TROL の抽出液中のたんぱく量（μg/ml）
PV：SOYA PROTEIN CONTROL の Protein Value（0.84）
W1：精秤した試料量（g）
W2：トリス-塩酸緩衝液量（g）
W3：抽出に供した試料量（g）
W4：抽出に供した SOYA PROTEIN CONTROL 量（mg）

2．大豆イソフラボン

食品中の大豆イソフラボンの定量は，厚生労働省が通知した「大豆イソフラボンを含む特定保健用食品等の取り扱いに関する指針（食安発第0823001号）」別紙[3] に記載されている高速液体クロマトグラフ（HPLC）法にて行った。

2-1 標 準 品

大豆イソフラボンは，ダイジン，グリシチン，ゲニスチンの配糖体，マロニル配糖体，アセチル配糖体，サクシニル配糖体およびアグリコンの合計15種類の大豆イソフラボン（表1，長良サイエンス，岐阜）について定性を行った。定量分析には，標準品としてダイジン，グリシチン，ゲニスチンの3種類を用いた。標品は70%エタノールに溶解し，濃度が 1 mg/5 ml になるように調製した。分析に用いるまでは－30℃で保存した。

2-2 試料の調製

液状食品は，大豆イソフラボンとして 1－10 mg が含まれる量の食品を 50 ml 容メスフラスコに正確に分取し，70%エタノール（v/v）で 50 ml に定容した。よく転倒混和し試験溶液とした。この方法により抽出されない食品サンプルについては，大豆イソフラボンアグリコンは100℃ 付近では分解されないという報告や[4]，酸性条件下で特に過熱による分解に対する耐性が増す[5] という報告から，40－50℃で加熱攪拌抽出を行った。

粉末状食品は，大豆イソフラボンとして 1－10 mg が含まれる量の食品を 50 ml ビーカーに精秤し，70%エタノール 25 ml を加えて30分間攪拌した。30分間室温で攪拌抽出した後，遠心分離して上清を 50 ml 容メスフラスコに移し，残渣についても 10 ml の 70%エタノールを加え，同様の抽出操作を更に 2 回行った。計 3 回分の上清を集め，70%エタノールで 50 ml に定容し試験溶液とした。試料は全て 0.45 μm のメンブレンフィルター（ADVANTEC, Cellulose Acetate 0.45 μm）で濾過した後，分析に供した。

2-3 定量分析

高速液体クロマトグラフ（HPLC）法の分析条件を以下に示す。

分析機器：高速液体クロマトグラフィー（島津製作所）
カ ラ ム：YMC-Pack ODS-AM-303（sizeφ4.6×250 mm）（ワイエムシイ社，京都）
移 動 相：Aアセトニトリル／水／酢酸混液（15:85:0.1 v/v/v）
Bアセトニトリル／水／酢酸混液（35:65:0.1 v/v/v）
濃度勾配：AからBまでの直線濃度勾配を50分間行った。移動相Aを20分間送液した後，次の分析を開始した。
流 速：1 ml/min
カラム温度：35℃
測定波長：254 nm
試料注入量：10 μl

2-4 食品中の大豆イソフラボン量の解析

試料中の大豆イソフラボン濃度は，分子量の比を用いて全てアグリコン当量として求めた。ダイゼインを例に，アグリコン等量の求め方を以下の式に示す。

例）TDe＝TADe×CD/AD×0.611※
TDe：ダイゼイン型大豆イソフラボンの濃度（アグリコン当量）[mg/l]
TADe：ダイゼイン型大豆イソフラボンのピーク面積
CD：大豆イソフラボン標準溶液中のダイジンの濃度[mg/l]
AD：大豆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
※ダイゼイン型のアグリコン換算係数。グリシテイン型は0.637，ゲニステイン型は0.625を乗じてそれぞれのアグリコン等量を求めた。

ダイゼイン型，グリシテイン型およびゲニステイン型も同様にアグリコンの濃度の総和を試料中の総大豆イソフラボン濃度（アグリコン当量）とし，一日または一回摂取目安量における試料中の大豆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。

試料中の大豆イソフラボン含量（アグリコン当量）
＝総大豆イソフラボン濃度（mg/l）×50/1000×1/ 試料採取量（g または ml）×（一日または一回摂取目安量）

結　　果

「健康食品」中の大豆たんぱく質および大豆イソフラボンの分析

1．大豆たんぱく質について

対象とした食品は，大豆たんぱく質を関与成分とする特定保健用食品（液状食品，粉末状食品）ならびにスポーツ選手を対象とした大豆たんぱく質強化食品（粉末

表2 食品の表示と大豆たんぱく質含有量

分類	名称（食品形態）	原材料名	1日または1回摂取目安量に関する表示	たんぱく質表示値（g）	大豆たんぱく質表示量（g）	分析値（mg）	表示量に対する割合（%）	CV値（%）
液状食品	調整豆乳（液状食品）	大豆（遺伝子組換えでない）	1本（200 ml）	8.8	6.7	7.7	114.9	18.9
	調整豆乳（液状食品）	大豆	1本（200 ml）	7.6	6.0	6.2	103.3	18.1
	大豆たんぱく飲料（液状食品）	大豆たんぱく，大豆	1本（195 g）	8.4	6.0	7.1	118.3	12.2
	清涼飲料水（液状食品）	粉末状大豆たんぱく（遺伝子組み換えでない）	1本（100 g）	7.1	6.0	6.3	105.0	7.4
粉末状食品	乾燥スープ（粉末状食品）	分離大豆たんぱく質	1袋（ 15 g）	9.0	7.0	7.9	112.9	9.1
	プロテインパウダー（粉末状食品）	大豆たんぱく	1食（ 21 g）	16.8	16.8	18.7	111.3	6.9
	大豆たんぱく食品（粉末状食品）	大豆分離たんぱく	1食（ 20 g）	16.9	16.9	15.2	89.9	2.0
	プロテインパウダー（粉末状食品）	大豆たんぱく（遺伝子組換え大豆は使用しておりません）	1食（ 20 g）	16.6	16.6	15.3	92.2	2.4
	プロテインパウダー（粉末状食品；ジュニア用）	粉末状大豆たんぱく	1食（ 20 g）	8.7	（表示なし）	4.7	−	8.3
	たんぱく食品（粉末状食品；ジュニア用）	大豆分離たんぱく	1食（ 20 g）	8.4	（表示なし）	5.5	−	2.5

状食品）である。表2に，食品の大豆たんぱく質に関する表示と分析値を示した。分析値は，平均値および変動係数（CV値（%））で示した。分析した食品中の大豆たんぱく質は，10品目中8品目には大豆たんぱく質量が表示されており，分析値の表示量に対する割合は90−118%であった。スポーツ選手を対象とした大豆たんぱく質強化食品には，一日摂取目安量当り 15−18 g の大豆たんぱく質が含まれていた。これは，特定保健用食品の一日摂取目安量の約2倍であった。ジュニア選手を対象とした大豆たんぱく質強化食品（2品目）には，表示されている一回摂取目安当りのたんぱく質量のおおよそ半分量である5 g の大豆たんぱく質が確認された。変動係数は液状食品で（6.2−7.7%：intra-assay），粉末状食品で（4.2−18.7%：intra-assay）であった。

2．大豆イソラボンについて

表3に各食品の大豆イソフラボン（アグリコン等量）の表示と分析値を示した。大豆たんぱく質を関与成分とする特定保健用食品の表示では，大豆イソフラボンの含有量は 8−30 mg／一日摂取目安量であるが，分析の結果，表示の90−122%の大豆イソフラボンが実測された。スポーツ選手を対象とした大豆たんぱく質強化食品5品目においては，大豆イソフラボン含量の表示はなされていなかったが，分析の結果，アグリコン当量として1食分当たり 20−44 mg の大豆イソフラボンが含まれていた。ジュニア選手を対象とした大豆たんぱく質強化食品（2品目）には，1食当たり 17−20 mg の大豆イソフラボンが含まれていた。変動係数（CV（%））は，液状食品では（2.1−9.6%: intra-assay），粉末状食品では（0.9−9.1%：intra-assay）であった。

大豆イソフラボンは，ダイゼイン型，グリシテイン型，ゲニステイン型の3種類に大別することができる。また，それぞれにアグリコンおよび配糖体が存在し，合計15種類の大豆イソフラボン化合物がある（表1）。今回対象とした健康食品中にも多種の大豆イソフラボンが確認された。表4に各食品中一日または一回摂取目安量に含まれる15種類の大豆イソフラボンの成分分析の結果を示した。解析の結果，豆乳には配糖体（ダイジン，ゲニスチン）とマロニル配糖体（マロニルダイジン，マロニルゲニスチン）が多く含まれていることが確認され，大豆たんぱく質強化食品には配糖体（ダイジン，ゲニスチン），マロニル配糖体（マロニルダイジン，マロニルゲニスチン），あるいはアグリコン（ダイゼイン，ゲニステイン）の多種の成分が混在していた。納豆に多く含まれることが知られるサクシニル配糖体は，少量であるが豆乳やプロテインパウダーに含まれていた。表5に対象とした食品に含まれる大豆イソフラボンの型別の割合を示した。今回対象とした食品にはゲニステイン型が約60%含まれており，次いでダイゼイン型が約30%，グリシチン型の割合は10%以下であった。

表3　食品の表示とイソフラボン含有量（アグリコン換算値）

分類	名　称（食品形態）	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（mg）	分析値（アグリコン当量）（mg）	表示量に対する割合（%）	CV値（%）
液状食品	調整豆乳（液状食品）	大豆（遺伝子組換えでない）	1本（200 ml）	大豆イソフラボンアグリコンとして30 mg	30	35	116.2	3.8
	調整豆乳（液状食品）	大豆	1本（200 ml）	大豆イソフラボンアグリコンとして16 mg	16	20	122.2	9.6
	大豆たんぱく飲料（液状食品）	大豆たんぱく，大豆	1本（195 g）	大豆イソフラボンアグリコンとして27 mg	27	24	90.3	4.2
	清涼飲料水（液状食品）	粉末状大豆たんぱく（遺伝子組み換えでない）	1本（100 g）	大豆イソフラボンアグリコンとして8－22 mg	8－22	9	（範囲内）	2.1
粉末状食品	乾燥スープ（粉末状食品）	分離大豆たんぱく質	1袋（ 15 g）	大豆イソフラボンアグリコンとして18mg を含む	18	20	109.8	7.0
	プロテインパウダー（粉末状食品）	大豆たんぱく	1食（ 21 g）	（表示なし）	－	44	－	0.5
	大豆たんぱく食品（粉末状食品）	大豆分離たんぱく	1食（ 20 g）	（表示なし）	－	43	－	8.3
	プロテインパウダー（粉末状食品）	大豆たんぱく（遺伝子組換え大豆は使用しておりません）	1食（ 20 g）	（表示なし）	－	23	－	0.9
	プロテインパウダー（粉末状食品；ジュニア用）	粉末状大豆たんぱく	1食（ 20 g）	（表示なし）	－	21	－	9.1
	たんぱく食品（粉末状食品；ジュニア用）	大豆分離たんぱく	1食（ 20 g）	（表示なし）	－	17	－	5.0

表4　イソフラボン成分量（アグリコン換算値）mg／1日または1回摂取目安量当たり

分類	名　称（食品形態）	原材料名	D	MD	AD	SD	De	Gl	MGl	AGl	SGl	Gle	G	MG	AG	SG	Ge	総イソフラボン（mg）
液状食品	調整豆乳（液状食品）	大豆（遺伝子組換えでない）	9.3	4.0	0.2	0.2	0.1	0.6	0.1	0.1	0.5	0.1	11.0	8.0	0.2	0.0	0.0	34.4
	調整豆乳（液状食品）	大豆	4.9	0.6	0.0	0.0	0.0	0.2	0.0	2.3	0.0	0.2	9.2	0.0	0.8	0.0	0.7	18.8
	大豆たんぱく飲料（液状食品）	大豆たんぱく，大豆	3.8	0.6	0.3	0.0	3.1	0.9	0.1	0.4	0.0	0.4	8.7	2.0	0.3	0.0	3.8	24.4
	清涼飲料水（液状食品）	粉末状大豆たんぱく（遺伝子組み換えでない）	1.1	0.5	1.7	0.0	0.6	0.1	0.0	0.0	0.0	0.0	4.1	0.0	0.0	0.2	0.7	8.9
粉末状食品	乾燥スープ（粉末状食品）	分離大豆たんぱく質	1.2	2.9	7.0	0.0	1.9	0.3	0.5	0.0	0.9	0.2	1.8	0.0	0.6	0.0	3.1	20.5
	プロテインパウダー（粉末状食品）	大豆たんぱく	2.1	4.6	0.5	0.2	4.9	0.4	0.8	0.9	0.0	0.6	6.1	13.7	0.8	0.0	8.7	44.2
	大豆たんぱく食品（粉末状食品）	大豆分離たんぱく	2.1	6.4	0.5	0.0	3.6	0.6	1.3	0.0	0.0	0.5	3.6	16.8	1.0	0.0	6.2	42.6
	プロテインパウダー（粉末状食品）	大豆たんぱく（遺伝子組換え大豆は使用しておりません）	1.5	2.1	0.5	0.0	1.4	0.3	0.4	0.8	0.0	0.1	4.5	7.1	1.5	0.0	2.4	22.6
	プロテインパウダー（粉末状食品；ジュニア用）	粉末状大豆たんぱく	1.8	2.3	0.2	0.0	1.7	0.4	0.4	0.5	1.0	0.2	3.9	6.0	0.7	0.0	2.8	22.0
	たんぱく食品（粉末状食品；ジュニア用）	大豆分離たんぱく	1.4	1.5	0.4	0.0	1.8	0.3	0.2	0.2	0.0	0.1	3.5	3.8	0.8	0.0	3.0	17.0

表5 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（遺伝子組換えでない）	40.3	4.0	55.7	100
	調整豆乳（特定保健用食品）	大豆	31.8	3.8	64.4	100
	大豆たんぱく飲料（特定保健用食品）	大豆たんぱく，大豆	31.8	7.4	60.8	100
	清涼飲料水（特定保健用食品）	粉末状大豆たんぱく（遺伝子組み換えでない）	43.5	1.4	55.1	100
粉末状食品	乾燥スープ（特定保健用食品）	分離大豆たんぱく質	65.7	5.7	28.6	100
	プロテインパウダー（粉末たんぱく飲料）	大豆たんぱく	27.3	6.2	66.5	100
	大豆たんぱく食品（栄養補助食品）	大豆分離たんぱく	29.6	5.5	64.9	100
	プロテインパウダー（たんぱく補給食品）	粉末状大豆たんぱく	28.9	7.0	64.1	100
	プロテインパウダー（たんぱく質含有食品；ジュニア用）	大豆たんぱく（遺伝子組換え大豆は使用しておりません）	27.5	11.4	61.1	100
	たんぱく食品（プロテインパウダー；ジュニア用）	大豆分離たんぱく	29.2	5.0	65.9	100

考　　察

本研究では，原材料に大豆あるいは大豆たんぱく質を含んだ「健康食品」中に含まれる大豆たんぱく質および大豆イソフラボンの定量分析を行った。また，それぞれの成分について一日または一食摂取目安量当たりの含有量を求め，大豆イソフラボンについては15種類の組成についても分析した。

大豆たんぱく質および大豆イソフラボンの含有量と表示

1．大豆たんぱく質に関して

対象とした健康食品中の大豆たんぱく質の分析値は表示の90－118%であり，表示を極端に逸脱しているものはなかった。今回分析した食品10品目のうち，ジュニア用のたんぱく質強化食品2品目には，大豆たんぱく質含量の表示がなかったが，表示されているたんぱく質の約半分量が大豆たんぱく質であることが明らかとなった。残りのたんぱく質は，原材料名に表示されている乳由来のホエイたんぱくだと考えられる。大豆たんぱく質の生理機能として，血清脂質やリポたんぱく質の減少に有効であることが報告されている[6]。大豆たんぱく質は小腸でペプチドに分解されるが，大豆から分離されたペプチドが生理機能を持つという研究報告も多く[7－10]，「健康食品」中に含まれるペプチドの定量分析も興味深い。

2．大豆イソフラボンに関して

厚生労働省は「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」[3]（2006年8月）のなかで，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や，アグリコンとしての含有量を表示することとしている。また，指針のなかでは大豆たんぱくを関与成分とする特定保健用食品において，大豆イソフラボン（アグリコンとして）の含有量の表示も義務づけられている。しかしながら，大豆たんぱくを強化した「いわゆる健康食品」については，一日当たりの摂取目安量について大豆イソフラボンアグリコンとして30 mgを超えないように設定する必要はない。これにより，今回対象とした，ジュニア用も含めたスポーツ選手向けの大豆たんぱく質強化食品すべてにおいて，大豆イソフラボンの表示はなされていなかった。今回，これらの食品中に含まれる大豆イソフラボンの定量を行った結果，ジュニア用の食品には1回の摂取目安量当たり16－20 mgの大豆イソフラボンが確認された。一方，成人を対象とした食品には，1回の摂取目安量当たり大豆イソフラボンアグリコンとして30 mg以上の大豆イソフラボンが含まれていることが明らかとなった。このため，大豆たんぱく質が強化された健康食品のうち，特にジュニア選手はこれらの食品を過剰に摂取しないよう配慮する必要があると考えられた。

大豆イソフラボンを含む食品には，含まれる大豆イソフラボン含有量や組成成分が異なることが先回の調査同様[11]確認されたが，大豆イソフラボンの型によって生体内では吸収性や生体内動態，生理作用が異なる可能性があり[12,13]，摂取する側の年齢，性別，個体特性等も併せて考慮する必要があると考えられた。

分析方法

1．大豆たんぱく質

本実験の大豆たんぱく質の定量には，Soya protein Assay Kit（Tepnel BioSystems）を用いたが，本キットは，酵素免疫測定法（ELISA法）の中でも競合法を原理としている。本実験の分析結果における変動係数（CV値

(%)) は，サンプル間で4.2－18.7%とばらついた結果となった。ELISA 法には，競合法とサンドイッチ法があるが，競合法は，目的物質にエピトープが一つあれば測定が可能であり，サンドイッチ法よりも低分子の物質を測定できるなどのメリットがある。しかし，競合法では測定時に目的物質と酵素標識抗原を共存して反応を行っているため，検出感度は二つのエピトープを反応させて定量するサンドイッチ法と比較すると劣る。今回のばらつきは，ELISA 法の測定原理に起因していることが要因の一つとして考えられる。

2．大豆イソフラボン

大豆イソフラボンにおける定量分析は，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」[3] の方法に基づいて行った。大豆たんぱく質を関与成分とする特定保健用食品である調製豆乳は上記の指針に記載されている方法では表示されている量の大豆イソフラボン量が検出されなかった（未記載データ）。この調製豆乳には原材料に乳化剤が含まれており，これが食品中の大豆イソフラボンの抽出効率を下げている可能性が考えられる。この食品サンプルを40－50℃で加熱撹拌すると抽出効率が改善されたため，加熱撹拌を30分間行って試料溶液とした。液状食品については，一品目を除くと CV 値は５%未満と安定した結果が得られたが，加熱撹拌を行った調製豆乳（特定保健用食品）の CV 値は9.6%であった。一方，粉末状食品の分析おいては CV 値が0.5－9.1%であった。液状食品と粉末状食品の CV 値の平均値を比較すると，それぞれ4.6%と5.1%であり２つの異なる形状の食品に対して大きな差は見られなかった。厚生労働省の報告に記載されている大豆イソフラボンの試験方法において，液状食品および粉末状食品では変動係数が小さいこと，また両者間の差が小さいことから，この二つのタイプの食品に対して大豆イソフラボンの分析方法の精度が高い事が確認された。

ま と め

原材料に大豆または大豆たんぱく質を含む「健康食品」を対象とし，一日あるいは一回摂取目安量当たりに含まれる大豆たんぱく質および大豆イソフラボンの定量を行った。また，大豆イソフラボンに関しては，15種類の大豆イソフラボンの成分の分析を行った。大豆たんぱく質は，表示があるものについて，特定保健用食品およびたんぱく質強化食品ともに90－118%が定量され，表示値から大きく逸脱した食品はなかった。表示がないジュニア用の大豆たんぱく質を強化したプロテインパウダーには，表示されたたんぱく質含有量のおおよそ1/2量の大豆たんぱく質が認められた。一方，大豆イソフラボンの表示がある食品に対する分析値は90－122%であり，これも表示値から大きく逸脱しているものはなかった。しかし，大豆イソフラボン含有量の表示のない大豆たんぱく強化食品に関して，２品目が厚生労働省で定められている上乗せ上限である大豆イソフラボンアグリコンとして30 mg を超えるものが確認された。また，15種類の大豆イソフラボンの成分分析より，豆乳には配糖体とマロニル配糖体が多く含まれていること，大豆たんぱく質強化食品には配糖体，マロニル配糖体，あるいはアグリコンの多種の成分が混在していることが明らかとなった。これらの結果より，本研究は大豆たんぱく質を原材料とした「健康食品」中の大豆たんぱく質および大豆イソフラボンの含有量が明らかとなり，大豆たんぱく質を強化した「健康食品」を適切に摂取するための表示の在り方を考察するための基礎資料となった。

謝 辞

本研究は平成20年度不二たん白質振興財団特定研究（主任研究者：石見佳子）および平成20年度厚生労働科学研究費補助金食品の安心安全確保推進事業（主任研究者：山田和彦）の一部として実施されました。本研究を進めるにあたり，ご支援をいただきました(独)国立健康・栄養研究所，食品保健機能プログラムの矢野友啓先生に深謝いたします。

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（受付：平成22年1月13日，受理：平成22年5月12日）

The Journal of Nutrition
Supplement: Equol, Soy, and Menopause

Dietary Equol and Bone Metabolism in Postmenopausal Japanese Women and Osteoporotic Mice[1,2]

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Abstract

Equol binds to the estrogen receptor with greater affinity than its precursor, daidzein, an isoflavone found in soybeans. Inter-individual differences in the ability to produce equol may lead to differential effects of isoflavone intervention on human health. Here, we review previously published work from our laboratory on equol producer status and bone health in humans and in a mouse model of osteoporosis. We performed a 1-y, double-blind, randomized trial to compare the effects of isoflavone (75 mg of isoflavone conjugates/d; equivalent to 47 mg/d of the aglycone form) with those of placebo on bone mineral density (BMD), fat mass, and serum isoflavone concentrations in 54 early postmenopausal Japanese women classified by their equol-producer phenotype. Isoflavone intervention increased the serum equol concentration in equol producers but not in nonproducers ($P < 0.04$). The annualized changes in BMD in the total hip and intertrochanteric regions in the isoflavone-treated equol producers (–0.46 and –0.04%, respectively) were less than in the nonproducers (–2.28 and –2.61%, respectively). The annualized change in fat mass was lower in the equol producers compared with the nonproducers in the isoflavone group. The annualized changes in BMD and fat mass did not differ between the equol producers and nonproducers in the placebo group. Equol also inhibited bone loss and fat accumulation in estrogen-deficient osteoporotic mice. Our data suggest that prevention of bone loss and fat accumulation in early postmenopausal women by isoflavones may depend on an individual's equol-producing capacity. J. Nutr. 140: 1373S–1376S, 2010.

Effects of the intestinal metabolites of daidzein, equol, on bone metabolism

Epidemiological studies indicate that women who have high soy intake have less risk for osteoporosis than those consuming a typical Western diet (1). Additionally, several recently published studies report that isoflavone supplementation decreases risk for osteoporosis (2–4). In general, these studies administered isoflavones (40–200 mg/d) for 1–2 y and measured bone mineral density (BMD).[3] The results from human studies are inconsistent (5). A recent meta-analysis of 10 randomized, controlled trials indicated that isoflavone intervention significantly attenuated bone loss in postmenopausal women (6,7). However, another meta-analysis concluded that isoflavones do not affect bone loss (8). One potential reason for these inconsistencies is individual differences in isoflavone metabolism. Recent studies suggest that the clinical effectiveness of isoflavones on bone metabolism might be due to individual differences in the ability to produce the daidzein metabolite, equol, in the intestine (9).

Equol is an isoflavone and a nonsteroidal estrogen (9). It binds to both estrogen receptors and induces transcription more strongly than other isoflavones, especially estrogen receptor-α (10). Moreover, equol is a chiral molecule, which exists as enantiomers R (+)-equol and S (–)-equol. In humans, the intestinal bacterial metabolism of daidzein to equol results in S-equol production only (11). Humans may be the only species that exhibits inter-individual variability in equol production. Several animals, including rats (12), mice (13), and chimpanzees (14), excrete equol. O-desmethylangolensin (O-DMA), another daidzein metabolite, is found in ~80–90% of the human

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the "Equol, Soy, and Menopause Research Leadership Conference" held in Washington, DC, June 16, 2009. The supplement coordinator for this supplement is Kara Lewis, Life Sciences Research Organization (LSRO) Senior Staff Scientist. The supplement is the responsibility of the guest editors to whom the Editor of *The Journal of Nutrition* has delegated supervision of both technical conformity to the published regulations of *The Journal of Nutrition* and general oversight of the scientific merit of each article. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. The Guest Editor for this supplement is Neil Shay. Guest Editor disclosure: Neil Shay declares no conflict of interest. Supplement Coordinator disclosure: Kara Lewis is currently under contract with and receives compensation from the supplement sponsor. She was also compensated for attending and organizing the Equol, Soy, and Menopause Research Leadership Conference and for organizing, writing, editing, or reviewing, and collection of supplemental manuscripts. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *The Journal of Nutrition*.

[2] Author disclosure: Y. Ishimi, no conflicts of interest.

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[3] Abbreviations used: BMD, bone mineral density; *Lc.*, *Lactococcus*; O-DMA, O-desmethylangolensin; OVX, ovariectomized.

First published online May 19, 2010; doi:10.3945/jn.110.124842.

population (15,16) and equol is found in ~30–50% (9,17–20). The variability in mammalian equol production is considered to be dependent on individual differences in the intestinal microbiota responsible for equol production (9,21). Intestinal bacteria play an important role in isoflavone metabolism; young infants with undeveloped gut microflora do not produce equol (22–24) and germ-free animals also do not produce equol or O-DMA (25–28). Because some reports suggested a lower disease risk for equol producers than for nonproducers (9), there is a growing interest in certain bacterial strains that can produce equol. A number of strains involved in daidzein metabolism have been identified (29–31). However, the identification of equol-producing bacteria is complicated (9,11,21,25,26).

Lactococcus (*Lc.*), 20–92 homologous to *Lc. Garvieae*, is the only lactic acid bacterium to date known to produce equol directly from daidzein without producing O-DMA (32). Interestingly, the strain, *Lc.* 20–92, can also cleave glycosidic bonds of daidzein. Here we review our previously published work on equol producer status and bone health in humans and a mouse model of osteoporosis.

Effects of equol on bone metabolism in animals

Estrogen deficiency is associated with increased bone turnover and acceleration of bone loss, which leads to an increased susceptibility to bone fracture. A number of studies have reported that the soybean isoflavones, genistein and daidzein, dosed dependently inhibit bone loss in both female and male osteoporotic animal models without causing notable effects on reproductive organs (33). Similarly, equol may inhibit bone loss due to ovariectomy. We have reported that administration of equol (0.5 mg/d subcutaneously) inhibited bone loss of the whole body and femur in ovariectomized mice without uterine hypertrophy (34). Although 17β-estradiol administration (0.03 μg/d subcutaneously) prevented ovariectomized-induced bone loss from all regions, uterine hypertrophy occurred in mice (34). These results suggest that similar to selective estrogen receptor modulators, isoflavones, including equol, inhibit bone loss without estrogenic activity in the reproductive organs of estrogen-deficient animals. It is now recognized that one of the mechanisms by which estrogen deficiency causes bone loss is via stimulation of osteoclast formation, a process enhanced by several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β. Furthermore, Nakamura et al. (35) recently reported apoptosis and *Fas* ligand expression upregulation in trabecular osteoclasts from wild type but not estrogen receptor knockout ($ER\alpha^{\Delta Oc/\Delta Oc}$) mice after estrogen treatment. These results support a model in which estrogen regulates the life span of mature osteoclasts via the induction of the *Fas/Fas* ligand system and help explain the osteoprotective function of estrogen and selective estrogen receptor modulators, including isoflavones.

The role of equol status in the effects of isoflavones on bone health in humans

Equol production depends on an individual's intestinal flora. Research has shown that ~30–50% of individuals in the population studied are capable of producing equol from daidzein (36). Lydeking-Olsen et al. (36) conducted a 2-y, randomized, placebo-controlled trial to investigate the effectiveness of isoflavone-supplemented soymilk (76 mg/d aglycone isoflavones content) for prevention of bone loss in postmenopausal women aged 41–75 y (mean age 58 y). The lumbar spine BMD for women who consumed soymilk with low levels of isoflavones (n = 22) decreased (4.2%; P = 0.01) by the end of the study; however, the lumbar spine BMD of women who consumed soymilk with isoflavones (n = 23) did not change compared with baseline. Equol producers (n = 10), defined by a cutoff level of 10 ng/mL (40 mmol/L) plasma equol, had a 2.4% increase in lumbar spine BMD compared with the 0.6% increase for equol nonproducers (n = 12) after the 2-y intervention with isoflavone-enriched soymilk.

We assessed the effects of equol-producing activity on BMD in postmenopausal Japanese women (37). Study participants were 68 healthy women aged 45–60 y who had undergone natural menopause within the previous 5 y, where menopause was defined as at least 12 mo beyond the last menstrual cycle. Fifty-four women (29/34 in the placebo, 25/34 in the isoflavone group) completed the 1-y intervention and their data were used for equol analysis. Study participants in the isoflavone group received a daily dose of 75 mg of isoflavone conjugates (38.3 mg daidzin, 0.2 mg malonyl-daidzin, 2.1 mg acetyldaidzin, 0.6 mg daidzein, 8.6 mg genistin, 0.6 mg acetylgenistin, 0.2 mg genistein, and 24.4 mg glycitin with glycitein) in capsule form (Fujiflavone P40, Fujicco; 47 mg aglycon form) with dextrin and those in the placebo group received capsules containing only

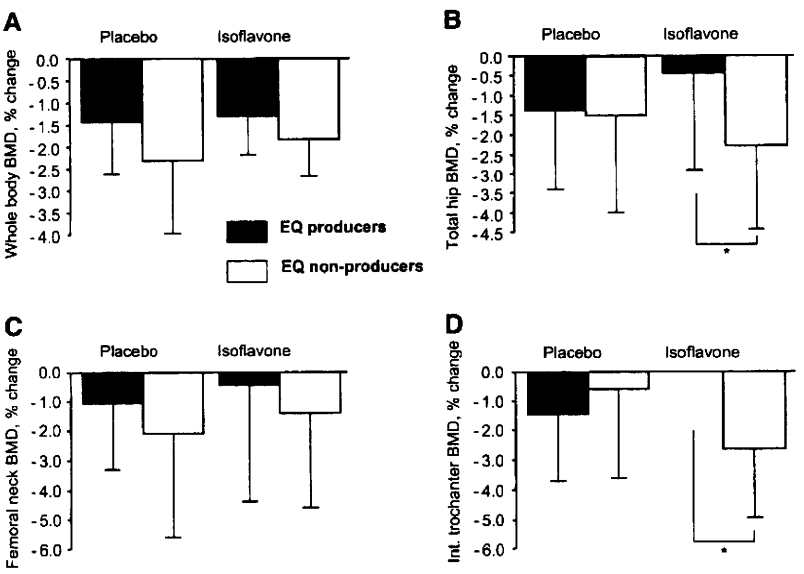

FIGURE 1 Percent changes in BMD of the whole body (*A*), hip (*B*), femoral neck (*C*), and the intertrochanter region (*D*) in postmenopausal Japanese women who were equol (EQ) producers or nonproducers after 1 y of isoflavone (75 mg/d) or placebo treatment. Values are means ± SD, n = 10–15. *Different from EQ producers, $P < 0.05$. Adapted from (37) with permission by Wolters Kluwer/Lippincott, Williams & Wilkins.

dextrin. Fifty-six percent of study participants were equol producers, as assessed by equol production in fecal suspension incubated with daidzein under anaerobic conditions. In the placebo group, there were 15 and 14 equol producers and nonproducers, respectively; and in the isoflavone group, there were 15 and 10 equol producers and nonproducers, respectively. Serum daidzein concentrations, as determined by reverse-phase HPLC, increased to 3-fold the baseline value in equol producers and nonproducers who consumed isoflavone for 1 y ($P = 0.002$). However, there was no difference in the serum daidzein concentration between equol producers and nonproducers. On the other hand, serum equol increased only in equol producers in the isoflavone group after 1 y ($P = 0.04$). Serum equol did not change in equol producers in the placebo group. Because equol is a metabolite of daidzein, it has a slower plasma clearance rate than daidzein (10). Therefore, whereas the serum levels of daidzein did not significantly differ between the equol producers and nonproducers, the equol levels were significantly higher in the equol producers. Equol producers tended to have lower serum concentrations than nonproducers ($P = 0.15$), but the longer half-life of equol in the bloodstream could explain why there were no significant differences in daidzein levels. After 1 y, serum genistein did not differ between equol producers and nonproducers in the isoflavone group.

In the isoflavone intervention group, the percent change in bone loss in the total hip (−0.46%) and the hip intertrochanteric region (−0.04%) of equol producers was lower ($P < 0.05$) than that of equol nonproducers (−2.28 and −2.61%, respectively) (Student's t test; **Fig. 1**) (38).

A 2-factor analysis of covariance revealed no significant main effect on the percent change in BMD in any region after 1 y. The percent change in bone loss in the total hip and the hip intertrochanteric region did not differ between equol producers and nonproducers in the placebo group. Equol producers had a significantly lower annualized change in fat mass than nonproducers in the isoflavone group, but this was not the case for the placebo group. Based on these results, the effects of isoflavone on bone and fat mass might depend on equol-producing activity in postmenopausal Japanese women (37). Isoflavone supplementation (47 mg/d aglycone equivalent) with a normal diet for 1 y did not affect serum levels of estrogen, follicle-stimulating hormone, luteinizing hormone, progesterone, triiodothyronine, thyroxine, and thyroid stimulating hormone in postmenopausal Japanese women (39).

Isolation of equol-producing bacteria from human feces

Uchiyama et al. (32) detected 3 equol-producing bacterial strains from human feces (32,39). They selected the *Lc.* 20–92 strain as the most appropriate bacteria for food usage, because it is homologous to *Lc. Garvieae*, which is widely used in Italian cheese. Using real time-PCR and the particular primer for *Lc. garvieae*, they found that *Lc.* 20–92 exists in the feces of postmenopausal Japanese women (32). *Lc. garvieae* was found in 35.3% of the postmenopausal Japanese women (39). Interestingly, women with *Lc. garvieae* were not always equol producers, suggesting that other bacteria may play a role in human equol production. Several other factors such as hydrogen gas and short chain fatty acids, which can affect the environmental conditions in the colon, may also be necessary for equol production.

Conclusions

Several factors such as race, age, diet, timing of exposure, and individual variations including genetic differences and variation in intestinal microflora influence the effects of isoflavones on bone health. The preventive effects of daidzein on bone loss in postmenopausal women might depend on an individual's equol-producing capacity. In research from our laboratory, supplementation of the normal diet for 1 y with an additional 47 mg/d of isoflavone aglycon equivalent did not have adverse effects in postmenopausal women. Further studies are required to address the numerous questions on the potential benefits, mechanisms of action, and safety of isoflavones for postmenopausal women.

Acknowledgment

The sole author had responsibility for all parts of the manuscript.

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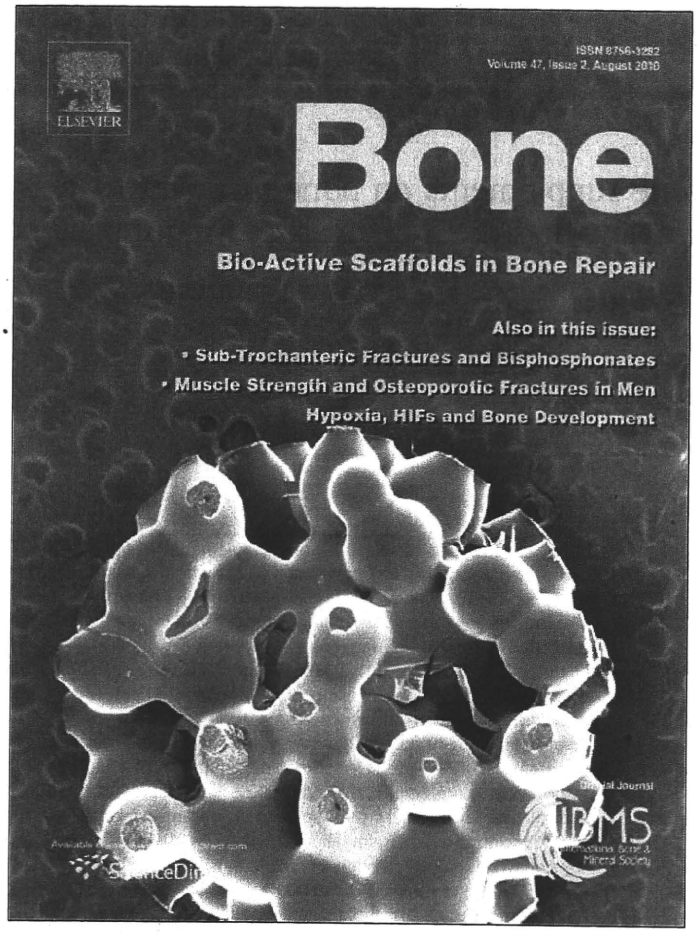

Bone 47 (2010) 413–423

Contents lists available at ScienceDirect

Bone

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Effects of soy isoflavone supplements on bone turnover markers in menopausal women: Systematic review and meta-analysis of randomized controlled trials

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ARTICLE INFO

Article history:
Received 29 October 2009
Revised 26 January 2010
Accepted 3 May 2010
Available online 7 May 2010

Edited by: Richard Eastell

Keywords:
Soy isoflavones
Bone turnover markers
Menopausal women
Systematic review
Meta-analysis

ABSTRACT

Introduction: Effects of soy isoflavone supplements on bone turnover markers remain unclear. This up-to-date systematic review and meta-analysis of randomized controlled trials (RCTs) was performed primarily to more completely and precisely clarify the effects on urinary deoxypyridinoline (DPD) and serum bone alkaline phosphatase (BAP) and secondarily to evaluate the effects on other bone turnover markers, compared with placebo in menopausal women.

Methods: PubMed, CENTRAL, ICHUSHI, and CNKI were searched in June 2009 for relevant studies of RCTs. Data on study design, participants, interventions, and outcomes were extracted and methodological quality of each included trial was assessed.

Results: From 3740 identified relevant articles, 10 (887 participants), 10 (1210 participants), and 8 (380 participants) RCTs were selected for meta-analysis of effects on DPD, BAP, and serum osteocalcin (OC), respectively, using Review Manager 5.0.22. Daily ingestion of an average 56 mg soy isoflavones (aglycone equivalents) for 10 weeks to 12 months significantly decreased DPD by 14.1% (95% CI: −26.8% to −1.5%; $P=0.03$) compared to baseline (heterogeneity: $P<0.00001$; $I^2=93\%$; random effects model). The overall effect of soy isoflavones on DPD compared with placebo was a significant decrease of −18.0% (95% CI: −28.4% to −7.7%, $P=0.0007$; heterogeneity: $P=0.0001$; $I^2=73\%$; random effects model). Subgroup analyses and meta-regressions revealed that isoflavone dose and intervention duration did not significantly relate to the variable effects on DPD. Daily supplementation of about 84 mg and 73 mg of soy isoflavones for up to 12 months insignificantly increased BAP by 8.0% (95% CI: −4.2% to 20.2%, $P=0.20$; heterogeneity: $P<0.00001$; $I^2=98\%$) and OC by 10.3% (95% CI: −3.1% to 23.7%, $P=0.13$; heterogeneity: $P=0.002$; $I^2=69\%$) compared with placebo (random effects model), respectively.

Conclusions: Soy isoflavone supplements moderately decreased the bone resorption marker DPD, but did not affect bone formation markers BAP and OC in menopausal women. The effects varied between studies, and further studies are needed to address factors relating to the observed effects of soy isoflavones on DPD and to verify effects on other bone turnover markers.

Introduction

Osteoporosis is a major health problem in postmenopausal women, who experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling [1,2]. The increased bone remodeling is associated with both decreased bone mineral density (BMD) and increased risk of fracture [3]. Together with BMD, bone turnover markers (BTMs) have been considered to be biomarkers for fracture risk [4]. BTMs can be used for the diagnosis and evaluation of therapy effects on osteoporosis [5], and include bone resorption markers (e.g. urine deoxypyridinoline (DPD), urine and serum type I collagen crosslinked N-telopeptide (NTX) and type I collagen crosslinked C-telopeptide (CTX)) and bone formation markers (e.g. serum bone alkaline phosphatase (BAP), osteocalcin (OC; or bone gamma-carboxyglutamate protein, BGP), and type I procollagen-N-propeptide (PINP)).

BTMs change earlier and to a larger extent than changes in BMD or risk of fracture. The decrease in BTMs in the early stages of treatment may reflect a reduction in the long-term risk of fracture [6,7]. Thus, a proper assessment of changes in BTMs may provide the earliest indication of whether to continue a particular treatment. Therapeutic effects of anti-osteoporosis drugs (e.g. bisphosphonates, raloxifene,

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doi:10.1016/j.bone.2010.05.001

and estrogen) can be assessed by DPD, NTX, CTX, BAP, or PINP; and the therapeutic effects are indicated only if the minimum significant change (MSC, %) in BTMs from baseline occurs during the course of treatment [5].

Although estrogen is known to change BTMs, it is associated with a higher risk of hormone-related cancer [8–10] and other unfavorable adverse events [11,12]. As a result, many women are searching for alternatives to estrogen for reduction of risk of osteoporosis. Phyto-estrogens may to some degree inhibit osteoporosis in postmenopausal women, owing to their oestrogenic activity [13]. Epidemiological studies indicate that soy food consumption may reduce the risk of fracture in postmenopausal women, particularly among those in the early years following menopause [14]. Consequently, many menopausal women use phyto-estrogens to maintain their bone mass because they are unlikely to cause the undesirable effects associated with steroid hormones [11,15]. The main dietary phyto-estrogens ingested are soy isoflavones, which have structures similar to that of estrogen [16]. A meta-analysis revealed that soy isoflavone intake for 3–24 months significantly increased spine BMD, compared with placebo in menopausal women [17]. The favorable effect became more significant when >90 mg/day of isoflavones were consumed, and ingestion for 6 months seemed to be enough to exert its beneficial effect on BMD. However, a subsequent meta-analysis revealed that a mean dose of 87 mg soy isoflavones for at least one year (12–24 months) did not significantly affect spine and hip BMD [18].

One meta-analysis of nine randomized controlled trials (RCTs) found that ingestion of soy isoflavones significantly decreased DPD by 2.08 nmol/mmol and increased BAP by 1.48 μg/L compared to controls in menopausal women [19]. We have identified several RCTs that addressed effects of soy isoflavones on bone turnover markers, but were not included in the above meta-analysis. Different RCTs reported data of bone turnover markers in different units and the values of each marker differed substantially across trials. In addition, the meta-analysis seems to combine the data of DPD and BAP without adjustment for different units reported.

The primary aims of this up-to-date systematic review and meta-analysis of RCTs were to more completely and more precisely clarify the effects of soy isoflavone supplements on urinary DPD and serum BAP compared with placebo, by combining their outcomes in terms of percentage changes from baseline in menopausal (including peri- and postmenopausal) women. The effects of soy isoflavones on other bone turnover markers were secondarily evaluated.

Materials and methods

Literature search

PubMed, CENTRAL, ICHUSHI, and CNKI were searched for published randomized controlled trials (RCTs) in June 2009, with the use of dozens of complex search strategies containing index terms. The best search strategy for each database that resulted in the most relevant RCTs was used in the final analysis (Appendix). The search strategies for PubMed and CENTRAL excluding PubMed were developed on the basis of Designing search strategies (section 6.4) described in the Cochrane Handbook for Systematic Reviews of Interventions (Version 5.0.1, updated September 2008), which accompanies with Review Manager 5.0.22 (Nordic Cochrane Center, Oxford, England). Reference lists of relevant RCTs and systematic reviews and meta-analyses were manually searched [19–21].

Inclusion and exclusion criteria

Studies were included for systematic review if they met all of the following criteria: *1)* included participants of menopausal (peri- or/and postmenopausal) women; *2)* evaluated intervention of soy supplements containing isoflavones and clearly described isoflavone dose; *3)* contained at least one relevant pair-wise comparison of intervention arms (i.e., soy isoflavone supplements vs. placebo, or both plus a non-estrogen add-on), and placebo used did not contain isoflavone or estrogen and was identical or similar in appearance and taste to comparative soy isoflavone supplements; *4)* reported outcomes for the effects on at least one of the bone turnover markers mentioned above (DPD, BAP, OC, NTX, CTX, and PINP); and *5)* was a parallel-group or crossover RCT. Duplicated reports or subgroup analysis of the primary study were excluded.

Meta-analysis based on means requires that data are at least approximately normally distributed, and the appropriate analysis of continuous data from crossover trials requires that neither carry-over nor period effects are thought to be a problem [22]. Thus, meta-analysis was performed to clarify effects of soy isoflavone supplements on bone turnover markers for which mean change from baseline and the SD/SE for normally distributed data were available in at least five RCTs. In addition, to be included in the meta-analysis, crossover RCT had to contain a washout period and did not have carry-over or period effects; alternatively, data for the first period were used for meta-analysis if there was no washout period. If there was more than one possible placebo, data for these placebo arms were combined to create one placebo arm. Similarly, if there was more than one dose of soy isoflavones, data for these arms were combined to create one isoflavone arm to compare to placebo. When two or more reports were published for the same trial, only the report with the largest sample size was included in this systematic review and meta-analysis.

Two researchers independently reviewed and evaluated the inclusion and exclusion of relevant RCTs for the systematic review and meta-analysis, and consensus was reached by discussion when there were disagreements.

Data extraction

Data on study design, number of participants, intervention, and outcomes for bone turnover markers were also independently extracted by two reviewers and were confirmed by each other. When necessary, data on outcomes for bone markers were obtained from graphs reported. If possible, we obtained necessary data not reported by contacting the authors. We calculated mean change (follow-up − baseline) and percentage change [(follow-up − baseline) ÷ baseline × 100%] from baseline in bone turnover markers, when the data were not directly reported. When the SDs of the mean changes were unavailable, we estimated them using the reported statistics (e.g., confidence intervals, standard errors, *t* values, *P* values, *F* values) comparing the mean changes; otherwise we conservatively imputed them by using the lowest correlation coefficient between the baseline and follow-up values calculated from included trials, in which SD (or SE) for change as well as for baseline and follow-up measurements were available [22]. SD for percentage change was then calculated by dividing SD for change with mean baseline value.

Quality assessment

We used the Jadad scale to assess the quality of included RCTs, a score of <3 indicating low quality [23]. We also used a 3-category grading system (A, B, and C) to denote the methodological quality of each study, detail as described in the evidence report [20]. Category A studies have the least bias and results are considered valid; category B studies are susceptible to some bias, but not sufficient to invalidate the results; and category C studies have significant bias that may invalidate the results (e.g., dropout rate >20%, missing baseline data, or irreconcilable apparent differences between data in figures, tables, and text). We defined category C as low quality. Concealment of treatment allocation in RCTs was assessed as

adequate, inadequate or unclear [24]. Two reviewers independently assessed the studies, and consensus was reached by discussion when there were disagreements.

Meta-analysis and statistical analysis

We performed a meta-analysis to determine the overall treatment effect of soy isoflavones on bone turnover markers, using the weighted mean difference method in Review Manager. The treatment effect of each trial was estimated as the mean difference between percentage changes from baseline in bone marker for each comparison intervention arm (i.e., the percentage change from the baseline for participants ingesting soy isoflavones minus that for placebo). Because the data required to perform a paired analysis in meta-analysis were unavailable in crossover trials, we incorporated crossover trials with parallel-group trials in the meta-analysis by using data from isoflavone periods and placebo periods as if the trial were a parallel-group trial of isoflavones and placebo [22]. When trials contained repeated measurements of bone markers, we used the data from the longest follow-up as long as the data from various time points had the same dropout rates; otherwise, the data set with the lowest dropout was used. The data sets for other time points were used for sensitivity analyses. For trials using multiple dosages of isoflavones compared to placebo, we combined the multiple isoflavones arms into a single arm for each of these trials; similarly, the data reported for each center in a multicenter trial were combined as if the data were from one center [22].

We used both a fixed effect model and 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 [22]. The I^2 statistic (0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity) was used for quantifying inconsistency across studies [22]. This describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance).

We conducted sensitivity analyses to evaluate the effects of degree of correlation between baseline and follow-up values (using the average of correlation coefficients calculated from included trials), time point of measurement (using data sets for other follow-up durations reported in trials with repeated measurements on participants), study quality (eliminating low-quality trials), and study design (excluding crossover trials). Since it is recommended that levels of bone resorption and formation markers are measured after 3 and 6 months of treatment, respectively [5], sensitivity analyses for bone resorption and formation markers were also performed by excluding trials with <3 months and <6 months intervention duration, respectively.

If at least 10 trials were available, subgroup analyses and meta-regressions were performed to investigate possible factors that might relate to varying effects of soy isoflavones on each bone marker across trials, on the basis of five pre-specified factors: region of participants, menopausal status, supplement type, isoflavone dose, and intervention duration [19]. We used a cutoff point of 75 mg/day in the subgroup analysis for isoflavone dosage, because a daily isoflavone intake of up to 75 mg (aglycone equivalent) is considered safe by the Japan Food Safety Commission. Tests for heterogeneity based on chi-squared statistics were performed to investigate differences between the two subgroups [22].

We examined potential publication bias by using funnel plots and by performing Egger's and Begg's tests to assess the asymmetry of funnel plots. Meta-regressions and tests for asymmetry of funnel plots were respectively performed with the use of user-written "metareg" and "metabias" commands for Stata 10.1 for Windows (StataCorp LP, College Station, Tex); "metainf" command was used to investigate the influence of each trial on the overall meta-analysis estimate.

Results

Search and selection

The flow information of the search and selection of RCTs were shown in Fig. 1. Among 3740 identified relevant articles, 37 articles met the 1st, 2nd, 4th, and 5th inclusion criteria for systematic review [11,25–60]. Continuously, 13 [26–28,31,33,35,40,43,45,46,51,54,59] articles were excluded because they did not meet the 3rd inclusion criteria for systematic review. Subsequently, one article [58] was excluded by applying the exclusion criteria for systematic review. Finally, 21 RCTs (reported in 23 articles) that cleared all the inclusion and exclusion criteria for systematic review were included [11,25,29,30,32,34,36–39,41,42,44,47–50,52,53,55–57,60]. Two RCTs were each reported in two [41,60] and another two articles [52,53], respectively.

One RCT [29] provided non-normally distributed DPD data, and one crossover RCT [47] without a washout period did not provide urine DPD data for the first period and data for all periods were

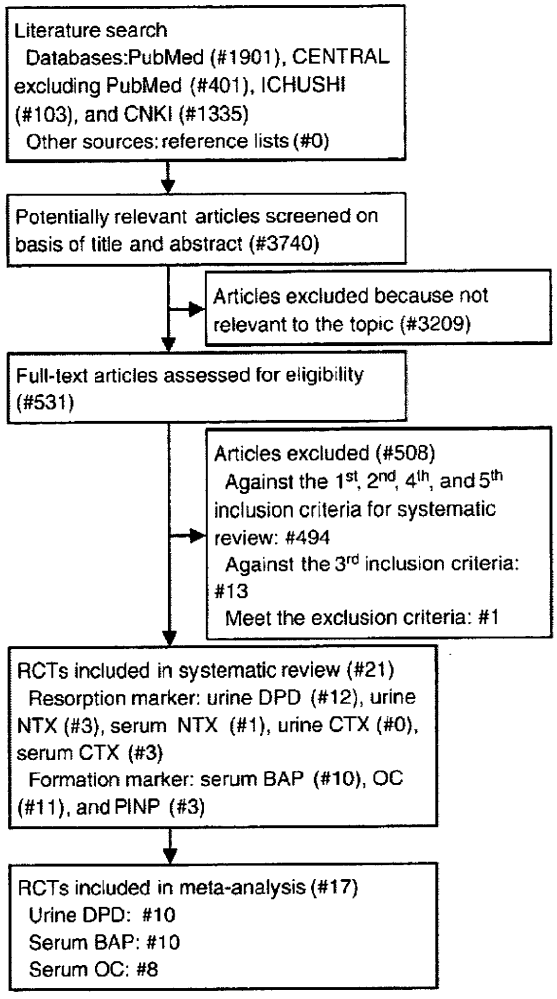

Fig. 1. Search and selection of randomized controlled trials (RCTs). #, number of records; BAP, bone alkaline phosphatase; CTX, type I collagen crosslinked C-telopeptide; DPD, deoxypyridinoline; NTX, type I collagen crosslinked N-telopeptide; OC, osteocalcin; PINP, type I procollagen-N-propeptide.

reported in median and percentile values. One RCT [42] contained serum OC data including pre- and postmenopausal women, one RCT [49] did not report SD/SE of the mean data for serum OC, and one crossover RCT [47] without a washout period did not provide serum OC data for the first period and data for all periods were reported in median and percentile values. After excluding these inappropriate data, ten [11,30,32,41,42,44,48,52,56,57], ten [11,29,30,34,36,38,41,44,52,57], and eight [11,25,34,37,48,52,55,57] trials were included in meta-analysis to clarify effects on DPD, BAP, and OC, respectively. Only three, one,

Table 1
Characteristics of 17 randomized placebo-controlled trials included in the meta-analysis.

Study	Design[a]	Duration	Participants[b]	Intervention[c]	Outcomes[d]
Albertazzi 2005 [25]	CO; R+, DB+, WD; JS = 5; A	6 wk×2	100/99 (1%) Post; mean age: 54 y; Italy	Arm1: placebo capsules without Ge Arm2: capsules containing 90 mg 98.9% pure Ge/d	OC: 32.04 (unit not described)
Brink 2008 [29]	P; R, DB+, WD; JS = 4	53 wk	300/237 (21%) Post; mean age: 53 y; Netherlands, Italy, France	Arm1: placebo biscuits and bars Arm2: biscuits and bars added with 110 mg SI (25–35% De, 60–75% Ge, 1–5% Gle)/d	DPD: 12.75/creatinine (non-normally distributed) BAP: 26.66
Brooks 2004 [30]	P; R, DB+, WD; JS = 4; A	16 wk	46/44 (4%) Post; mean age: 53 y; Canada	Arm1: placebo muffin of whole-wheat flour Arm2: muffin of 25 g soy flour containing 41.9 mg SI (37% De, 61% Ge, 2% Gle)/d Arm3: flaxseed muffin	DPD: 9.27 BAP: 15.41
Dalais 2003 [32]	P; R+, DB+, WD; JS = 5	3 mo	106/77 (27%) Post; mean age: 60 y; Australia	Arm1: placebo casein protein Arm2: 40 g soy protein containing 69 [118] mg SI (32% De, 64% Ge, 4% Gle)/d	DPD: 14.86
Harkness 2004 [34]	CO; R+, DB, WD; JS = 4	6 mo×2	20/19 (5%) Post; mean age: 71 y; USA	Arm1: placebo capsules Arm2: capsules containing 110 mg SI (40% De, 52% Ge, 9% Gle)/d	BAP: 26.26 OC: 12.60
Kenny 2009 [36]	P; R, DB+, WD; JS = 4	3, 12 mo	131/97 (26%) Post; mean age: 73 y; USA	Arm1: control protein (50% casein, 25% whey, and 25% egg) + placebo tablets Arm2: control protein + tablets containing 105 mg SI (40% De, 52% Ge, 9% Gle)/d Arm3: 18 g alcohol-washed soy protein/d + placebo tablets Arm4: alcohol-washed soy protein + SI tablets	BAP: 24.17
Knight 2001 [37]	P; R+, DB+, WD; JS = 5; A	12 wk	24/24 (17%) Post; mean age: 54 y; Australia	Arm1: isoflavone-free casein-based placebo powder Arm2: 60 g TakeCare™ powder containing 77.4 [134.4] mg SI (33% De, 64% Ge, 4% Gle)/d	OC: 5.97
Kreijkamp-Kaspers 2004 [38]	P; R+, DB+, WD; JS = 5; A	12 mo	202/175 (24%) Post; mean age: 67 y; Netherlands	Arm1: placebo total milk protein Arm2: 25.6 g soy protein containing 99 mg SI (41% De, 53% Ge, 6% Gle)/d	BAP: 12.8 μg/L
Marini 2007 [41]	P; R+, DB+, WD; JS = 5	1, 2 y	389/389 (10, 22%) Post; mean age: 55 y; Italy	Arm1: placebo tablets Arm2: tablets containing 54 mg 98% pure Ge/d	DPD: 21.41 BAP: 10.35 μg/L
Morabito 2002 [11]	P; R, DB+; JS = 3	6, 12 mo	90/90 Post; mean age: 52 y; Italy	Arm1: placebo tablets Arm2: tablets containing 54 mg ~98% pure Ge/d Arm3: HRT	DPD: 25 BAP: 9.57 μg/L OC: 12.67
Mori 2004 [42]	P; R, DB, WD; JS = 3	4 wk	102/67 (34%) Pre and Post; age: 40–63 y; Japan	Arm1: placebo tablets (vehicle only) Arm2: tablets containing 14.4 [40] mg SI (58% De, 13% Ge, 29% Gle)/d Arm3: vitamin tablets (C and E)	DPD: 42.88 mmol/day
Nikander 2004 [44]	CO; R+, DB+, WD; JS = 5	3 mo×2 (washout = 2 mo)	66/55 (11%) Post; mean age: 55 y; Finland	Arm1: placebo tablets Arm2: tablets containing 114 mg SI (36% De, 6% Ge, 58% Gle)/d	DPD: 19.4 BAP: 14.3 μg/L
Uesugi 2002 [48]	P; R, DB, WD; JS = 3	4 wk	23/23 (0%) Peri; mean age: 52 y; Japan	Arm1: placebo capsules Arm2: capsules containing 38.4 [61.8] mg SI (52% De, 11% Ge, 37% Gle)/d	DPD: 10.9 OC: 7.9 μg/mL
Wu 2006a [52], b [53]	P; R, DB+, WD; JS = 4	6, 12 mo	136/128, 108 (6, 21%) Post; mean age: 55 y; Japan	Arm1: placebo capsules Arm2: capsules containing 47 [75] mg SI (54% De, 13% Ge, 34% Gle)/d Arm3: placebo capsules + walking Arm4: SI capsules + walking	DPD: 7.40 BAP: 29.11 OC: 9.93
Xu 2007 [55]	P; R, DB; JS = 2	3 mo	96/37 (61%) Post; mean age: 45–65 y; China	Arm1: placebo capsules (corn flour) Arm2: calcium capsules Arm3: capsules containing isoflavone of pueraria root Arm4: capsules containing 66 mg 97% pure SI/d Arm5: calcium capsules + SI capsules	OC: 4.29
Yamori 2002 [56]	P; R, DB; JS = 2	3, 10 wk	40/unknown Post; mean age: 53 y; Brazil	Arm1: placebo composed only of sesame Arm2: 6 g soybean germ and sesame containing 22.7 [37.3] mg SI (54% De, 15% Ge, 30% Gle)/d	DPD: 6.4
Ye 2006 [57]	P; R+, SB, WD; JS = 3	6 mo	90/78 (13%) Post; mean age: 52 y; China	Arm1: placebo capsules (starch) Arm2: capsules containing 84 mg SI (52% De, 15% Ge, 33% Gle)/d Arm3: capsules containing 126 mg SI/d	DPD: 7.39 BAP: 32.23 OC: 5.47

[a] CO, crossover; R+, randomized by appropriate method (gives 2 points to Jadad scale); DB+, double-blinded by appropriate method (2 points); WD, withdrawals and dropouts described (1 point); JS, Jadad scale (1–5 points); A, adequate concealment of treatment allocation; P, Parallel; R, randomized (1 point); DB, double-blinded (1 point); SB, single-blinded.

[b] Randomize/analyzed number (dropout rate) of participants; Post, Pre, and Peri, post-, pre-, and perimenopausal women, respectively.

[c] Ge, genistein; SI, soy isoflavones (aglycone equivalents, bracketed are values including glycoside forms); De, daidzein; Gle, glycitein; HRT, hormone-replacement therapy.

[d] Baseline mean values of bone turnover markers. OC, serum osteocalcin (ng/mL, unless specified); DPD, urine deoxypyridinoline (nmol/mmol creatinine, unless specified); BAP, serum bone alkaline phosphatase (U/L, unless specified).

zero, three, and three RCTs cleared the inclusion and exclusion criteria for systematic review and reported effects on urine NTX [36,44,49], serum NTX [50], urine CTX, serum CTX [25,39,60], and serum PINP [29,39,44], respectively. Thus, meta-analyses for these bone turnover markers were not performed due to the small number of available relevant studies.

Characteristics of the studies

The characteristics of 17 trials included in the meta-analysis are summarized in Table 1. Four trials were assessed as having "adequate" concealment of treatment allocation, and the remaining trials were assessed as "unclear" due to insufficient information. One trial did not clearly report the number of post-treatment participants analyzed [56], and in that case we considered them to be the same as at baseline. Only one trial did not clearly describe the form of isoflavones used [55]; we assumed them to be aglycone equivalents for calculating average dosage of isoflavones for all included trials. For the two crossover design trials [25,34], only data from the first treatment period were used for the meta-analysis. For another crossover trial that utilized a washout period, neither carry-over nor period effects were detected and data from all treatment periods were used as if the trial was of parallel-group design [44]. In trials with repeated measurements, data for follow-up durations of 12 months [11,36,41], 10 weeks [56], and 6 months [52] were used. For one study that used two doses of isoflavones [57], data for both doses were combined to create one isoflavone arm to compare to placebo. Data for the flaxseed intervention in a 3-arm trial were excluded [30]; only data for arm1 and arm2 reported in two 2×2 factorial design trials were used, due to possible interaction between the effects of the two factors [36,52,53]; and only comparative data for arm1 and arm4 reported in a 5-arm trial were used [55].

Participants in the comparison groups had similar physical activity patterns and habitual dietary intakes of soy isoflavones, calcium, and vitamin D. Most of the studies were designed to maintain the participants' usual diets, lifestyles and body weights. Adverse events were generally similar for both the isoflavone and placebo groups and no serious adverse events were noted in the included trials, although they were not well addressed in several trials.

Overall effect of soy isoflavone supplements on urine DPD

Four correlation coefficients (0.75–0.79) between baseline and follow-up values for urine DPD calculated from two RCTs [32,34], were similar and averaged 0.77. Meta-analysis of the 10 trials with 887 participants using the fixed effect model resulted in significant heterogeneity ($P=0.0001$; $I^2=73\%$), no trial seemed to clearly influence the overall combined effect (Fig. 2). Meta-analysis using the random effects model revealed that daily ingestion of an average of 56 (14–114) mg (aglycone equivalent) soy isoflavones for 10 weeks to 12 months significantly decreased DPD by −18.0% (95% CI: −28.4% to −7.6%, $P=0.0007$) compared with placebo (Fig. 3). Of the 10 included trials, 9 trials resulted in negative mean difference in percentage change from baseline between isoflavone and placebo arms. Soy isoflavones decreased DPD from baseline in all 10 included trials; whereas, placebo decreased DPD only in 5 of 10 trials. The combined percentage change from baseline for isoflavones and placebo were −14.1% (95% CI: −26.8% to −1.5%, $P=0.03$; heterogeneity $P<0.00001$; $I^2=93\%$; random effects model) and 2.0% (95% CI: −2.6% to 6.7%, $P=0.39$; heterogeneity $P=0.07$; $I^2=44\%$; random effects model) using generic variance method in Review Manager, respectively.

The following sensitivity analyses were performed: *1)* assuming the level of correlation coefficient between baseline and follow-up values to be the average (0.77); *2)* using data sets of other time points for trials with repeated measurements on participants [11,41,52,53,56,57]; *3)* excluding one trial of <3 months (10 weeks) intervention duration [56]; *4)* eliminating low-quality trials [32,42,56]; and *5)* excluding one trial of crossover design [44]. None resulted in significantly different overall effects of isoflavones on DPD.

Results of subgroup analyses of the effects of isoflavones on DPD based on the five pre-specified factors (region of participants, menopausal status, supplement type, isoflavone dose, and intervention duration) are shown in Table 2. Using the fixed effects model, none of the five factors resulted in significant subgroup differences. Subgroup analyses of trials with postmenopausal women and trials that used soy isoflavone extracts resulted in significant overall effect of isoflavones on DPD using either the fixed effect model or the random effects model, whereas trials with perimenopausal women and trials using soy foods containing isoflavones did not result in significant effects. Meta-regressions analyzing each of or all of the five

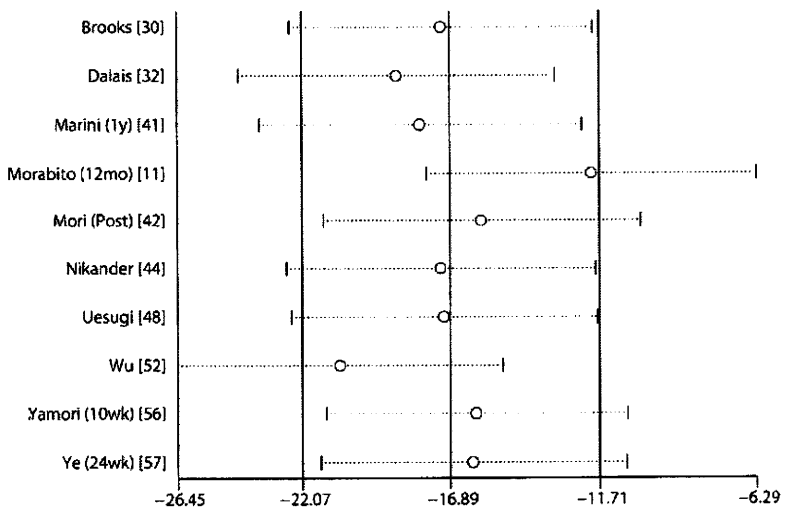

Fig. 2. Influence of each study on the overall effect on urine DPD (%). The vertical center line and the two lines on both sides of it respectively denote the combined overall effect and 95% CI for all included studies (fixed effect model). The open circles (O) and horizontal dotted lines respectively denote the combined overall effect and 95% CI when each study is omitted, thereby demonstrating the influence of this study on the overall effect.

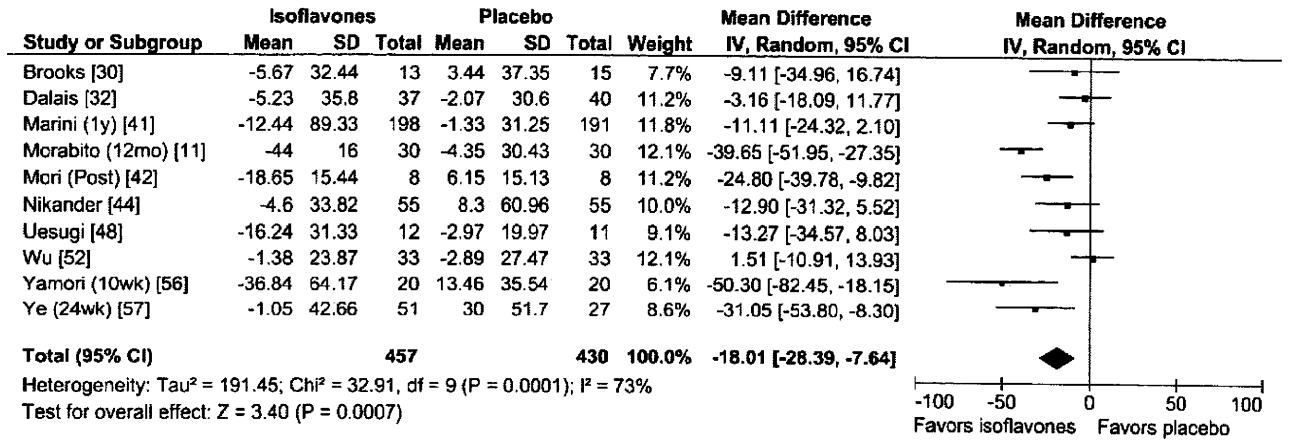

Fig. 3. Effects of soy isoflavones on urine DPD (%). Mean difference, mean percentage changes (%) of urine deoxypyridinoline (DPD) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight); horizontal line denote the 95% CI; ♦, combined overall effect.

pre-specified factors did not reveal these factors to be significantly associated with the varying effects of isoflavones on DPD across included trials.

The funnel plots (Fig. 4) and Egger's ($P=0.58$) and Begg's ($P=0.245$) tests did not indicate obvious publication bias.

Overall effect of soy isoflavone supplements on serum BAP

Eight correlation coefficients (0.70–0.97) for serum BAP calculated from four RCTs [34,38,41,44], were similar and averaged 0.84. Meta-analysis of the 10 trials with 1210 participants using the fixed effects model resulted in significant heterogeneity ($P<0.00001$; $I^2=98\%$), and revealed that daily ingestion of an average of 84 (42–114) mg (aglycone equivalent) soy isoflavones for 3 to 12 months significantly increased serum BAP by 12.0% (95% CI: 10.5% to 13.6%, $P<0.00001$). However, meta-analysis using the random effects model resulted in a non-significant increase of 8.0% (95% CI, −4.2% to 20.2%; $P=0.20$) in BAP (Fig. 5).

Sensitivity analyses did not result in significantly different overall effects of soy isoflavones on BAP, regardless of whether they assumed the correlation coefficient between baseline and follow-up values to be the average (0.84), used data sets of other time points for trials with repeated measurements on participants [11,36,41,52,53,57], excluded trials with <6 months intervention duration [30,44], eliminated low-quality trials [29,36,38] or excluding one trial with a crossover design [44].

Subgroup analysis of the effects of isoflavones on serum BAP based on one (menopausal status) of the five pre-specified factors was not possible. Subgroup analysis based on the factor of isoflavone dose revealed that isoflavones significantly increased serum BAP by 20.3% (95% CI: 4.6% to 36.1%, $P=0.01$; heterogeneity $P<0.00001$; $I^2=92\%$; random effects model) in RCTs used ≤75 mg/d dose [11,30,41,52], but no significant effect of isoflavones was detected in the RCTs used >75 mg/d dose. Subgroup analyses based on the remaining three pre-specified factors (region of participants, supplement type, and intervention duration) and one *post hoc* factor (participants' age: ≥70 years vs. <70 years) did not reveal subgroups with significant effect on serum BAP. Meta-regressions analyzing the pre-specified factor of isoflavone dose revealed that the isoflavone dose used was significantly related to the effects on serum BAP across included trials, either on a two-subcategory basis (≤75vs. >75 mg/d; $P=0.013$) or on a continuous dose basis ($P=0.041$). Meta-regressions analyzing each of the remaining three pre-specified factors and one *post hoc* factor did not reveal these factors to be significantly associated with the varying effects of isoflavones on serum BAP across included trials.

Table 2
Subgroup analyses of the effects of soy isoflavones on urine deoxypyridinoline (DPD).

Variables	Trials	Sample size	*P* value for heterogeneity	Fixed effect model		Random effects model	
				WMD (95% CI), %	*P* value	WMD (95% CI), %	*P* value
Total	10	887	0.0001	−16.89 (−22.06, −11.71)	<0.00001	−18.01 (−28.39, −7.64)	0.0007
Participants' region					[0.51]		
Asian [42,48,52,56,57]	5	223	0.005	−14.87 (−22.77, −6.98)	0.0002	−20.71 (−37.25, −4.18)	0.01
Western [11,30,32,41,44]	5	664	0.002	−18.41 (−25.27, −11.55)	<0.00001	−15.95 (−30.89, −1.02)	0.04
Menopause status					[0.73]		
Perimenopausal [48]	1	23	Not applicable	−13.27 (−34.57, 8.03)	0.22	−13.27 (−34.57, 8.03)	0.22
Postmenopausal [11,30,32,41,42,44,52,56,57]	9	864	<0.0001	−17.11 (−22.45, −11.78)	<0.00001	−18.57 (−29.91, −7.23)	0.001
Supplement type					[0.29]		
Isoflavone extracts [11,41,42,44,48,52,57]	7	742	0.0003	−18.23 (−23.97, −12.49)	<0.00001	−18.53 (−30.61, −6.45)	0.003
Soy foods with isoflavones [30,32,56]	3	145	0.03	−11.01 (−23.00, 0.99)	0.07	−17.80 (−43.12, 7.53)	0.17
Isoflavone dose					[0.64]		
≤75 mg/d [11,30,32,41,42,48,52,56]	8	699	<0.0001	−16.41 (−21.96, −10.85)	<0.00001	−17.38 (−29.72, −5.05)	0.006
>75 mg/d [44,57]	2	188	0.22	−20.09 (−34.41, −5.77)	0.006	−20.70 (−38.31, −3.09)	0.02
Intervention duration					[0.13]		
<3 months [42,48,56]	3	79	0.17	−24.70 (−36.15, −13.25)	<0.0001	−25.93 (−42.48, −9.38)	0.002
≥3 months [11,30,32,41,44,52,57]	7	808	0.0001	−14.88 (−20.68, −9.07)	<0.00001	−14.91 (−27.74, −2.08)	0.02

WMD, weighted mean difference in percentage change (%) from baseline between soy isoflavones and placebo (i.e., mean value for soy isoflavones minus that for placebo); *P* value, test for overall effect of each subgroup; bracketed *P* values, test for subgroup differences.

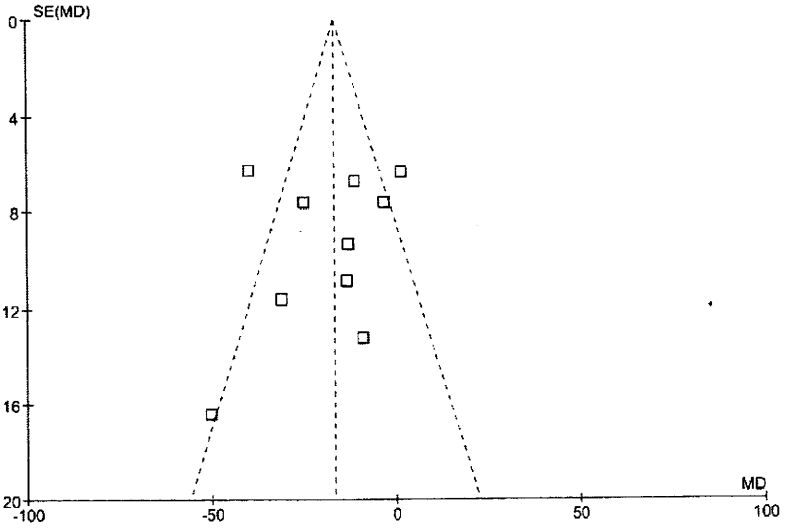

Fig. 4. Funnel plots of effects of soy isoflavones on urine DPD (%). MD, mean percentage changes (%) of urine deoxypyridinoline (DPD) from baseline for soy isoflavones minus that for placebo; SE(MD), standard error of MD. The vertical center broken line and two broken lines on both sides of it respectively denote the combined overall effect and 95% CI (fixed effect model).

The funnel plots and Egger's ($P=0.60$) and Begg's ($P=0.18$) tests did not indicate obvious publication bias.

Overall effect of soy isoflavone supplements on serum OC

Three correlation coefficients (0.34–0.89) for serum OC were calculated from two RCTs [25,34] and averaged 0.68. Meta-analysis of the 8 trials with 380 participants using the fixed effect model resulted in significant heterogeneity ($P=0.002$; $I^2=69\%$), and revealed that daily ingestion of an average of 73 (38–110) mg (aglycone equivalent) soy isoflavones for 6 weeks to 12 months non-significantly increased serum OC by 4.6% (95% CI: −0.98% to 10.2%, $P=0.11$). Meta-analysis using the random effects model also resulted in a non-significant increase of 10.3% (95% CI, −3.1% to 23.7%; $P=0.13$) in OC (Fig. 6). Sensitivity analyses did not result in significantly different overall effects of soy isoflavones on OC, whether they assumed the level of correlation coefficient between baseline and follow-up values to be the average (0.68), used data sets of other time points for trials with repeated measurements on participants [11,52,53,57], excluded trials with <6 months intervention duration [25,37,48,55], eliminated low-quality trials [55], or excluded one trial of crossover design [44]. The funnel plots and Egger's ($P=0.36$) and Begg's ($P=0.46$) tests did not indicate obvious publication bias.

Effect of soy isoflavone supplements on NTX, CTX, and PINP

No significant effects of soy isoflavone supplements on urine NTX [36,44,49] and serum NTX [50] were revealed in three and one RCTs, respectively. Two RCTs did not reveal significant effects of soy isoflavone supplements on serum CTX [25,39]. However, one RCT revealed that serum CTX decreased significantly over the 3-year period compared with baseline ($P<0.001$) and second year ($P<0.01$) in the group ingesting 54 mg/day of pure genistein, and there was no significant decrease of serum CTX at 2 and 3 years for the placebo group [60]; between-group analyses showed that genistein aglycone significantly decreased serum CTX at year 2 and 3 compared with placebo ($P<0.001$). Moreover, a treatment × time interaction was also highly significant ($P<0.0001$). No significant effects of soy isoflavone supplements on serum PINP were detected in three RCTs [29,39,44].

	Isoflavones			Placebo				Mean Difference	Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**	**IV, Random, 95% CI**
Brink [29]	7.01	25.91	118	2.81	21.83	119	10.8%	4.20 [-1.90, 10.30]	
Brooks [30]	-4.59	44.98	13	4.87	61.65	15	5.1%	-9.46 [-49.10, 30.18]	
Harkness [34]	-3.43	20.47	10	-9.14	18.28	9	9.1%	5.71 [-11.71, 23.13]	
Kenny (12mo) [36]	-1.93	19.12	26	-6.89	14.92	22	10.4%	4.96 [-4.68, 14.60]	
Kreijkamp-kaspers [38]	-4.72	8.71	88	-3.88	8.71	87	11.1%	-0.84 [-3.42, 1.74]	
Marini (1y) [41]	32.69	13.81	198	-0.97	13.69	191	11.1%	33.66 [30.93, 36.39]	
Morabito (12mo) [11]	25	7	30	-4	28.64	30	10.3%	29.00 [18.45, 39.55]	
Nikander [44]	1.4	17.12	55	5.4	15.65	55	10.8%	-4.00 [-10.13, 2.13]	
Wu [52]	8.09	19.24	33	-0.31	15.4	33	10.6%	8.40 [-0.01, 16.81]	
Ye (24wk) [57]	5.87	13.13	51	6.4	13.9	27	10.8%	-0.53 [-6.89, 5.83]	
Total (95% CI)			**622**			**588**	**100.0%**	**8.01 [-4.15, 20.18]**	

Heterogeneity: Tau² = 345.98; Chi² = 398.05, df = 9 (P < 0.00001); I² = 98%
Test for overall effect: Z = 1.29 (P = 0.20)

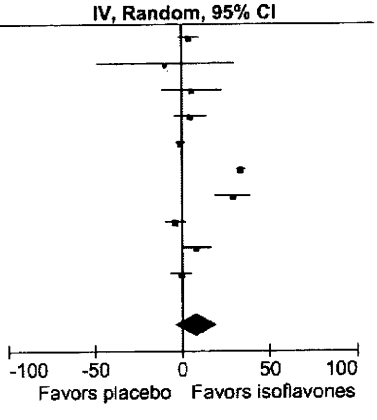

Fig. 5. Effects of soy isoflavones on serum BAP (%). Mean Difference, mean percentage changes (%) of serum bone alkaline phosphatase (BAP) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight); horizontal line denote the 95% CI; ◆, combined overall effect.

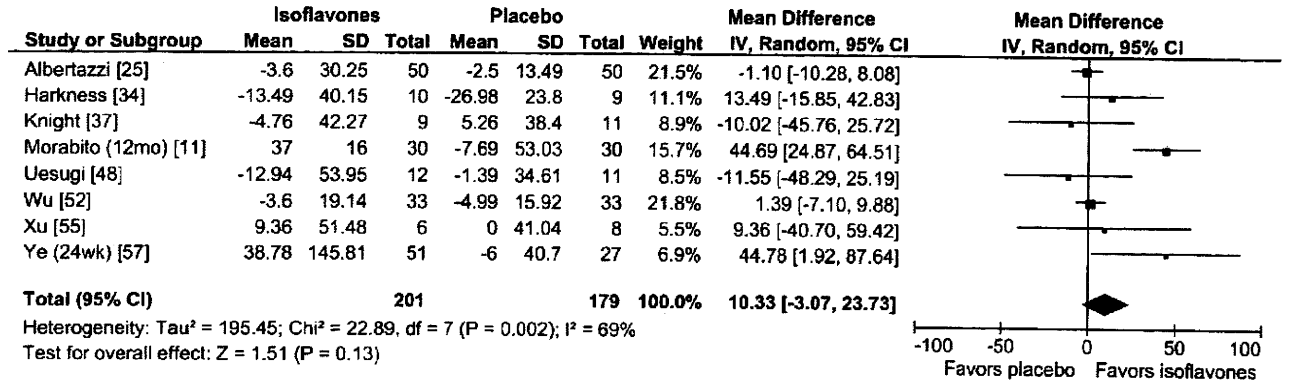

Fig. 6. Effects of soy isoflavones on serum OC (%). Mean Difference, mean percentage changes (%) of serum osteocalcin (OC) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight); horizontal line denote the 95% CI; ◆, combined overall effect.

Discussion

Overall effect of soy isoflavone supplements on urine DPD

The present meta-analysis found that daily ingestion of about 56 mg soy isoflavones (aglycone equivalent) for 10 weeks to 12 months significantly decreased the bone resorption marker urinary DPD by 14.1% compared to baseline in menopausal women. The overall effect of isoflavones on DPD was a significant decrease of 18.0% compared with placebo. Sensitivity analyses indicated that the effect of soy isoflavones on DPD was robust. Although the magnitude of effect of soy isoflavones on DPD was weaker than that of anti-osteoporosis drugs for which a MSC of 29.6% is necessary for claiming a therapeutic effect and the effects varied across trials, soy isoflavone supplements did significantly decrease urine DPD in a moderate manner. A daily intake of 56 mg soy isoflavones is approximately equivalent to 1.2 times the amount consumed habitually in Japan (mean: 47.2 mg/day) [61]. Postmenopausal women experience a sharp decrease in estrogen concentration that leads to an increased rate of bone remodeling [1,2], and the increased bone remodeling is associated with both decreased BMD and increased risk of fracture [3]. Furthermore, the decrease in bone turnover markers in the early stages of treatment may reflect a reduction in the long-term risk of fracture [6,7]. Thus, our meta-analysis indicated that soy isoflavone supplements might be able to increase BMD and decrease risk of fracture in menopausal women.

The directions of the overall effect of soy isoflavone supplements on urine DPD and results of subgroup analyses on the basis of menopausal status (perimenopausal vs. postmenopausal) and type of soy isoflavone supplements (soy isoflavone extracts vs. soy foods with isoflavones) were consistent with those demonstrated in a previous meta-analysis [19]. These indicated that soy isoflavone supplements, especially when ingested in extract form and when ingested by postmenopausal women, exert significant effects in decreasing the bone resorption marker DPD. Our meta-analysis has more completely and more precisely clarified the effect of soy isoflavones on DPD by including additional three RCTs [41,52,57] and by limiting the comparison to placebo and combining outcomes of percentage change from baseline. By using the overall effect of soy isoflavones on DPD in terms of percentage change from baseline, the magnitude of the effect can be compared between different institutions using different units and can be compared with other anti-resorption agents. The interpretation of the differences in effects on DPD between subgroups on the basis of the two pre-specified factors was limited by the small numbers of RCTs in each subgroup. In addition, our subgroup analyses failed to detect influences of region of participants, isoflavone dosage, and intervention duration that were addressed in the previous meta-analysis [19]. Further research is needed to clarify the particular subgroups, doses and other factors that may increase or decrease the magnitude of the effects of soy isoflavone supplements on urine DPD.

The mechanism by which soy isoflavones decrease bone resorption is not well understood, but it may result from their chemical and biological similarity to mammalian estrogens, which are used as antiresorptive drugs [5]. Estrogen deficiency induces a rapid and sustained increase in skeletal remodeling that is reflected by a 50–100% mean increase in formation and resorption markers [62]. Hormone (estrogen) replacement therapy induces a rapid decrease in bone resorption markers that can be seen as early as 2 weeks with a plateau reached within 3–6 months.

Overall effect of soy isoflavone supplements on serum BAP and OC

Our meta-analysis showed that ingestion of soy isoflavone supplements up to one year did not significantly affect the bone formation markers serum BAP and OC. Sensitivity analyses did not reveal significant overall effects on these two formation markers. The overall effect on serum BAP was not consistent with that reported in the previous meta-analysis [19], which found that soy isoflavones significantly increased serum BAP. The explanation for this difference might be due to that the previous meta-analysis included only five RCTs [11,27,28,30,44], among which two trials were not placebo-controlled [27,28]; in contrast, our meta-analysis included ten randomized placebo-controlled trials. Subgroup analyses and meta-regressions revealed that soy isoflavone dose was related to the various effects on serum BAP across included trials. Soy isoflavones significantly increased serum BAP in subgroup RCTs that used $\leq$75 mg/d dose [11,30,41,52], but not in the subgroup RCTs that used >75 mg/d dose. The significant effect on serum BAP detected in the subgroup including four RCTs was predominantly influenced by two of the included trials [11,41] that used 54 mg/d pure genistein (Fig. 5). This dose of pure genistein seemed to possess stronger effects in increasing the bone formation marker serum BAP than other soy isoflavones with different chemical forms. One interpretation might be that genistein exhibits a stronger binding affinity for estrogen receptors than other soy isoflavones. Because of the low number of RCTs that evaluated the effects of pure genistein on serum BAP and other bone turnover markers, the effects of pure genistein need further study.

The decrease in bone formation markers by estrogen is delayed, reflecting the physiologic coupling of formation to resorption and a plateau is usually achieved within 6–12 months [63]. Because changes in the levels of bone formation markers will appear later than the changes in bone resorption markers, it is recommended that levels be measured at the initiation of treatment and 6 months thereafter [5].

This might explain why our meta-analysis did not reveal significant effects of soy isoflavones on BAP and OC, because two of ten trials and four of the eight trials respectively included in meta-analysis for BAP and OC had intervention durations less than 6 months. The limited number of trials of ≥6 months duration might also have led to insufficient statistical power to detect significant effects of soy isoflavones on BAP and OC.

Effect of soy isoflavone supplements on NTX, CTX, and PINP

Very few RCTs contained data for NTX, CTX, and PINP, and generally failed to detect significant effects of soy isoflavone supplements on these bone turnover markers. Although a follow-up study revealed that ingesting 54 mg/day of pure genistein over 2 and 3 years significantly decreased serum CTX compared with placebo [60], only 138 (an approximate 65% dropout rate) of the original 389 participants were included in the analysis. The effect of pure genistein on serum CTX needs further study. Our meta-analysis was unable to statistically summarize the effects of soy isoflavone supplements on NTX, CTX, and PINP, because of the small numbers of available relevant RCTs. Further study is needed to clarify the effects on these bone turnover markers.

Limitations of this meta-analysis

One limitation of this meta-analysis was the existence of the significant heterogeneity in the effects of soy isoflavone supplement on urine DPD (Fig. 3), serum BAP (Fig. 5) and OC (Fig. 6). Although we presented the results based on the random effects model [22,64], the effects of soy isoflavones on these bone turnover markers were highly variable between studies. Results of subgroup analyses on the basis of the two pre-specified factors (type of soy isoflavone supplements and participants' menopausal status) indicated that these two factors might account for the various effects on urine DPD across trials. However, subgroup analyses of the remaining pre-specified factors (participants' region, isoflavone dose, and intervention duration) and meta-regressions of the five pre-specified factors did not reveal that these factors were associated with the various effects on urine DPD across trials. Subgroup analyses and meta-regressions analyzing the pre-specified factor of isoflavone dose revealed that isoflavone dose was significantly related to the various effects on serum BAP between trials; whereas, subgroup analyses and meta-regressions analyzing each the remaining pre-specified factors and one *post hoc* factor did not reveal these factors to be associated with the various effects across trials.

The heterogeneity of results across trials, which cannot currently be well explained, might also be induced by differences in habitual dietary intake of soy isoflavones [53], interval since menopause [65], chemical forms and proportions of individual soy isoflavones [66–68], 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 to the peripheral circulation [68]. Asian and Western populations are reported to have differences in the capacity of intestinal flora to convert daidzein to its metabolite, equol [69]. Equol is easily absorbed and possesses substantial estrogenic activity because of its affinity for both the estrogen α and β receptors [67]. Equol has been suggested to be the single most important factor that influences the clinical efficacy of soy isoflavones in preventing bone loss [70]. However, the role of equol production on bone should be studied further. Because of the limited number of randomized placebo-controlled trials and insufficient available data, our meta-analysis was unable to evaluate the possible influences of habitual dietary intake of soy isoflavones, interval since menopause, participants' ethnicity, chemical forms and proportions of individual soy isoflavones, blood isoflavone concentration, urinary isoflavone excretion, and equol producer status.

Another limitation was that we incorporated one crossover trial [44] (for which the data required to perform a paired analysis were unavailable) with the remaining parallel-group trials in the meta-analyses of effects on serum BAP and OC, by using data from isoflavone periods and placebo periods as if the trial were a parallel-group trial of isoflavones and placebo [22]. This gave rise to a unit-of-analysis error, but the crossover trial did not show period or carry-over effects. However, sensitivity analyses excluding this crossover trial did not change the effects on serum BAP and OC. Moreover, this analysis was conservative, given that the trial was underweighted rather than over-weighted. While some argue against inclusion of crossover trials in this way, this unit-of-analysis error might be regarded as less serious than some other types of unit-of-analysis errors [22].

In conclusion

The present meta-analysis revealed that soy isoflavone supplements significantly decreased the bone resorption marker urinary DPD and showed no significant effects on the bone formation markers serum BAP and OC in menopausal women. However, the significant effect of soy isoflavones on DPD was moderate compared to estrogen or bisphosphonates, and studies should be performed to assess the possible interactions between isoflavones and anti-osteoporosis drugs. Further studies also need to address factors relating to the observed effects of soy isoflavones on urine DPD and to verify effects on other bone turnover markers.

Conflict of interest statement

KT contributed to the study search and selection, data extraction, meta-analysis, and preparation of draft of the manuscript; SM contributed to study selection, data extraction, and statistical analysis; YI and SW contributed to study design; all authors contributed to the final version of the manuscript. None disclose any conflicts of interest. The sponsor of this study had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Acknowledgments

This study was funded in part by the Fuji Foundation for Protein Research, Osaka, Japan. The foundation awards grants for research related to soy protein, under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bone.2010.05.001.

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