TADe：ダイゼイン型イソフラボンのピーク面積
CD　：イソフラボン標準溶液中のダイジンの濃度［mg/l］
AD　：イソフラボン標準溶液クロマトグラム上のダイジンのピーク面積

グリシテイン型及びゲニステイン型イソフラボンアグリコンの濃度も同様に求め，その総和を試料中の総イソフラボン濃度（アグリコン当量）とし，試料中のイソフラボン含有量（アグリコン当量）を下記の式により求めた。グリシテイン型は0.637，ゲニステイン型は0.625を乗じてアグリコン当量を求めた。

試料中のイソフラボン含量（アグリコン当量）［mg/100 g または mg/100 ml］
＝総イソフラボン濃度（mg/l）×50/1,000×1/試料採取量（g/ml）×100

試料の測定は2連で行い，平均値で示した。CV値（%）は，測定内0.7%，測定間2.8%であった。なお，対象食品に表示されている総イソフラボン量のアグリコン換算値は，分析で求めたイソフラボン成分の構成比をもとに分子量の比から求めた。

結　　果

1．表　　示

表2に対象とした食品中の，名称，大豆イソフラボンに関する原材料名，一日または一回あたりの摂取目安量に関する表示，大豆イソフラボン含有量に関する表示，そのアグリコン換算値を示した。

厚生労働省の通知では，特定保健用食品のほか，錠剤，カプセル剤，粉末剤，液状剤の形状のいわゆる健康食品のうち，大豆イソフラボンを含む食品には大豆イソフラボンアグリコンとしての含有量を表示すること，また一日あたりの摂取目安量を大豆イソフラボンアグリコンとして30 mgを超えないようにとされた。大豆イソフラボンを含む特定保健用食品には大豆イソフラボンアグリコンとしての含有量の表示があったが，その他の食品では錠剤型の食品一品目と煎り黒大豆にアグリコン表示があったのみで，多くはアグリコン表示がなかった。また，一日あたりの摂取目安量のアグリコン換算値は，錠剤の形状をした食品4品目中3品目で若干ではあるが30 mgを超えていた。固体状食品のうち，錠剤の形状をした食品には，美容サポート食品，健康補助食品の表示が，また，菓子には栄養食品などの表示があった。なお，20品目中11品目に「遺伝子組換えでない」の表示があった。

2．「健康食品」中の大豆イソフラボン量

表2に各食品の一日または一回摂取目安量当たりの大豆イソフラボンの表示量とそのアグリコン換算値及び分析値（アグリコン当量）を示した。

液状食品のうち豆乳類及びお茶では，摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は20～29 mgであり，表示量のアグリコン換算値ともほぼ一致した。一方，清涼飲料水（液状剤型）には表示量に対して大豆イソフラボン分析値が1/3のものがあった。

粉末状食品はコーヒーミックス，コラーゲン食品，きなこ，青汁豆乳であり，一回摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は，6～18 mgであった。表示量に対して分析値が著しく逸脱している食品はなかった。

固体状食品のうち錠剤型の食品では，一日の摂取目安量あたりに含まれる大豆イソフラボンアグリコンの分析値は3～46 mgの範囲であり，このうち大豆胚芽抽出物（錠剤型）では，分析値が表示量の2/3のものがあった。黒豆茶，煎り黒大豆，菓子の一回摂取目安量に含まれる大豆イソフラボンアグリコンの分析値は4～19 mgであり，表示量に対して分析値が著しく逸脱している食品はなかった。

3．「健康食品」中の大豆イソフラボン成分の解析

表3に各食品100 gあたりに含まれる12種類の大豆イソフラボンの成分分析の結果を示す。

大豆イソフラボン含量が最も多い食品は，大豆イソフラボン加工食品の表示がある錠剤型の食品で，100 gあたり1.6～4.5 gの大豆イソフラボンが含まれていた。次いで大豆発酵抽出物の表示がある錠剤型等が100 gあたり0.5 g程度のイソフラボンを含有していた。その他の通常の食品は100 gあたり数十ミリグラムのイソフラボンが含まれていた。

イソフラボンの成分分析では，全ての食品に12種類のイソフラボン成分が含まれていたが，食品によって成分に特徴があり，大きく分けて豆乳型，大豆抽出物添加型，大豆型に分類された。

豆乳型食品は配糖体のダイジン，ゲニスチン及びマロニル配糖体のマロニルダイジン，マロニルゲニスチンを主に含有する食品で，豆乳及び大豆菓子がこれに該当する。これらの配糖体とマロニル配糖体は全体の80%以上を占めていた。豆乳に大豆胚芽抽出物または大豆イソフラボンが添加されている食品には，これらに加えてグリシチンが含まれていた。

大豆抽出物添加型食品は，原材料に大豆胚芽抽出物あるいは大豆イソフラボンの表示がある食品で，形状によらず，全体の約90%が配糖体のダイジン及びグリシチンで占められる食品が多かった。一方，原材料に大豆胚芽抽出物の表示がある錠剤型の食品のうち，アグリコン型のゲニステインのみが主成分である食品が1品目あった。

大豆型食品は，比較的多種類のイソフラボン成分を含

表2　食品の表示とイソフラボン含有量（アグリコン換算値）

形態	名　　称	原　材　料　名	1日または1回摂取目安量に関する表示	表　　示　　量	表示量のアグリコン換算値[1)] (mg)	分析値（アグリコン当量） (mg)
液状食品	調整豆乳（特定保健用食品 大豆たんぱく質）	大豆（遺伝子組換えでない）	1日1本 (200 ml)	大豆イソフラボンアグリコンとして16 mg	16	20
	豆乳飲料（特定保健用食品 大豆イソフラボン）	黒大豆（遺伝子組換えでない）大豆イソフラボン	1日1本 (125 ml)	大豆イソフラボンアグリコンとして25 mg	25	29
	まるごと大豆飲料	大豆（遺伝子組換えでない）	1本 (125 ml)	大豆イソフラボン 50 mg	32	27
	豆乳飲料	大豆　大豆イソフラボン	1本 (125 ml)	イソフラボン 30 mg	19	20
	黒豆茶	黒大豆（遺伝子組換えでない）　大豆イソフラボン	1本 (500 ml)	大豆イソフラボン 25 mg（アグリコン換算）	25	24
	清涼飲料水：液状剤型	焙煎大豆粉末（イソフラボン含有）	1本 (50 ml)	大豆イソフラボン 40 mg	26	8
粉末状食品	コーヒーミックス（栄養機能食品　ビタミンD）	黒豆（大豆） 大豆イソフラボン	1杯分 (11 g)	イソフラボン 10 mg	6.3	9.4
	コラーゲン食品	大豆胚芽抽出物	1日5 g	大豆イソフラボン配合	記載なし	6
	きな粉調製品	黒大豆（遺伝子組換えでない）	1袋 (12 g)	イソフラボン 13.2 mg	10	14
	大豆加工食品（青汁豆乳）	青大豆粉末 大豆胚芽抽出物	1袋 (18 g)	大豆イソフラボン 15.14 mg	10	11
	黒ごまきなこ	大豆（国内産）	1杯分 (24 g)	大豆イソフラボン 24.24 mg	20	18
固体状食品	大豆イソフラボン加工食品：錠剤型（美容サポート食品）	大豆抽出物（遺伝子組換えでない）	1日6粒 (1.26 g)	大豆イソフラボン 50 mg	31	46
	大豆イソフラボン加工食品：錠剤型（健康補助食品）	大豆抽出物（遺伝子組換えでない）	1日4粒 (1 g)	大豆イソフラボン 60 mg	38	45
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	1日8粒 (2.0 g)	大豆胚芽抽出物125 mg（イソフラボンとして50 mg）	32	33
	コエンザイムQ10含有食品：軟カプセル型（栄養機能食品 ビタミンE）	大豆イソフラボン	1日2粒 (900 mg)	イソフラボン 3.7 mg	2.3	3
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	1日5粒 (1.25 g)	イソフラボンアグリコン 9 mg	9	5.9
	菓子A（栄養食品）	大豆粉	1本 (30 g)	イソフラボン 22 mg	14	14
	菓子B（栄養食品）	大豆粉	1本 (30 g)	イソフラボン 20 mg	13	16
	黒豆茶	黒大豆（遺伝子組換えでない）	1包 (15 g)	イソフラボン 7.4 mg	4.6	4.2
	煎り黒大豆	黒大豆（遺伝子組換えでない）	10 g	大豆イソフラボン 15 mg（アグリコン換算）	15	15

[1)] 表示量のアグリコン換算値は、分析により求めたイソフラボン成分の割合を基に分子量の比から求めた。

有している食品で，特に配糖体のダイジン，グリシチン，ゲニスチンに加え，アセチルグリシチンを多く含んでいた。これには，きな粉やお茶が該当した。

なお，今回は分析の対象とはしていないが，粉末状及び固体状食品には，サクシニルゲニスチンと溶出時間が一致する小さなピークが検出されるものが多かった。

表3　食品100 g 当りのイソフラボン成分量（アグリコン換算値）[1)]　mg/100 g（100 ml）

形態	名称	原材料名	D*	MD*	AD*	De*	Gl*	MGl*	AGl*	Gle*	G*	MG*	AG*	Ge*	総イソフラボン
液状食品	調整豆乳（特定保健用食品　大豆たんぱく質）	大豆（遺伝子組換えでない）	2.4	1.7	0.3	0.0	0.0	0.0	0.0	0.0	3.5	2.9	0.0	0.1	11.0
	豆乳飲料（特定保健用食品　大豆イソフラボン）	黒大豆（遺伝子組換えでない） 大豆イソフラボン	9.1	1.1	0.1	0.6	1.6	0.1	0.6	0.0	7.2	1.4	0.4	0.8	23.0
	まるごと大豆飲料	大豆（遺伝子組換えでない）	4.1	3.4	0.4	0.1	0.5	0.0	1.4	0.0	4.7	5.4	0.0	0.7	20.8
	豆乳飲料	大豆　大豆イソフラボン	6.0	0.3	0.0	0.2	3.6	0.0	0.4	0.2	3.7	0.7	0.0	0.5	15.8
	黒豆茶	黒大豆（遺伝子組換えでない） 大豆イソフラボン	2.3	0.7	0.0	0.0	1.2	0.0	0.2	0.1	0.0	0.0	0.1	0.0	4.8
	清涼飲料水：液状剤型	焙煎大豆粉末（イソフラボン含有）	5.9	0.2	0.0	0.3	2.9	0.2	0.5	0.3	4.4	0.5	0.0	0.5	15.8
粉末状食品	コーヒーミックス（栄養機能食品　ビタミンD）	黒豆（大豆）　大豆イソフラボン	39.1	0.3	0.0	3.4	34.0	0.1	4.2	0.2	12.3	0.4	0.0	0.5	94.4
	コラーゲン食品	大豆胚芽抽出物	47.0	0.1	0.0	2.9	45.5	0.0	6.7	3.1	11.4	1.1	0.0	0.8	118.6
	きな粉調製品	黒大豆（遺伝子組換えでない）	18.9	2.2	0.2	32.2	2.0	0.4	32.8	3.3	18.6	0.9	0.0	6.2	117.6
	大豆加工食品（青汁豆乳）	青大豆粉末　大豆胚芽抽出物	13.2	0.2	1.9	0.7	8.0	0.2	2.7	3.4	9.9	19.6	0.2	0.7	60.8
	黒ごまきなこ	大豆（国内産）	7.2	0.0	0.1	1.5	3.1	1.0	7.3	33.5	14.4	0.7	0.1	5.5	74.5
固体状食品	大豆イソフラボン加工食品：錠剤型 （美容サポート食品）	大豆抽出物（遺伝子組換えでない）	1511.0	0.0	2.6	6.3	1821.1	6.8	33.3	0.5	248.1	5.8	0.0	2.2	3637.8
	大豆イソフラボン加工食品：錠剤型 （健康補助食品）	大豆抽出物（遺伝子組換えでない）	1954.3	13.0	2.9	24.8	1953.4	0.8	84.0	1.3	427.9	8.0	0.0	25.2	4495.6
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	656.6	8.4	1.4	26.1	647.9	1.8	105.7	2.3	155.3	16.1	0.1	6.5	1628.0
	コエンザイム Q10 含有食品：軟カプセル型 （栄養機能食品　ビタミンE）	大豆イソフラボン	113.8	0.0	0.1	5.2	139.8	3.3	18.7	11.6	38.5	1.9	0.0	1.1	334.0
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物（遺伝子組換えでない）	0.6	2.6	1.0	0.9	0.6	2.4	10.0	2.6	0.4	0.0	0.0	448.9	470.1
	菓子A（栄養食品）	大豆粉	6.9	5.1	0.7	0.5	0.1	0.0	0.0	1.6	13.9	13.7	2.6	0.8	45.7
	菓子B（栄養食品）	大豆粉	9.6	6.0	1.1	1.6	0.0	0.0	0.0	0.1	18.6	12.8	2.8	2.2	55.0
	黒豆茶	黒大豆（遺伝子組換えでない）	3.5	0.0	0.3	0.4	1.3	1.6	13.3	1.3	4.6	0.7	0.0	1.2	28.1
	煎り黒大豆	黒大豆（遺伝子組換えでない）	25.4	0.3	0.4	0.7	3.3	8.5	75.0	5.4	25.7	0.2	0.0	6.3	151.3

1) アグリコン換算値は分子量の比から求めた。

豆乳型食品に多く含まれる成分、大豆抽出物添加型食品に多く含まれる成分を示す

*表1を参照

4．「健康食品」中の型別イソフラボン含有量（重量%）

大豆イソフラボンは大きく分けてダイゼイン型，グリシテイン型，ゲニステイン型に分類される（表1）。食品中の12種類のイソフラボン成分の合計量のうち，各型のイソフラボンが占める割合（重量%）を表4に示した。豆乳型食品は，ダイゼイン型とゲニステイン型が約90%を占めた。大豆抽出物添加型は錠剤型の食品に多く，ダイゼイン型とグリシテイン型が約90%を占めていたが，中にはゲニステイン型のみが含まれている錠剤型食品があった。大豆抽出物が添加された豆乳飲料は3種の型をほぼ等しく含んでいた。一方，大豆型食品は，ダイゼイン型，グリシテイン型，ゲニステイン型をほぼ等しく含

表4　食品中の型別イソフラボンの割合（重量比）

形態	名　　称	原　材　料　名	ダイゼイン型（%）	グリシテイン型（%）	ゲニステイン型（%）	総量（%）
液状食品	調整豆乳 （特定保健用食品　大豆たんぱく質）	大豆 （遺伝子組換えでない）	40.5	0.5	59.0	100
	豆乳飲料 （特定保健用食品　大豆イソフラボン）	黒大豆（遺伝子組換えでない） 大豆イソフラボン	47.2	10.1	42.7	100
	まるごと大豆飲料	大豆（遺伝子組換えでない）	38.4	9.5	52.1	100
	豆乳飲料	大豆　大豆イソフラボン	42.2	26.6	31.2	100
	黒豆茶	黒大豆（遺伝子組換えでない） 大豆イソフラボン	64.0	32.7	3.3	100
	清涼飲料水：液状剤型	焙煎大豆粉末 （イソフラボン含有）	41.1	24.5	34.5	100
粉末状食品	コーヒーミックス （栄養機能食品　ビタミンD）	黒豆（大豆） 大豆イソフラボン	45.3	40.7	13.9	100
	コラーゲン食品	大豆胚芽抽出物	42.1	46.6	11.2	100
	きな粉調製品	黒大豆 （遺伝子組換えでない）	45.4	32.7	21.9	100
	大豆加工食品（青汁豆乳）	青大豆粉末　大豆胚芽抽出物	26.2	23.7	50.1	100
	黒ごまきなこ	大豆（国内産）	11.8	60.4	27.9	100
固体状食品	大豆イソフラボン加工食品：錠剤型 （美容サポート食品）	大豆抽出物 （遺伝子組換えでない）	41.8	51.2	7.0	100
	大豆イソフラボン加工食品：錠剤型 （健康補助食品）	大豆抽出物 （遺伝子組換えでない）	44.4	45.4	10.3	100
	大豆イソフラボン加工食品：錠剤型	大豆胚芽抽出物 （遺伝子組換えでない）	42.5	46.5	10.9	100
	コエンザイムQ10含有食品：軟カプセル型（栄養機能食品　ビタミンE）	大豆イソフラボン	35.6	51.9	12.4	100
	大豆発酵抽出物：錠剤型	大豆胚芽抽出物 （遺伝子組換えでない）	1.1	3.3	95.6	100
	菓子A （栄養食品）	大豆粉	28.8	2.7	67.5	100
	菓子B （栄養食品）	大豆粉	33.3	0.5	66.2	100
	黒豆茶	黒大豆 （遺伝子組換えでない）	14.9	62.0	23.1	100
	煎り黒大豆	黒大豆 （遺伝子組換えでない）	17.7	61.0	21.3	100

□豆乳型食品に多く含まれるイソフラボン型、■大豆抽出物添加型食品に多く含まれるイソフラボン型を示す

有していた。

考　　察

大豆イソフラボンを含む「健康食品」に着目し，一日または一回の摂取目安量に含まれる大豆イソフラボン量を求めるとともに，12種類のイソフラボン成分量を比較した。

これらの食品には，大豆イソフラボンまたは大豆たんぱく質を関与成分とする特定保健用食品といわゆる健康食品が含まれる。これらを，形状により，液状食品，粉末状食品，固体状食品に分類し，各々に適した方法に従って大豆イソフラボンの抽出を行い，HPLC 法にて定量及び成分分析を行った。

1．大豆イソフラボンの含有量と表示

分析した食品のなかで重量あたり総イソフラボン含量が最も多かったのは，主原料が大豆胚芽抽出物である錠剤型の大豆加工食品であり，次いで多いのがきな粉，煎り黒大豆，大豆菓子などの大豆を丸ごと使用した食品で，最も少ないのは豆乳などの液状食品であった。豆乳は，イソフラボンが浸水などの加工中に流出することやおからに移行することから含有量が低下すると考えられる。

表2より，一日または一回の摂取目安量に含まれる大豆イソフラボン（アグリコン換算）は液状食品，粉末状食品とも表示量とほぼ一致した。液状食品のなかで，液状剤型食品では表示量の 1/3 しか大豆イソフラボンが含まれていないものがあった。一方，固体状食品のうち錠剤型の食品では 3～46 mg と幅があり，錠剤型の食品 5 品目中 3 品目で 30 mg を超えるものがあること，また含有量が表示量の 2/3 の食品があった。これらのことから，「健康食品」のうち，液状剤型及び錠剤型のいわゆる健康食品には，表示量と含有量が一致しない食品があることが明らかになった。これらの食品は形状及び重量あたりのイソフラボン含有量が高いことから，過剰摂取にも注意を要すると考えられた。

厚生労働省は，平成18年 8 月に「大豆イソフラボンを含む特定保健用食品などの取扱いに関する指針」において，平成19年 4 月以降に製造される大豆イソフラボンを含む特定保健用食品及びイソフラボンが強化，濃縮されたいわゆる健康食品については，大豆イソフラボンアグリコンとして一日の摂取量が 30 mg を超えないように設定すること，またアグリコンとしての含有量を表示することとした。本調査において，これらの食品にアグリコン表示のないものや，分析値が 30 mg を超える食品が散見されたが，この理由として，食品の多くが平成18年12月～平成19年 4 月に購入されたものであることから，時期的に指針に十分に対応していない可能性が考えられた。なお，平成18年 3 月に国民生活センターが実施した，「大豆イソフラボンを多く含むとうたった「健康食品」」に関する調査では，24銘柄中14銘柄が，最大摂取目安量当たりの大豆イソフラボン量（アグリコンとして）が 30 mg を超えていた[4]。

2．大豆イソフラボンの成分分析

大豆イソフラボンの成分分析では，12種類の組成により豆乳型，大豆抽出物添加型，大豆型に分類された。豆乳型にはダイゼイン型とゲニステイン型のイソフラボンが多く，そのなかでも水溶性の配糖体とマロニル配糖体が多くを占めた。この結果は先行研究とよく一致している[5]。大豆抽出物添加型は食品の形状によらず，大豆胚芽抽出物，大豆抽出物あるいは大豆イソフラボンが添加された食品で，ダイジンとグリシチンが全体の80％以上を占めた。大豆胚芽（胚軸）には配糖体のダイジン及びグリシチンが豊富であることから，これらの食品には同様の成分が多く含まれていることとよく一致する[6]。大豆型の食品はきな粉，焙煎黒大豆で，ダイゼイン型，グリシテイン型，ゲニステイン型のイソフラボンをおおよそ等しく含んでおり，なかでも配糖体のダイジン，グリシチン，ゲニスチンに加え，アセチルグリシチンを多く含んでいた。きな粉や焙煎大豆は大豆を高温で焙煎するため，熱によって容易に分解されるマロニル配糖体は殆んど検出されなかった。また，水分が少ない状態で高温加熱処理を行うため，アセチル配糖体が多い。これに比べ，大豆菓子は豆乳と類似したイソフラボン組成を持っていたことから，焙煎ほど高温で加工されたものではないと考えられた。

3．「健康食品」中の大豆イソフラボンの生体利用性

大豆イソフラボンは成分によって吸収性や生物活性が異なることが報告されている。アグリコン型のイソフラボンは腸管で直接吸収されるが，配糖体は腸内細菌によってアグリコンに代謝されてから吸収される。ヒトにおける単回投与試験では，アグリコンの吸収は早く，吸収量も多いことが報告されている[7]。これらのことから，アグリコンのみを含む錠剤を単独で摂取した場合は，大豆食品を食事とともに摂取する場合とは異なる体内動態を示すとともに，生物活性も異なる可能性があると考えられる。

一方，配糖体のダイジンはアグリコンのダイゼインに代謝され吸収されるが，一部はさらにエクオールまたは *O*-デスメチルアンゴレンシン（*O*-DMA）に代謝されてから吸収される[8]。腸内細菌叢はヒトによって個人差があり，エクオール産生菌を保有しているのは日本人閉経後女性では約50％であると報告されている[9]。エクオールはイソフラボンのなかでも最もエストロゲン様作用が強いことから，大豆イソフラボンの臨床的な作用はエクオール産生能に依存するとの報告も多い[10]。Duncan ら

は，エクオール産生者は非産生者に比べて，乳がんの罹患率が低いことを報告している[11]。我々も，閉経後女性を対象にした大豆イソフラボンの介入試験において，エクオール産生者では非産生者に比べて骨量の減少率が低いことを確認している[12]。したがって，ダイゼイン型のイソフラボンを多く含む食品は，摂取側の腸内細菌叢の違いにより異なる効果を発揮する可能性がある。

本調査では，ゲニステインのみを含む錠剤型の食品が1品目あった。ゲニステイン型のイソフラボンは，他の成分とは異なり，チロシンキナーゼ活性やトポイソメラーゼ活性を阻害する作用を持っているため[13]，細胞増殖に影響を与えることや[14]，乳がんリスクを低下させる可能性が報告されている[15]。一方，大豆イソフラボンは17β-エストラジオールに構造が類似しているため，そのレセプターに対して親和性を示すが，ゲニステインは，ダイゼインやグリシテインに比べてエストロゲンレセプターへの親和性が高く，エストロゲン様作用も強い[16]。また，特にβレセプターへの親和性が高いことから，βレセプターに特異的な作用を示す可能性もある。これらのことから，ゲニステイン型のイソフラボンを多く含む食品は他のイソフラボン型を含む食品と異なる健康効果を示す可能性がある。

以上より，今回の調査は今後のイソフラボンの適正表示の提案に役立つと考えられた。また，大豆イソフラボンを含む「健康食品」の有効性と安全性は，含まれるイソフラボン成分の違いによる吸収性や生物活性を把握し，摂取する側の年齢，性別，個体特性等も考慮して評価する必要があると考えられた。

ま と め

大豆イソフラボンを含む「健康食品」に着目し，一日または一回の摂取目安量に含まれる大豆イソフラボン量を求めるとともに，12種類のイソフラボン成分量を比較した。これらを，形状により，液状食品，粉末状食品，固体状食品に分類し，厚生労働省の「大豆イソフラボンを含む特定保健用食品等の取扱いに関する指針」の方法に従い，高速液体クロマトグラフィー法（HPLC法）により定量した。豆乳などの液状食品の摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は20～29 mgであり，表示量のアグリコン換算値ともほぼ一致した。液状剤型食品には表示に対して大豆イソフラボン分析値が1/3のものがあった。きな粉などの粉末状食品の1回摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は，6～18 mgであり，表示量に対して分析値が著しく逸脱している食品はなかった。固体状食品のうち錠剤型食品の1回の摂取目安量あたりに含まれる大豆イソフラボン（アグリコン換算）は3～46 mgの範囲であり，このうち一品目では，分析値が表示量の2/3のものがあった。

一方，12種類の大豆イソフラボンの成分分析により，これらの食品は豆乳型，大豆抽出物添加型，大豆型に分類された。このように大豆イソフラボンを含む食品は，含まれるイソフラボンの量や組成に違いがあることから，その吸収性や生物活性が食品によって異なる可能性があること，またその評価の際には，摂取する側の年齢，性別，個体特性等も考慮する必要があると考えられた。

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Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

Kyoko Taku PhD[1], Melissa K Melby PhD[2], Jun Takebayashi PhD[3], Shoichi Mizuno PhD[4], Yoshiko Ishimi PhD[3], Toyonori Omori PhD[5], Shaw Watanabe PhD[2]

[1]*Information Center, National Institute of Health and Nutrition, Tokyo, Japan*
[2]*Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan*
[3]*Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo, Japan*
[4]*Center for Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan*
[5]*Director for Research Coordination and Evaluation, National Institute of Health and Nutrition, Tokyo, Japan*

This study was conducted to clarify the effect of ingesting soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. PubMed, CENTRAL, ICHUSHI, CNKI, Wanfang Data, CQVIP, and NSTL were searched for randomized controlled trials published in English, Japanese, or Chinese reporting the effects of soy isoflavone extracts on lumbar spine or hip BMD in menopausal women. Trials were identified and reviewed for inclusion and exclusion eligibility. Data on study design, participants, interventions, and outcomes were extracted. Eleven, seven, five, and five trials were finally selected for estimation of the effects on spine, femoral neck, hip total, and trochanter BMD, respectively. Meta-analysis including data from1240 menopausal women revealed that daily ingestion of an average of 82 (47–150) mg soy isoflavones (aglycone equivalent) for 6–12 months significantly increased spine BMD by 22.25 mg/cm^2 (95% CI: 7.62, 32.89; p=0.002), or by 2.38% (95% CI: 0.93, 3.83; p=0.001) compared with controls (random-effects model). Subgroup analyses indicated that the varying effects of isoflavones on spine BMD across trials might be associated with study characteristics of intervention duration (6 vs. 12 months), region of participant (Asian vs. Western), and basal BMD (normal bone mass vs. osteopenia or osteoporosis). No significant effects on femoral neck, hip total, and trochanter BMD were found. Soy isoflavone extract supplements increased lumbar spine BMD in menopausal women. Further studies are needed to address factors affecting the magnitudes of effect on spine and to verify the effect on hip.

Key Words: meta-analysis, isoflavones, dietary supplements, menopause, bone density

INTRODUCTION

Osteopenia and osteoporosis are major health problems in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling.[1,2] The yearly decline in bone mineral density (BMD) of the lumbar spine and hip in postmenopausal women is reported to be at least 1% and up to 2.4%.[1,3] Although hormone replacement therapy (HRT) has positive effects in increasing BMD in postmenopausal women with low bone mass,[1,4] it is associated with a higher risk of hormone-related cancer[5-7] and other unfavorable adverse events.[8,9]

Epidemiological studies indicate that women who have high soy intake have a lower risk of osteoporosis than women who consume a typical Western diet.[10-12] Consequently, many menopausal women use phytoestrogens to maintain their BMD because they are unlikely to cause the undesirable effects associated with steroid hormones.[8,13] The primary dietary phytoestrogens ingested are soy isoflavones, which have structures similar to that of estrogen.[14]

A meta-analysis of randomized controlled trials (RCTs) has estimated the effect of ingesting soy isoflavones on lumbar spine BMD.[15] This included 10 RCTs of both soy isoflavone tablets and isolated soy protein containing isoflavones, and revealed a significant increase of BMD by 20.6 mg/cm^2 (magnitude in term of percentage and effect on hip not presented) resulting from soy isoflavones. Given the result in units of mg/cm^2, whether the magnitude of increase can prevent the naturally occurring postmenopausal bone loss remains unclear. Subgroup analysis of three trials testing isoflavone tablet revealed no significant effect, however one trial testing soy isoflavone extract was mistakenly included in the isolated soy

Corresponding Author: Dr Kyoko Taku, Information Center, National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan.
Tel: +81-3-3203-5721; Fax: +81-3-3202-3278
E-mail: takuk@nih.go.jp
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protein subgroup.[16] In addition, two[17] and three[18] comparisons from the same trial respectively with two and three soy isoflavone groups compared to the same control group were included simultaneously as separate studies in the meta-analysis. This is not recommended because it is considered to induce a serious unit-of-analysis problem.[19] Another recently published meta-analysis included 10 RCTs of soy isoflavones supplementation of at least one year duration (four RCTs testing isoflavones extracts), and did not find significantly beneficial effects of soy isoflavones on spine and hip BMD.[20]

Supplements of soy isoflavone extracts were easily ingested by the people who want to benefit from soy isoflavones, but are unable to usually consume and/or do not like to intake products of soy protein or soy foods containing isoflavones. In addition, the beneficial effects of soy protein might require synergistic reactions between isoflavones and other soy components.[15] Thus, clarifying the effects of extracted soy isoflavones (not as a constituent part in soy protein) is of more clinically important. However, both the two meta-analyses failed to reveal significant effects of soy isoflavone extracts in subgroup analysis, which might be due to the fact that only data from four RCTs were included.[15, 20] We have identified 12 RCTs of soy isoflavone extracts (not of soy protein or foods containing isoflavones) that reported effects on spine BMD in menopausal women,[8, 16-18, 21-29] and performed the present meta-analysis to clarify the effects of soy isoflavone extract both in terms of change (mg/cm^2) and percentage change (%) from baseline for lumbar spine and hip BMD, without influence on the same parameters by soy protein per se or other components in soy protein.

MATERIALS AND METHODS

PubMed (1966–2008), CENTRAL (1966–2008), ICHUSHI (1983–2008), and CNKI (1979–2008) were searched for relevant studies that had been published by September 2008. We also searched Wanfang Data, CQVIP and NSTL, which are other major search engines in China. Reference lists of relevant studies were manually searched. Studies were eligible for inclusion if they met all of the following criteria: (1) randomized parallel-group controlled trials published in English, Japanese, or Chinese; (2) trials with a crossover design that contained data for the first period;[19,30] (3) tested the effects of ingesting supplements of soy isoflavone extracts (not of soy protein or foods containing isoflavones) on lumbar spine or hip (femoral neck, total hip, or trochanter) BMD in menopausal women; and (4) BMD data were measured by dual X-ray absorptiometry. When duplicate data were reported for the same study subjects, only the article with the largest sample was included.[19] Two reviewers independently reviewed and evaluated the studies, and consensus was reached by discussion when there were disagreements.

Data on study design, number of participants, interventions, and outcomes for BMD were also independently extracted by two reviewers and confirmed by each other. When necessary, data on outcomes for BMD were obtained from graphs. If possible, we obtained necessary data not reported in the articles by contacting to the authors. We calculated mean change (follow-up − baseline) and percentage change [(follow-up − baseline) ÷ baseline × 100%] from baseline in BMD, when the data were not directly available. We primarily determined missing SD of the changes if statistical analyses comparing the changes themselves were presented (e.g., confidence intervals, standard errors, t values, p values, F values). Alternatively, we imputed them by computing mean correlations between the baseline and final values from included trials in which SD for change, as well as for baseline and final measurements were available.[19] Standard deviation for percentage change was calculated by dividing SD for change with mean baseline value.

We used the Jadad scale to assess the quality of included RCTs, a score of < 3 indicating low quality.[31] We also used a 3-category grading system (A, B, C) to denote the methodological quality of each study.[32] Category A studies have the least bias and results are considered valid; B studies are susceptible to some bias, but not sufficient to invalidate the results; and C studies have significant bias that may invalidate the results. We arbitrarily defined category C as of low quality. Concealment of treatment allocation in RCTs was assessed as adequate, inadequate or unclear.[33] Two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

We performed meta-analysis to determine the overall treatment effect of soy isoflavones on BMD, using the weighted mean difference method in Review Manager (version 5.0.20; Nordic Cochrane Center, Oxford, England). Treatment effect of each trial was estimated as the mean difference between changes (or percentage changes) from baseline in BMD for each comparison group (i.e., the change from the baseline for participants ingesting soy isoflavones minus that for controls). When data of more than one time points for the same trial were reported in one article or reported separately in two articles, we primarily used the data set for the short duration in order not to induce unit-of-analysis error. The data set for other time points were used for sensitivity analysis to prevent reporting bias. For trials had more than one isoflavone group compared with one control group, we combined the multiple isoflavones groups into a single group for each of these trials without inducing unit-of-analysis error.[34]

We used both a fixed effect model or a random effects model to calculate weighted mean differences (WMD), 95% CIs for each comparison, a combined overall effect with p-value, and the p-value for testing heterogeneity ($p < 0.1$ was considered significant); when there was significant heterogeneity across included trials, the results based on the random effects model were shown.[19,30,35]

We conducted sensitivity analyses to evaluate the effects of degree of correlation between baseline and final values, time point of measurement (using data for long duration instead of data for short duration in trials with multiple time points of evaluation), study design (selecting only placebo-controlled trials), and study quality (eliminating low-quality trials). If at least 10 trials were available, subgroup analyses and meta-regressions were performed to investigate possible factors that might related to varying effects of soy isoflavones on BMD across trials, on the basis of pre-specified factors of intervention

duration, isoflavone dosage, region of participants, and basal BMD.[15,20] We used a cut-off point of 75 mg/day in subgroup analysis for isoflavone dosage, because daily isoflavone intake of up to 75 mg (aglycone form) is considered safe by the Japan Food Safety Commission. Significant tests based on test for heterogeneity, chi-squared statistics, were performed to investigate differences between two subgroups.[19,34] We examined potential publication bias by using funnel plots and by performing Egger's test to assess the asymmetry of funnel plots. Meta-regressions and Egger's test were respectively performed with the use of user-written "metareg" and "metabias" commands for Stata 10.1 for Windows (StataCorp LP, College Station, Tex).

RESULTS

The search strategy (Figure 1) yielded 16 potentially appropriate reports of RCTs to be included in the meta-analysis. After excluding one article[36] reporting only duplicate femoral data that had appeared in another article,[25] and two articles[37,38] describing a smaller sample than that analyzed in another article,[23,17] 13 articles on 12 trials were included for meta-analysis.[8, 16-18, 21-29] Two articles

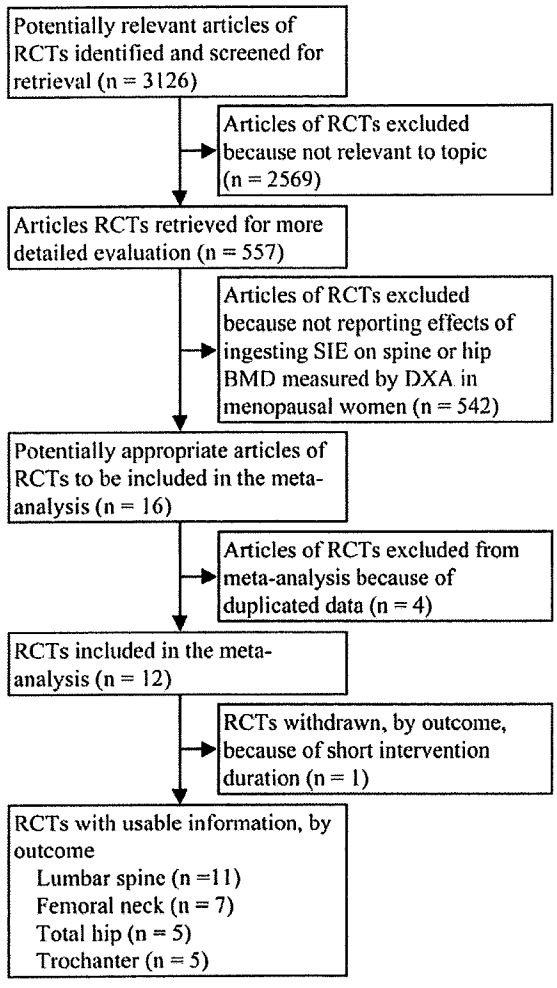

Figure 1. Search and selection of trials. Abbreviations: RCTs, randomized controlled trials; BMD, bone mineral density; SIE, soy isoflavone extracts, DXA, dual X-ray absorptiometry.

reported outcomes for durations of six months[27] and one year[28] for the same trial participants.

The characteristics of 12 trials are summarized in Table 1. Two articles for each trial contained data for two time points.[21,25] Three trials tested two isoflavone groups[17,22,24] and one tested three isoflavone groups[18] compared with one identical control group. One trial did not address the form and composition of soy isoflavones tested,[18] we assumed the dose as aglycone equivalent to calculate the mean dosage. Four, six, and two trials included participants of normal bone mass (T-score > −1 SD, corresponds to BMD > 0.937g/cm^2), low bone mass or osteopenia (−1 SD ≥ −2.5, corresponds to 0.937 g/cm^2 ≥ BMD ≥ 0.772 g/cm^2), and osteoporosis (T-score < −2.5 SD, corresponds to BMD < 0.772 g/cm^2) on the basis of averaged basal spine BMD, respectively.[39] Only one trial was assessed as "adequate" for concealment of treatment allocation,[22] and the remaining trials were assessed as "unclear" due to insufficient information. Participants in the comparison groups had similar dietary intakes of soy isoflavones, calcium, and vitamin D and physical activities. Most of the studies were designed to maintain the participants' usual diets, lifestyle and body weight. Adverse events were generally similar for both the isoflavone and control groups and no serious adverse events were noted in the included trials, although they were not well addressed in several trials.

Because bone is a slowly responding organ, a complete bone remodeling cycle takes up to 6 months, and therefore a study duration of less than 6 months is not sufficient to evaluate the effect of any intervention on bone BMD.[28] Thus, one 3-month trial of low-quality that reported negative effect of soy isoflavones on spine BMD was then withdrawn.[26] From 3126 relevant articles identified, 11,[8,16-18,21-25,27-29] 7,[8,17,22-25,27,28] 5,[16,17,22,27-29] and 5[17,22-24,27,28] trials were finally selected for estimating the effects on lumbar spine, femoral neck, total hip, and trochanter BMD, respectively (Figure 1, Table 1). Fourteen correlation coefficients between baseline and follow-up values were calculated from 5 reports of 4 trials,[17,23,24,27,28] which were consistent and resulted in a mean value of 0.98 (0.96–1).

Meta-analysis of the 11 trials with 1240 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$), and revealed that daily ingestion of an average of 82 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year significantly increased lumbar spine BMD by 12.08 mg/cm^2 (95% CI: 9.83, 14.33 mg/cm^2; $p < 0.001$), or by 1.47% (95% CI: 1.21, 1.74%; $p < 0.001$) compared with controls. Meta-analysis using the random effects model, revealed an significant overall effect of soy isoflavones in increasing spine BMD by 20.25 mg/cm^2 (95% CI: 7.62, 32.89 mg/cm^2, $p = 0.002$), or by 2.38% (95% CI: 0.93, 3.83%, $p = 0.001$; Figure 2). Of the11 selected trials, 7 trials revealed significant positive mean difference between changes or percentage change from baseline in spine BMD for isoflavone and control groups (favors isoflavone). The mean difference was negative at 27-week time point and was positive at 53-week time point in one trial,[21] the mean difference at 2-year duration was about two times of that at 1-year time point;[25] whereas, the

Table 1. Characteristics of included randomized controlled trials

Study	Design[†]	Follow-up	Participants[‡]	Intervention[§]	Baseline mean BMD outcomes (g/cm^2)[¶]	Jadad scale	Quality category
Brink 2008[21]	P; R, DB+, WD	27, 53 wk	N: 300/237 (21%) PoW; mean age: 53 y; TSM = 33 (12–60) mo; non-osteoporotic (spine Z-score ≥ 2); Netherlands, Italy, France	110 mg IAE [25–35% De, 60–75% Ge, 1–5% Gle] vs. placebo	L1–4: 0.990, mean (SD) T-score = −0.0 ± 1.1	4	C (dropout > 20%)
Chen 2003[22]	P; R+, DB+, WD	1 y	N: 203/175 (14%) PoW; mean age: 54.2 y; TSM = 4.1 (1–10) y; Hong Kong	40 and 80 mg IAE [46% De, 15% Ge, 39% Gle] vs. placebo	L1–4: 0.860; FN: 0.682; TH: 0.819; Tr: 0.605	5	A
Dong 2008[23]	P; R, WD	12 mo	N: 60/52 (13%) PoW; mean age: 54.7 y; TSM = 6.2 (≥ 1) y; T-score < −1.5 China	100 mg IC [66 mg IAE: 39% De, 61% Ge, 1% Gle] + calcium vs. calcium only (control)	L2–4: 0.756; FN: 0.719; Tr: 0.552	2	B
Gao 2006[18]	P; R	24 wk	N: 50/50 PoW; age: 48–62 y; TSM ≥ 1y; China	60, 90, and 150 mg IF vs. no-treatment (control)	L1–4: 0.974	1	B
Harkness 2004[16]	CO; R+, DB, WD	6 mo × 2	N: 20/19 (5%) PoW; mean age: 70.6 y; TSM = 19.1 (> 8) y; T-score < 2.5; USA	110 mg IAE [40% De, 52% Ge, 9% Gle] vs. placebo	L1–4: 0.881; TH: 0.800	4	B
Huang 2006[24]	P; R, OL, WD	1 y	N: 43/42 (2%) PoW; mean age: 52.4 y; TSM = 4.4 (1–13) y; Taiwan	100 and 200 mg IAE [29% De, 71% Ge] vs. regular diet only (control)	L1–4: 0.881; FN: 0.812; Tr: 0.715	2	B
Marini 2007[25]	P; R+, DB+, WD	12, 24 mo	N: 389/389 (10, 22%) PoW; mean age: 54.5 y; TSM = 63 mo (≥ 1 y); femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.840; FN: 0.670	5	A, C (dropout > 20%)
Morabito 2002[8]	P; R, DB+	1 y	N: 90/90 PoW; mean age: 51.5 y; TSM = 6.5 (≥ 1) y; femoral neck BMD < 0.795 g/cm^2 (−1.0 T-score); Italy	54 mg pure Ge vs. placebo	L: 0.925; FN: 0.688	3	A
Uesugi 2003[26]	P; R, WD	3 mo	N: 22/21 (4%) PoW; mean age: 53.7 y; TSM = 6 (5–10) y; non-osteoporosis; Japan	62 mg IC [38 mg IAE: 52% De, 11% Ge, 37% Gle] vs. placebo	L2–4: 1.040	2	C (unclear analyzed N)
Wu 2006a[27], b[28]	P; R, DB+, WD	6, 12 mo	N: 136/128, 108 (6, 21%); mean age: 54.4 y; TSM = 3.2 (1–5) y; Japan	75 mg IC [47 mg IAE: 54% De, 13% Ge, 34% Gle] vs. placebo	L2–4: 0.899; FN: 0.672; TH: 0.782; Tr: 0.595	4	A, C (dropout > 20%)
Xin 2006[29]	P; R, DB	6 mo	N: 76 MW; age: 45–55 y; TSM ≤ 5 y; China	50 mg pure De + calcium vs. calcium only (control)	L2–4: 0.715; TH: 0.643	2	C (unclear analyzed N)
Ye 2006[17]	P; R+, SB, WD	6 mo	N: 90/84 (7%) PoW; mean age: 52.3 (1–5) y; TSM = 2.6 (1–5) y; China	84 and 126 mg IAE [52% D(e), 15% G(e), 33% Gl(e)] vs. placebo	L1–4: 0.864; FN: 0.702; TH: 0.800; Tr: 0.588	3	B

[†]CO, crossover; DB, double-blinded (gives 1 point to Jadad scale); DB+, double-blinded by appropriate method (gives 2 point); OL, open-labeled; P, Parallel; R, randomized (give 1 point); R+, randomized by appropriate method (gives 2 point); SB, single-blinded; WD, withdrawals and dropouts described (gives 1 point).

[‡]BMD, bone mineral density; N, randomize/analyzed number (dropout rate) of participants; MW, menopausal women; PoW, postmenopausal women; TSM, averaged time since menopause.

[§]IAE, isoflavone aglycone equivalents; IC, isoflavone conjugate containing glycoside and aglycone forms; IF, isoflavones (form and composition unknown); D(e), daidz(e)in; De, daidzein; Ge, genistein; G(e), genist(e)in; Gl(e), glycit(e)in; Gle, glycitein.

[¶]FN, femoral neck; L, lumbar spine; TH, total hip; Tr, trochanter.

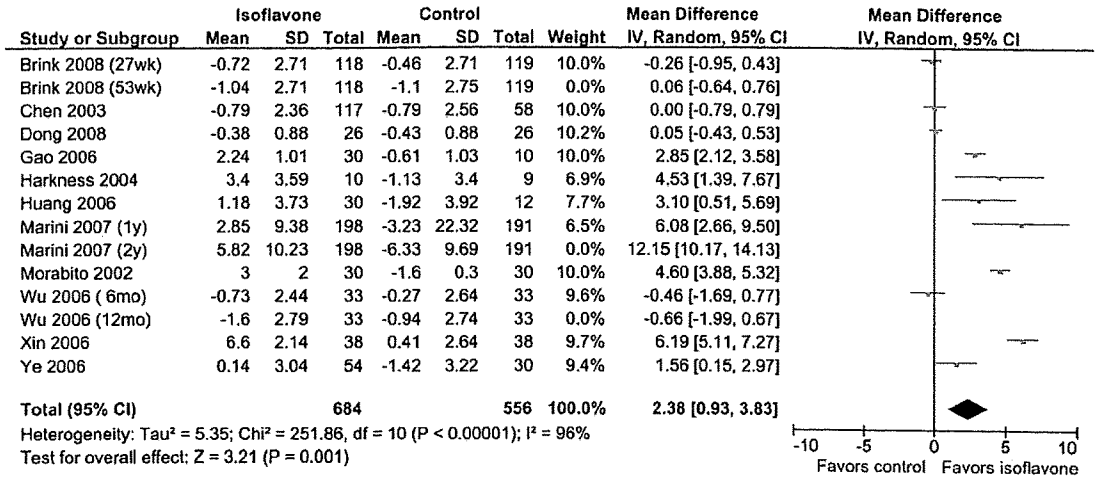

Figure 2. Effects of soy isoflavones on spine BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% Weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

mean difference for 6 months duration[27] was similarly negative to that for 1 year duration.[28]

Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, using data sets of longer duration instead of short duration for trials with two time points of measurements, selecting only placebo-controlled trials, and eliminating low-quality trials (Jadad scale < 3 or Category C) did not result in significantly different overall effects of soy isoflavones on spine BMD.

Results of subgroup analyses of the effects of soy isoflavones on spine BMD were shown in Table 2. Each subgroup analysis resulted in significant heterogeneity and revealed significant effect of soy isoflavones in increasing spine BMD compared with controls using the fixed effect model. Results based on fixed effect model revealed that effects of soy isoflavones on spine BMD in subgroups of 6 months duration and of Asian region were significantly different with the effects in subgroup of 1 year duration and of Western region, respectively. Two subgroups of each subgroup analysis using the random effects model, show similarly significant effects of soy isoflavones in increasing spine BMD, except for a subgroup of participants with normal bone mass at baseline. Meta-regressions analyzing each of or all of the four pre-specified categorical study characteristics (intervention duration, isoflavone dosage, region of participants, and basal spine BMD), did not reveal that these pre-specified factors were significantly associated with the varying effects of soy isoflavones on spine BMD across trials. The funnel plots (Figure 3) and Egger's test of effects of soy isoflavones on spine BMD among the 11 trials (p = 0.251 and p = 0.267 for effects in terms of change and percentage change, respectively) did not indicate any obvious publication bias.

Meta-analysis of the 7 trials with 868 participants using the fixed effect model resulted in significant heterogeneity ($p < 0.001$). Meta-analysis using the random effects model, revealed that daily ingestion of an average of 76 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly increased femoral neck BMD by 10.24 mg/cm^2 (95% CI: −3.73, 24.20 mg/cm^2, p = 0.15), or by 1.48% (95% CI: −0.54, 3.50%, p = 0.15) compared with controls. Sensitivity analysis assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5, did not result in significantly different overall effects of soy isoflavones on femoral neck BMD. Whereas, sensitivity analysis using data sets of longer duration for trials with two time points of measurements, found that ingestion of soy isoflavones for 6 months to 2 years tended to increase femoral neck BMD by 16.89 mg/cm^2 (95% CI: −2.34, 36.11 mg/cm^2, p = 0.09), or by 2.45% (95% CI: −0.31, 5.21, p = 0.08; Figure 4) compared with controls (random effects model). Sensitivity analyses selecting only placebo-controlled trials and eliminating low-quality trials were not performed because of the small number of available trials.

Meta-analysis of the 5 trials with 420 participants using the fixed effect model resulted in non-significant heterogeneity ($p \geq 0.1$), revealed that daily ingestion of an average of 74 (47–110) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change total hip BMD by 2.45 mg/cm^2 (95% CI: −1.41, 6.30 mg/cm^2, p = 0.21), or by 0.05% (95% CI: −0.53, 0.63%, p = 0.86) compared with controls. Sensitivity analyses assuming the level of correlation coefficient between baseline and follow-up values to be 0.75 and 0.5 and using data sets of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on total hip BMD.

Meta-analysis of the 5 trials with 419 participants revealed that daily ingestion of an average of 85 (47–150) mg (aglycone equivalent) soy isoflavones for 6 months to one year non-significantly change trochanter BMD by −0.40 mg/cm^2 (95% CI: −6.58, 5.78 mg/cm^2, p = 0.90), or by −0.07% (95% CI: −1.15, 1.02%, p = 0.91) compared with controls (random effects model). Sensitivity analyses assuming the level of correlation coefficient between

Table 2. Subgroup analyses of the effects of soy isoflavones on spine BMD†

Variables	No. of trials	Sample size	*p* for heterogeneity	Fixed effect model			Random effects model	
				WMD (95% CI)	*p*-value	*p*-value (diff)	WMD (95% CI)	*p*-value
Intervention duration								
6 months	6[16-18, 21, 27, 29]	522	< 0.00001	17.72 (14.03, 21.41) mg/cm^2	< 0.00001	= 0.0002	18.74 (1.25, 36.23) mg/cm^2	0.04
			< 0.00001	1.81 (1.40, 2.21) %	< 0.00001	= 0.03	2.31 (0.16, 4.47) %	0.04
1 year	5[8, 22-25]	718	< 0.00001	8.74 (5.90, 11.58) mg/cm^2	< 0.00001		22.64 (1.54, 43.74) mg/cm^2	0.04
			< 0.00001	1.23 (0.88, 1.58) %	< 0.00001		2.52 (0.17, 4.87) %	0.04
Isoflavone dose								
≤ 75 mg/d	6[8, 22, 23, 25, 27, 29]	818	< 0.00001	11.70 (9.10, 14.30) mg/cm^2	< 0.00001	= 0.57	20.79 (1.48, 40.09) mg/cm^2	0.03
			< 0.00001	1.53 (1.20, 1.85) %	< 0.00001	= 0.59	2.59 (0.26, 4.92) %	0.03
> 75 mg/d	5[16-18, 21, 24]	422	< 0.00001	13.21 (8.73, 17.69) mg/cm^2	< 0.00001		19.49 (2.64, 36.34) mg/cm^2	0.02
			< 0.00001	1.37 (0.91, 1.83) %	< 0.00001		2.10 (0.31, 3.90) %	0.02
Region of participants								
Asian	7[17, 18, 22-24, 27, 29]	535	< 0.00001	9.01 (6.44, 11.59) mg/cm^2	< 0.00001	< 0.00001	15.06 (0.89, 29.23) mg/cm^2	0.04
			< 0.00001	1.17 (0.86, 1.49) %	< 0.00001	= 0.0006	1.85 (0.16, 3.54) %	0.03
Western	5[8, 16, 21, 25]	705	< 0.00001	21.97 (17.34, 26.60) mg/cm^2	< 0.00001		31.46 (0.56, 62.37) mg/cm^2	0.05
			< 0.00001	2.20 (1.71, 2.68) %	< 0.00001		3.56 (0.13, 6.99) %	0.04
Basal spine BMD								
Normal bone mass	3[18, 21, 24]	319	< 0.00001	12.31 (7.42, 17.20) mg/cm^2	< 0.00001	= 0.92	17.06 (−7.55, 41.66) mg/cm^2	0.17
			< 0.00001	1.27 (0.78, 1.76) %	< 0.00001	= 0.33	1.78 (−0.74, 4.29) %	0.17
Osteopinia or osteoporosis	8[8, 16, 17, 22, 23, 25, 27, 29]	921	< 0.00001	12.02 (9.48, 14.55) mg/cm^2	< 0.00001		21.70 (5.43, 37.97) mg/cm^2	0.009
			< 0.00001	1.56 (1.24, 1,87) %	< 0.00001		2.64 (0.69, 4.60) %	0.008

†BMD, bone mineral density; WMD, weighted mean difference; *p*-value, test for overall effect of each subgroup; *p*-value (diff), test for subgroup differences.

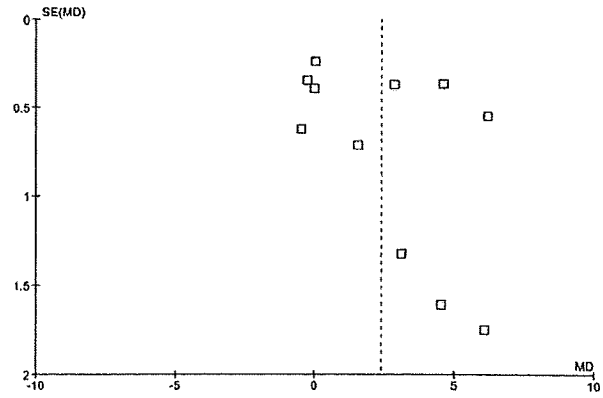

Figure 3. Funnel plots of effects of soy isoflavones on spine BMD (%). MD, weighted mean difference between percentage changes (%) of spine bone mineral density (BMD) from baseline for isoflavone and control groups; SE (MD), standard error of MD; fixed, fixed effect model.

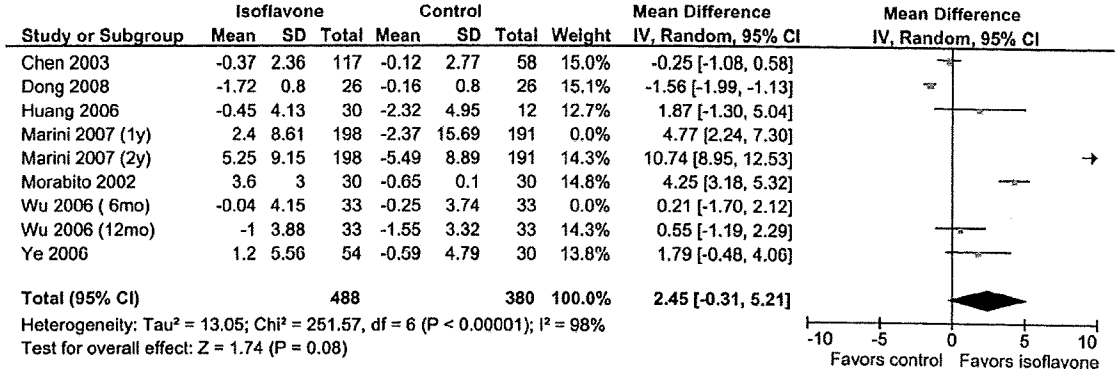

Figure 4. Effects of soy isoflavones on femoral neck BMD (%). Mean Difference, weighted mean difference between percentage changes (%) of femoral neck bone mineral density (BMD) from baseline for isoflavone and control groups; random, random effects model. Horizontal lines denote the 95% CI. Data sets for long duration not included in the meta-analysis were signed 0.0% weight. ■ Point estimate (size of the square corresponds to its weight); ♦ Combined overall effect.

baseline and follow-up values to be 0.75 and 0.5 and using data set of longer duration for trials with two time points of measurements, did not result in significantly different overall effects of soy isoflavones on trochanter BMD.

DISCUSSION

The present meta-analysis found that ingestion of about 82 mg of extracted soy isoflavones (in the aglycone form) per day for 6 months to 1 year significantly increased lumbar spine BMD by 2.38% compared with controls without isoflavones, in menopausal women. Results of sensitivity analyses indicated that the effect of soy isoflavone extracts in increasing lumbar spine BMD was robust. This magnitude of beneficial effect of soy isoflavones appears to almost completely offset naturally occurring postmenopausal bone loss. Effect of soy isoflavones in increasing femoral neck BMD seems to take more time than spine BMD. Our meta-analysis did not reveal significant effects on total hip and trochanter BMD, which might be due to the limited number of five trials.

An intake of 82 mg soy isoflavones/day (in the aglycone form) is approximately equivalent to 1.7 times the amount consumed habitually in Japan (mean: 47.2 mg/day).[40] The mechanism mediating the improvement of BMD at these skeletal sites by soy isoflavones is not well understood, but it may be a result of their chemical and biological similarity to mammalian estrogens, which are known to increase BMD in menopausal women.[1,4]

Results of subgroup analyses indicated that the varying effects of soy isoflavone extracts on spine BMD across the 11 trials were associated with study characteristics of intervention duration, region of participants, and basal BMD. The heterogeneity of effects of soy isoflavones on spine BMD across the 11 trials might also be induced by differences in habitual dietary intake of soy isoflavones,[28] time since menopause,[3] intervention duration,[25] isoflavone dosage,[17,41] chemical forms and proportions of individual soy isoflavones,[42-44] and participants' ethnicity. Isoflavone glycosides are not absorbed intact across the enterocytes of healthy adults, and their bioavailability requires initial hydrolysis by intestinal β-glucosidases for uptake into the peripheral circulation.[44] Asian and Western populations are reported to have differences in the capacity of intestinal flora to convert daidzein to its metabolite, equol.[45] Equol is easily absorbed and possesses substantial estrogenic activity because of its affinity for both the estrogen α and β receptors.[43] Equol is suggested to be the single most important factor that influences the clinical efficacy of soy isoflavones in preventing bone

loss.[46] Because of the limited number of trials and insufficient data available, our meta-analysis was also unable to evaluate possible influences on the varying effects of soy isoflavones on spine BMD across trials of dietary intake of soy isoflavones, time since menopause, chemical forms and proportions of individual soy isoflavones, blood isoflavone concentration, urinary isoflavone excretion, and equol producer status.

Since there was significant heterogeneity in effects of soy isoflavones on spine BMD, we preferably presented the results by incorporating heterogeneity into the random effects model in this meta-analysis. A random effects meta-analysis model involves an assumption that the effects being estimated in the different studies are not identical, but follow some distribution. The model represents our lack of knowledge about why real, or apparent, treatment effects differ by considering the differences as if they were random.[19]

The magnitude of effect of soy isoflavone extracts in increasing spine BMD by 20.25 mg/cm^2 revealed in our present meta-analysis, were consistent with the results (by 20.6 mg/cm^2) from the previous meta-analysis that included 10 RCTs testing both extracted soy isoflavones and isolated soy protein containing isoflavones.[15] Thus, soy isoflavones ingested either alone in extracted form or as constituent part of isolated soy protein have been demonstrated to exert a mild but significant effect in increasing lumbar spine BMD in menopausal women. Our meta-analysis also revealed that ingestion of soy isoflavones for 6 months appears to be enough to exert beneficial effect on spine BMD in menopausal women. The present meta-analysis did not reveal influences of isoflavone dosage on the effect on spine BMD, possibly due to the fact that trials tested various forms and compositions of soy isoflavones likely possessing different bioavailability and effects on bone mass; other explanations might be the limited number of trials or of some other factors inducing the heterogeneity.

CONCLUSION

The effect of soy isoflavones in increasing spine BMD in menopausal women are not as strong as those of approved pharmacologic therapies involving estrogen or bisphosphonates.[1,4,47,48] However, the present meta-analysis revealed that soy isoflavone extract supplements did result in a significant improvement of lumbar spine BMD with good tolerance and no induction of notable adverse events. Our meta-analysis suggested that soy isoflavone supplements can be used not only to offset the bone loss that occurs naturally in women after menopause, but are also applicable for complementary or alternative use in patients with postmenopausal osteopenia or osteoporosis who are unable to tolerate the side effects of estrogen or/and bisphosphonate therapies. Furthers studies are needed to address factors affecting the magnitudes of the effect of soy isoflavones on spine BMD and to verify the effect on hip BMD.

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AUTHOR DISCLOSURES

Kyoko Taku, Melissa K. Melby, Jun Takebayashi, Shoichi Mizuno, Yoshiko Ishimi, Toyonori Omori and Shaw Watanabe, disclose no conflicts of interest.

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Original Article

Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials

Kyoko Taku PhD[1], Melissa K Melby PhD[2], Jun Takebayashi PhD[3], Shoichi Mizuno PhD[4], Yoshiko Ishimi PhD[3], Toyonori Omori PhD[5], Shaw Watanabe PhD[2]

[1]*Information Center, National Institute of Health and Nutrition, Tokyo, Japan*
[2]*Nutritional Education Program, National Institute of Health and Nutrition, Tokyo, Japan*
[3]*Food Function and Labeling Program, National Institute of Health and Nutrition, Tokyo, Japan*
[4]*Center for Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan*
[5]*Director for Research Coordination and Evaluation, National Institute of Health and Nutrition, Tokyo, Japan*

大豆異黃酮抽取物的補充劑對停經後婦女骨質密度的效果：隨機對照試驗的後設分析

本研究旨在確認攝取大豆異黃酮抽取物(並非大豆蛋白或含有異黃酮的食品)對停經後婦女骨質密度(BMD)的效果。我們從 PubMed，CENTRAL，ICHUSHI，CNKI，Wanfang Data，CQVIP，和 NSTL 檢索，以英語，日語，或中文發表，並報告大豆異黃酮抽取物對停經後婦女腰椎或髖關節 BMD 效果的隨機對照試驗論文。依照納入和排除標準，對試驗論文進行鑑別和評閱來判定是否採用。有關研究設計，對象，介入，和結果的數據被抽取出進行分析。最終分別有 11、7、5、和 5 個試驗被採用來評估對腰椎、大腿骨頸部、髖關節全體、和股骨大轉子 BMD 的效果。包括 1240 名停經後婦女的後設分析(隨機效果模型)顯示，與對照組相比，每日平均攝取 82 (47-150) mg 的大豆異黃酮(苷元當量)持續 6-12 個月，顯著地提高腰椎 BMD 22.25 mg/cm^2 (95%信賴區間: 7.61，32.89；p=0.002)，或提高 2.38% (95%信賴區間: 0.93，3.83；p=0.001)。亞組分析顯示，不同試驗間大豆異黃酮對腰椎 BMD 的效果各異，可能與介入期間(6 或 12 個月)，對象的區域(亞洲或西方)，和基礎 BMD(正常骨質或骨質減少症或骨質疏鬆症)的研究特徵相關。我們的後設分析沒有發現對大腿骨頸部，髖關節全體，和股骨大轉子 BMD 的效果。大豆異黃酮抽取物的補充劑提高了停經後婦女的腰椎 BMD。需要更深入的研究去闡明影響其對腰椎效果程度的因素，以及驗證其對髖關節的效果。

關鍵字：後設分析、異黃酮、膳食補充劑、停經、骨密度

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Soybean Isoflavones in Bone Health

Yoshiko Ishimi

Project for Bio-index, Nutritional Epidemiology Program, National Institute of Health and Nutrition, Tokyo, Japan

Abstract

Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. It has been reported that isoflavones play an important role in the prevention of hormone-dependent diseases, including osteoporosis, cardiovascular diseases, cancer, and postmenopausal syndrome. There are many researches indicating isoflavones prevent bone loss caused by estrogen deficiency in animal models. Furthermore, it has been demonstrated that a combination of isoflavone treatment and exercise cooperatively prevented bone loss in the estrogen-deficient status. Epidemiological studies demonstrated the relationship between the lower incidence of osteoporosis in Asian women and a diet rich in soy foods. Although a number of observational studies confirm the findings from the animal studies, the results from intervention studies are still controversial. One of the potential reasons for these inconsistencies could be individual differences in the isoflavone metabolism. Recently, it has been suggested that the clinical effectiveness of isoflavones might partly depend on the ability to produce equol, a gut bacterial metabolite of daidzein showing stronger estrogenic activity than the predominant isoflavones. Several candidate bacteria responsible for equol production have been suggested, for example *Lactococcus* 20-92 strain. From these findings, food factors enhancing equol production have received great deal of attention recently. On the other hand, safety assessment of isoflavones has been conducted by the Japanese Food Safety Commission. Further studies are required to address the numerous questions on the potential benefits, mechanisms of action, and safety of isoflavones.

Soybeans have been consumed in Asia since ancient times. Compared with Caucasians, the low incidence of heart disease, reproductive cancers, hip fracture, and climacteric symptoms in Asians has been considered to be associated with their high intake of soy foods. Recent growing interest in health and diet has led to an increased focus on soy foods and their functional components, e.g. isoflavones. Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. Isoflavones exert beneficial health effects by acting as antioxidants, tyrosine kinase and topoisomerase inhibitors as well as estrogenic activity. It has been reported that they play an important role in the prevention of chronic diseases, including osteoporosis, cardiovascular diseases, hormone-dependent cancer, and postmenopausal syndrome [1–5].

Osteoporosis is a skeletal disorder in which bone strength is compromised by the loss of bone density and bone quality. It is the leading cause of increased morbidity and functional loss in the elderly. Particularly postmenopausal women suffer from osteoporosis, which is part of the postmenopausal syndrome [6]. Although the treatment of postmenopausal osteoporosis is hormone replacement therapy, the reported side effects, such as development of hormone-dependent breast and uterine cancers [7], have prompted the use of alternative therapies. Soybean isoflavones have received considerable attention as alternatives to hormone replacement therapy, since the risk for side effects of isoflavone treatment seem to be low compared with hormone replacement therapy [8].

Epidemiological studies indicate that women who have high soy intake have less risk for osteoporosis than women who consume a typical Western diet [8]. A recent meta-analysis of 10 randomized controlled trials (RCTs) indicated that isoflavone intervention significantly attenuated bone loss in postmenopausal women [9]. However, the results from human studies are still controversial [10]. One of the potential reasons for these inconsistencies could be individual differences in isoflavone metabolism. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to their ability to produce the metabolite equol in the intestine [11].

This chapter focuses on the current topics of research on isoflavones and their relationship to bone health.

Isoflavones and Their Metabolites

The major isoflavones contained in soy-based food products are daidzein, genistein, and glycitein (fig. 1). They exist mainly in the glycoside, acetyl glycoside, or malonyl glycoside forms in soy foods; hence, it becomes necessary to hydrolyze the glycosidic bonds for intestinal absorption to enable physiological activities. These glycosidic bonds are hydrolyzed by glycosidase produced by intestinal microflora such as *Lactobacilli*, *Bacteroides* and *Bifidobacteria* [12]. Furthermore, intestinal microflora affects the metabolism, wherein isoflavones are metabolized to equol, or O-desmethylangolensin (O-DMA) from their precursor daidzein (fig. 1).

Recent studies suggest that the clinical effectiveness of isoflavones might be due to their ability to produce metabolites, such as dihydrodaidzein, tetrahydrodaidzein, equol and O-DMA in the intestine [13]. Particularly, equol, a metabolite of daidzein, has received considerable attention because its biological activities differ from those of its precursor [11]. The metabolites of genistein and glycitein were also primarily found in human urine (genistein: dihydrogenistein, 6′-OH-O-DMA [13], 4-hydroxyphenyl-2-propionic acid and phloroglucinol; glycitein: dihydroglycitein, 5′-methoxy-O-DMA and 6-methoxy-equol). However, the physiological activities of these metabolites are still unclear.

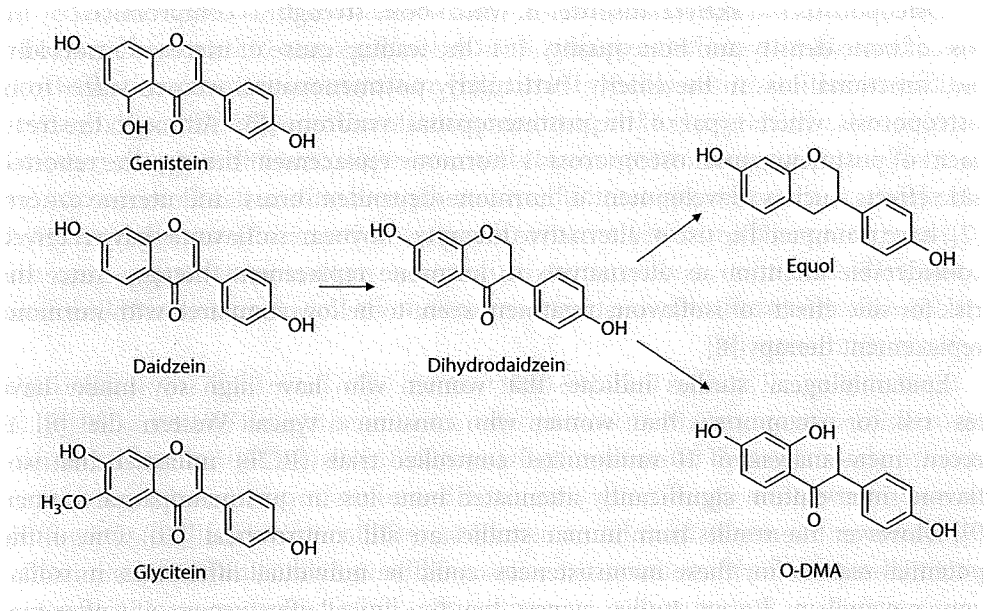

Fig. 1. Molecular structures of isoflavone aglycone and daidzein metabolites.

Equol has higher estrogenicity, stronger antioxidative efficacy, and exhibits anti-androgenic properties [11]. Moreover, equol is a chiral molecule, which exists as enantiomers R (+)-equol and S (–)-equol. In humans, the metabolism of daidzein to equol results in the production of only S-equol. Interindividual variability in equol production may be unique to humans; all the animals including rats, mice, and chimpanzees, tested systematically excrete equol. Although O-DMA was found in 80–90% of a human population, equol was found in only 30–50% of the population [14]. This is because of individual differences in the intestinal microbiota responsible for equol production.

Intestinal bacteria play a key role in isoflavone metabolism; young infants with undeveloped gut microflora do not produce equol, while germ-free animals also do not produce equol or O-DMA. Since some reports suggested a lower disease risk for equol producers than for nonproducers [11], there is a growing interest in certain bacterial strains that can produce equol. A number of strains involved in daidzein metabolism were identified. However, the identification of equol-producing bacteria is complicated. To date, only one lactic acid bacterium *(Lactococcus* 20-92 homologous to *Lactococcus garvieae)* has been identified that produces equol directly from daidzein without producing O-DMA [15]. Interestingly, the strain *Lactococcus* 20-92 can also cleave glycosidic bonds of daidzin; Uchiyama et al. [15] detected *L. garvieae* in the Italian cheese Toma Piemontese.