHPx (U/mg Hb)	105.98	116.87	-32.90	111.72
NO (μ mole/L)	0.11	4.32	2.89	9.87
Selenium (μg/L)	-0.12	0.18	-0.23	0.24

Abbreviation: HDL-C, high-density lipoprotein-cholesterol;
LDL-C, low-density lipoprotein-cholesterol;
SOD, superoxide dismutase;
GSHPx, glutathione peroxidase; and
NO, nitric oxide

* Significantly different, $P<0.05$ in univariate regression analysis

Table 3. The Correlation of the difference of plasma lipid, lipoprotein concentrations, micronutrients, superoxide dimutase (SOD), glutathion peroxidaes (GSHPx), nitric oxide (NO), and selenium before and after isoflavones supplementation for 6 months among postmenopausal subjects

EST-D (pg/ml)	HDL-D (mg/dL)	LDL-D (mg/dL)	Chol-D (mg/dL)	Tg-D (mg/dL)	Bone-D (mg/cm^2)	Selenium-D (μg/L)		
EST-D (pg/ml)	0.033	-0.276	-0.152	0.307	-0.019	0.058		
HDL-D (mg/dL)		-0.482*	-0.120	0.113	-0.018	-0.027		
LDL-D (mg/dL)			0.878**	-0.222	0.107	-0.025		
Chol-D (mg/dL)				0.106	0.093	-0.077		
Tg-D (mg/dL)					-0.010	-0.135		
EST-D (pg/ml)	Reti-D (μg/mL)	Toco-D (μg/mL)	Lyco-D (μg/dL)	Bcar-D (μg/dL)	GSH-D (U/mg Hb)	SOD-D (U/mg Hb)	NO-D (μ mole/L)	Bone-D (mg/cm2)
EST-D (pg/ml)	0.338	0.241	0.365	0.280	-0.058	0.119	0.477	-0.019
Reti-D (μg/mL)		0.507**	0.083	0.128	0.009	0.151	0.037	-0.061
Toco-D (μg/mL)			0.091	0.339	-0.312	-0.145	0.310	0.225
Lyco-D (μg/dL)				0.391*	-0.411*	0.237	-0.026	-0.060
Bcar-D (μg/dL)					-0.232	-0.039	-0.031	0.097
GSH-D (U/mg Hb)						-0.110	-0.043	0.025
SOD-D (U/mg Hb)							-0.036	0.0007
NO-D (μ mole/L)								0.149
Selenium-D (μg/L)	-0.208	0.079	-0.034	-0.054	-0.031	-0.035	0.148	-0.691*

Abbreviation: Bone-D, difference of bone density;
HDL-D, difference of high density lipoprotein-cholesterol;
LDL-D, difference of low density lipoprotein-cholesterol;
SOD-D, difference of superoxide dismutase; GSH-D, difference of glutathione peroxidas
NO-D, difference of nitric oxide; Reti-D, difference of retinol;
Toco-D, difference of α-tocopherol; Lyco-D, difference of lycopene;
Bcar-D, difference of β-carotene; EST-D, difference of estradiol;
CHO-D, difference of cholesterol; and TG-D difference of triglyceride;
Selenium-D, difference of selenium;
** Significantly different, P<0.01 in correlation analysis
* Significantly different, P<0.05 in correlation analysis

Table 4. Stepwise multiple regression analysis of bone density for micronutrient, lipid profile, lipid protein and antioxidant enzymes before and after isoflavone supplementation for 6 months among 37 postmenopausal subjects

	Bone density			
	Baseline		6 month	
Variable	β	SE	β	SE
Age			2.20	2.47
EST (pg/ml)	-2.44*	0.87	-1.90	1.49
Reti (μg/mL)	57.62	40.67		
Toco (μg/mL)	2.59	2.68	5.64	3.84
Lyco (μg/dL)	660.80**	152.29		
Bcar (μg/dL)	-179.77**	32.65	-130.28**	34.65
Tg (mg/dL)	0.45*	0.17		
SOD (U/mg Hb)			84.94**	25.81
NO (μ mole/L)			-21.87+	10.30
Se (μg/L)	-0.20+	0.11	-0.21	0.23

Abbreviation: EST, estradiol; Reti , retinol; Toco, α-tocopherol; Lyco, lycopene; Bcar, β-carotene; Tg, triglyceride; SOD, superoxide dissimulates; NO, nitric oxide; Se, selenium;

+ 0.05<P<0.1, paired t test * P<0.05, paired t test

** P<0.01, paired t test

Table 5. Stepwose multiple regression analysis of differences of bone density before and after the isoflavone supplementation for 6 months among the postmenopausal subjects

	Model I		Model II		Model III	
Variables	β	SE	β	SE	β	SE
Age	-1.26*	0.52	-1.27*	0.54	-1.15*	0.51
Estradiol-D (μg/L)	0.49	0.32	0.51	0.34	0.39	0.36
Retinol-D (μg/mL)	-14.05	21.72	-16.73	22.35		
β-carotene-D (μg/mL)					1.64	10.88
Cholesterol-D (mg/dL)	-0.09	0.08			-0.10	0.08
LDL-CD (mg/dL)			-0.06	0.08		
GSH-D (U/mgHb)	156.18*	58.48	152.58*	60.45	161.56*	61.80
NO-D (μ mole/L)	1.40+	0.69	1.30+	0.72	1.42+	0.72
Se-D (μg/L)	-0.42**	0.08	-0.42**	0.08	-0.40**	0.08

Abbreviation: Estradiol-D, difference of estradiol;
Retinol-D, difference of retinol;
β-carotene-D, difference of β-carotene;
LDL-CD, difference of low density lipoprotein-cholesterol;
Cholesterol-D, difference of cholesterol;
GSH-D, difference of glutathione;
NO-D, difference of nitric oxide;
Se-D, difference of selenium;

+ Borderline significantly different, $0.05<P<0.1$

* Significantly different, $P<0.05$

** Significantly different, $P<0.01$