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

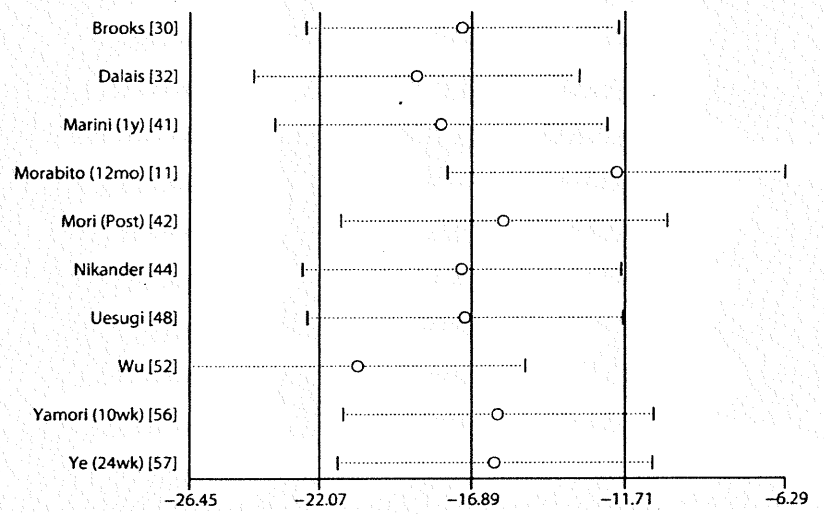

Fig. 2. Influence of each study on the overall effect on urine DPD (%). The vertical center line and the two lines on both sides of it respectively denote the combined overall effect and 95% CI for all included studies (fixed effect model). The open circles (○) and horizontal dotted lines respectively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

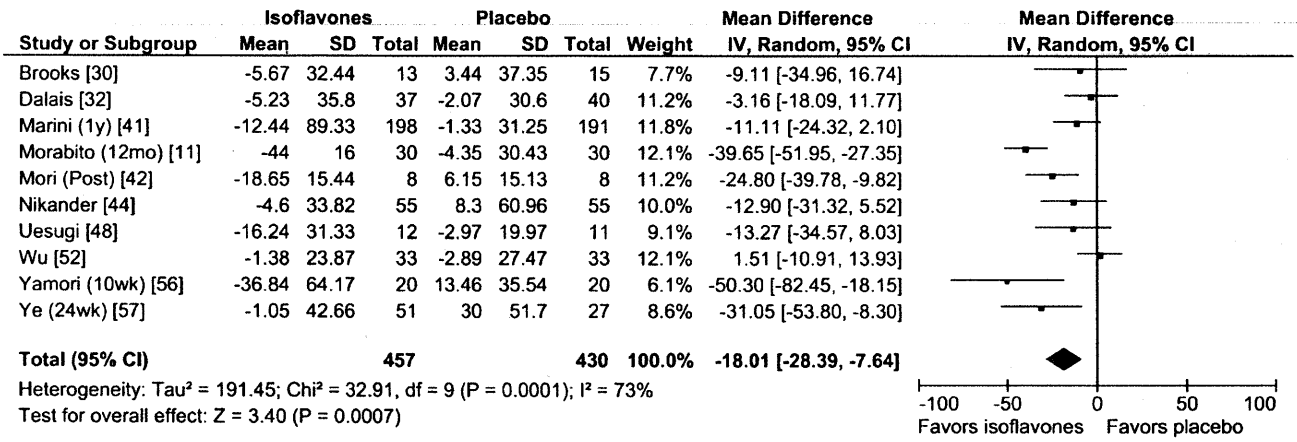

Fig. 3. Effects of soy isoflavones on urine DPD (%). Mean difference, mean percentage changes (%) of urine deoxypyridinoline (DPD) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight); horizontal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

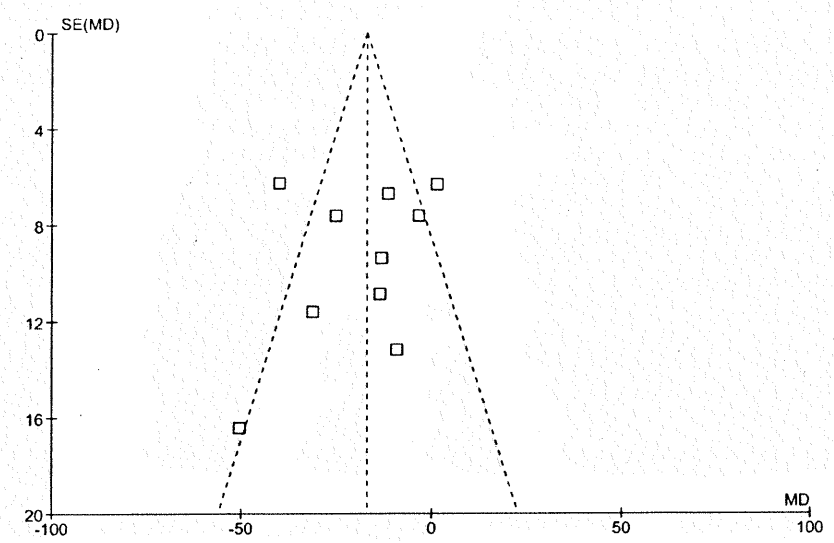

Fig. 4. Funnel plots of effects of soy isoflavones on urine DPD (%). MD, mean percentage changes (%) of urine deoxypyridinoline (DPD) from baseline for soy isoflavones minus that for placebo; SE(MD), standard error of MD. The vertical center broken line and two broken lines on both sides of it respectively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

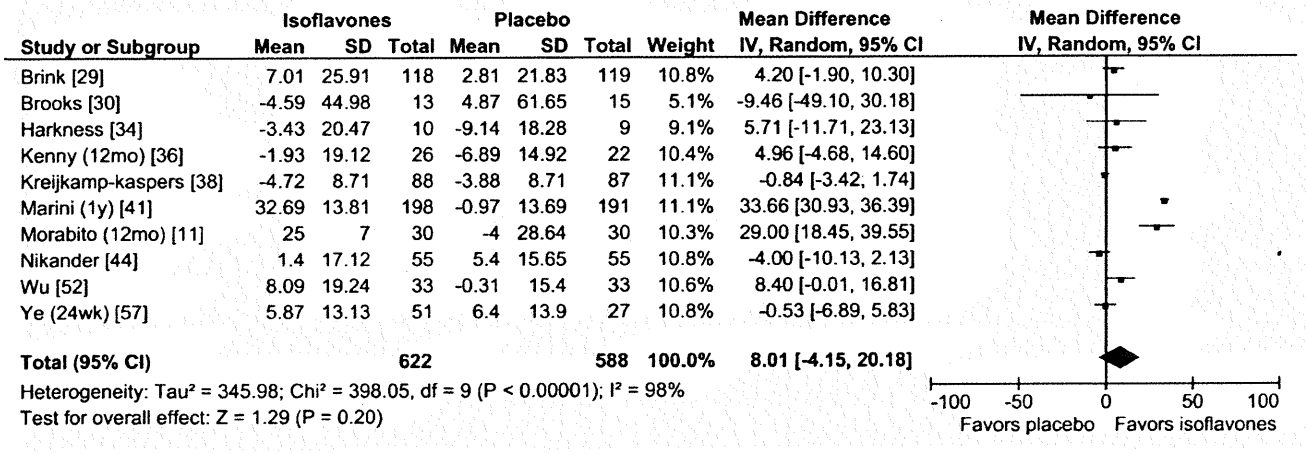

Fig. 5. Effects of soy isoflavones on serum BAP (%). Mean Difference, mean percentage changes (%) of serum bone alkaline phosphatase (BAP) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight);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

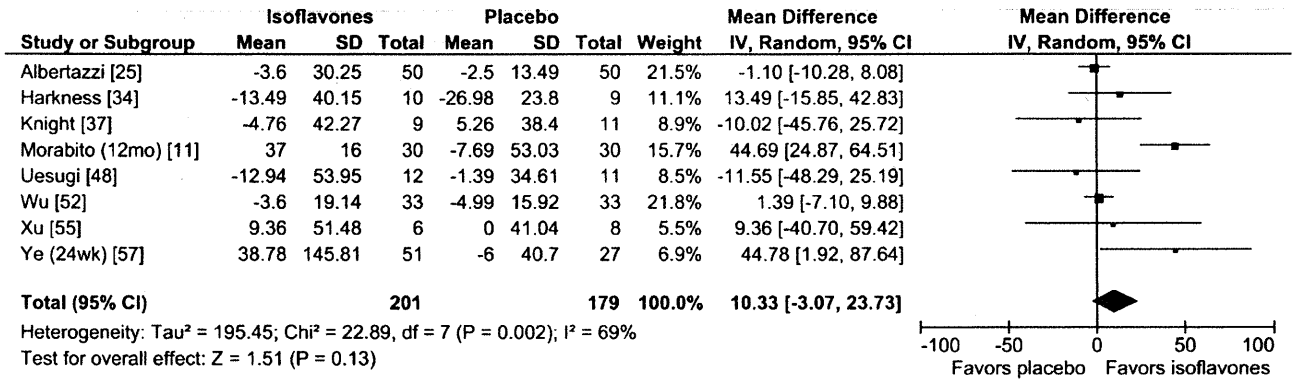

Fig. 6. Effects of soy isoflavones on serum OC (%). Mean Difference, mean percentage changes (%) of serum osteocalcin (OC) from baseline for soy isoflavones minus that for placebo; Random, random effects model. ■, effect estimate of each study (size of the square corresponds to its Weight); horizontal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

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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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METs in Adults While Playing Active Video Games: A Metabolic Chamber Study

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ABSTRACT

MIYACHI, M., K. YAMAMOTO, K. OHKAWARA, and S. TANAKA. METs in Adults While Playing Active Video Games: A Metabolic Chamber Study. *Med. Sci. Sports Exerc.*, Vol. 42, No. 6, pp. 1149–1153, 2010. **Purpose:** Active video game systems controlled through arm gestures and motions (Nintendo Wii Sports) and video games controlled through force plate (Wii Fit Plus) are becoming increasingly popular. This study was performed to determine the energy expenditure (EE) during Wii Fit Plus and Wii Sports game activities. **Methods:** Twelve adult men and women performed all the activities of Wii Sports (five activities: golf, bowling, tennis, baseball, and boxing) and Wii Fit Plus (63 activities classified as yoga, resistance, balance, and aerobic exercises). Each activity was continued for at least 8 min to obtain a steady-state EE. Because EE was assessed in an open-circuit indirect metabolic chamber consisting of an airtight room (20,000 or 15,000 L), subjects were freed of apparatus to collect expired gas while playing the games. MET value was calculated from resting EE and steady-state EE during activity. **Results:** The mean MET values of all 68 activities were distributed over a wide range from 1.3 METs (Lotus Focus) to 5.6 METs (single-arm stand). The mean MET values in yoga, balance, resistance, and aerobic exercise of Wii Fit Plus and Wii Sports were 2.1, 2.0, 3.2, 3.4, and 3.0 METs, respectively. Forty-six activities (67%) were classified as light intensity (<3 METs), and 22 activities (33%) were classified as moderate intensity (3.0–6.0 METs). There were no vigorous-intensity activities (>6.0 METs). **Conclusions:** Time spent playing one-third of the activities supplied by motion- and gesture-controlled video games can count toward the daily amount of exercise required according to the guidelines provided by the American College of Sports Medicine and the American Heart Association, which focus on 30 min of moderate-intensity daily physical activity 5 $d \cdot wk^{-1}$. **Key Words:** ENERGY EXPENDITURE, HUMAN CALORIMETER, METABOLIC EQUIVALENTS, Wii

Adults in developed countries are currently recommended to take more than a half hour of moderate to vigorous physical activity each day (6). However, many individuals spend many hours sitting in front of their TV playing video games. More than half of American adults (53%) play video games, and about one in five adults (21%) play every day or almost every day (9). This type of sedentary behavior is causally linked to chronic diseases and obesity (5,13).

The active video game systems controlled through arm gestures and motions (Wii Sports; Nintendo Inc., Kyoto, Japan) as well as the video games controlled through force plate (Wii Fit Plus; Nintendo Inc.) are becoming increasingly popular. These systems may attenuate a sedentary lifestyle and permit video game enthusiasts to increase their energy expenditure (EE), which is associated with prevention of obesity and lifestyle-related diseases (7,10). Several studies indicated that playing new-generation active computer games involves significantly greater EE than playing sedentary computer games but does not use as much energy as playing sport itself (3,4,8). The energy spent while playing active Wii Sports games was not of sufficiently high intensity to contribute toward the recommended daily amount of exercise (3,4,8). However, EE for these activities may have been underestimated because measurements were obtained using the Intelligent Device for Energy Expenditure and Activity (IDEEA) system (3) or indirect calorimeter with a facemask connected directly to an analyzer (4,8). The IDEEA does not detect arm or trunk movements well, considering the principle for physical activity evaluation (4,15), and therefore may underestimate EE. During measurement of EE with a facemask, the subjects' movements were tightly restricted (4,8). This may result in misleading conclusions regarding whether sufficient EE can be obtained while playing any mode of Wii Sports or Wii Fit Plus. Therefore, further research is needed to understand the energy load of the new modes of computer interaction and game play.

The present study was performed to determine EE and MET during various modes of activity in Wii Sports and Wii Fit Plus software using an open-circuit indirect metabolic chamber. The metabolic chamber can correctly

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measure whole-body EE and MET intensities while subjects are freely playing the game.

METHODS

Subjects. Twelve Japanese men (n = 7) and women (n = 5) participated in this study. All subjects were adults (25–44 yr) and were free of chronic diseases that could affect metabolism or daily physical activity. They had not engaged in regular intensive sports or physical activity for the past year. Informed consent was obtained from all subjects. The study protocol was approved by the ethical committee of the National Institute of Health and Nutrition.

Experimental design. Each subject completed metabolic chamber measurement under three different protocols on three different days: sitting rest, Wii Fit Plus balance and resistance exercises, Wii Fit Plus yoga and aerobic exercises, and Wii Sports. The order of these protocols was randomly assigned for each subject. Resting metabolic rate was evaluated immediately before performing activities of Wii Fit Plus balance and resistance exercises in the morning. Subjects abstained from meals and drink, except water, for at least 5 h before entering the metabolic chamber. Weight, height, and body composition analyzed by bioelectrical impedance were measured immediately before each session.

Wii Fit Plus software contains various activities consisting of 18 modes of yoga, 15 modes of resistance exercise, 16 modes of balance exercise, and 14 modes of aerobic exercise. Wii Sports software includes five activities: golf, bowing, tennis, baseball, and boxing. Each activity was continued for at least 8 min to obtain the steady-state EE separated by appropriate rest periods. Although game lengths of each activity were initially from 1 to 4 min, personal skills, fitness, and type of game resulted in fluctuations in the game lengths. The games in all activities were restarted immediately over and over again for 8 min. All subjects began each activity at the beginner level, and they performed these in an active fashion.

Metabolic chamber. The open-circuit indirect metabolic chamber used consisted of an airtight room (20,000 or 15,000 L) equipped with a bed, a desk, a chair, a TV with a video game player, a telephone, and a toilet. Thus, subjects were freed of apparatus to collect expired gas while playing the games. The temperature and the relative humidity in the room were controlled at 25°C and 55%, respectively. The oxygen (O_2) and carbon dioxide (CO_2) concentrations of the air supply and exhaust were measured by mass spectrometry. For each experiment, the gas analyzer (ARCO-1000A-CH; Arco System, Kashiwa, Japan) was initially calibrated using a certified gas mixture and atmospheric air. The flow rate exhausted from the chamber was measured by pneumotachography (FLB1; Arco System). The flowmeter was calibrated before each measurement, and the flow rate was maintained at 60 $L \cdot min^{-1}$ ambient temperature pressure (ATP). O_2 consumption and CO_2 production ($\dot{V}O_2$ and $\dot{V}CO_2$, respectively) were determined from the flow rate of exhaust from the chamber and the concentrations of the inlet and outlet air of the chamber, respectively (12). EE was estimated from $\dot{V}O_2$ and $\dot{V}CO_2$ using Weir's (14) equation. The accuracy and the precision of our metabolic chamber for measuring EE as determined by the alcohol combustion test were 99.2% ± 0.7% (mean ± SD) over 6 h and 99.2% ± 3.0% over 30 min (2).

Each activity was continued for at least 8 min. The metabolic chamber continuously analyzed O_2 and CO_2 concentrations for each gas and flow rate five times per minute and calculated EE for each minute. The EE increased progressively in the first 2–3 min of each activity, and then steady-state EE was obtained from 3 to 8 min. Therefore, we defined the mean value of EE for the last 5 min as steady-state EE of each activity. This increase in EE within a few minutes and the subsequent steady-state EE indicated that our metabolic chamber method has sufficient sensitivity. MET value was calculated from resting and steady-state EE during the activity.

Data calculation and analysis. All data are expressed as the means ± SD. Data were analyzed using one-way repeated-measures ANOVA with corrected *post hoc* paired t-test. We used the Statistical Package for the Social Sciences for Windows (SPSS Inc., Chicago, IL) for statistical analyses, and $P < 0.05$ was taken to indicate statistical significance.

RESULTS

The characteristics of the study subjects were as follows: age = 34 ± 6 yr, height = 167.4 ± 7.6 cm, body weight = 64.3 ± 15.0 kg, and percent fat = 22.3% ± 3.9%. Figure 1 shows the MET intensities during gaming. There were no significant differences in MET values between men and women. Therefore, mean MET values of each activity were calculated from the data of both sexes combined. The mean MET values of all 68 activities were distributed over a wide range from 1.3 METs (Lotus Focus: balance exercise) to 5.6 METs (single-arm stand: resistance exercise). The mean MET values in yoga, balance, resistance, and aerobic exercise of Wii Fit Plus and Wii Sports were 2.1 ± 0.6, 2.0 ± 0.6, 3.2 ± 1.2, 3.4 ± 0.9, and 3.0 ± 0.9 METs, respectively. The MET values of yoga and balance exercise were significantly lower than those of resistance and aerobic exercise of Wii Fit Plus or Wii Sports. Forty-six activities (67%) were classified as light intensity (<3 METs), and 22 activities (33%) were classified as moderate intensity (3.0–6.0 METs). There was no activity with intensity >6.0 METs.

The MET values of playing Wii Sports versions of activities were markedly lower than those of actual sports activities reported previously as follows (1): golf = 3.0–4.5 METs, bowling = 3.0, tennis = 5.0–7.0 METs, baseball = 5.0 METs, and boxing = 6.0–12.0 METs. However, the MET values of the Wii Fit Plus versions of yoga and

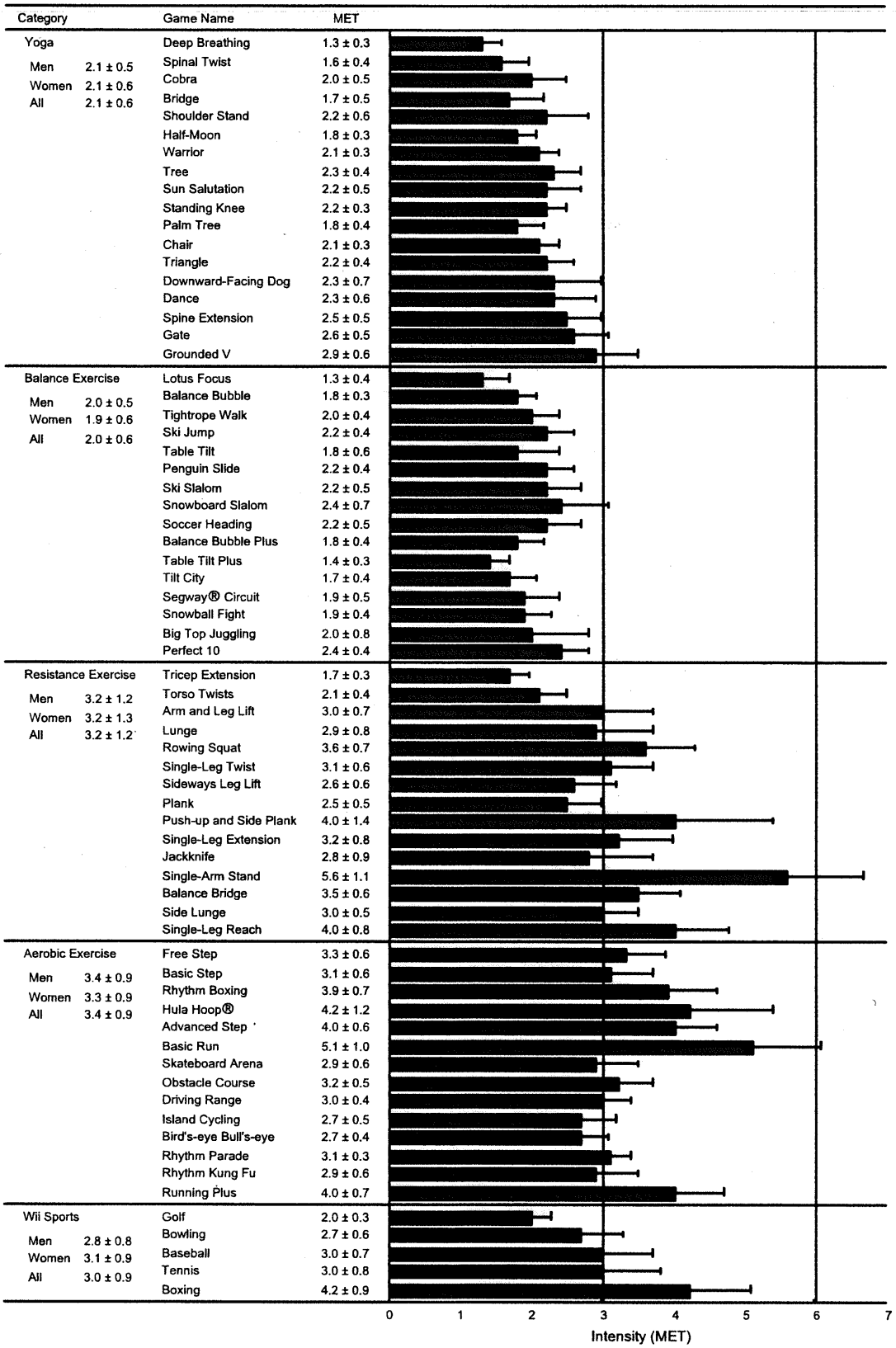

FIGURE 1—Mean values ± SD of METs while playing Wii Fit Plus and Wii Sports.

resistance exercise were similar to actual yoga (2.5 METs) and resistance exercise (3.0 METs) (1).

DISCUSSION

We determined EE and MET values during Wii Sports and Wii Fit Plus game activities using an open-circuit indirect metabolic chamber. The main findings of the present study were as follows. First, the mean MET values in yoga, balance, resistance, and aerobic exercise of Wii Fit Plus and Wii Sports were 2.1, 2.0, 3.2, 3.4, and 3.0 METs, respectively. Second, 46 activities (67%) were classified as light intensity (<3 METs), and 22 activities (33%) were classified as moderate intensity (3.0–6.0 METs). There were no vigorous-intensity activities (>6.0 METs). These findings suggest that time spent playing one-third of the activities supplied by motion- and gesture-controlled video games can partially count toward the daily amount of exercise required according to the guidelines provided by the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) (6).

The ACSM or AHA physical activity guidelines (6) focus on 30 min of moderate-intensity daily physical activity 5 $d \cdot wk^{-1}$ or vigorous-intensity aerobic activity for a minimum of 20 min for 3 $d \cdot wk^{-1}$. Moderate and vigorous physical activities were generally defined as intensities of 3.0–6.0 and >6.0 METs, respectively (6). Twenty-two (33%) of the 68 activities in Wii Fit Plus and Wii Sports were classified as moderate-intensity activities on the basis of MET intensity. Taken together, the observations of the present study suggest that the time spent playing Wii Fit Plus or Wii Sports can partially count toward the daily amount of exercise required according to the guidelines provided by the ACSM and the AHA (6). On the other hand, Graves et al. (3) concluded that Wii Sports games were not sufficiently vigorous to meet the guidelines for daily physical activity in children. We speculate that this discrepancy may be associated with differences in age of subjects and of measurement methods in EE and MET values (15).

Wii Sports gaming or Wii Fit Plus aerobic exercise involved less EE than authentic sports or exercises (1) because playing these active video games involved little horizontal locomotion. However, these light to moderate activities may contribute to increased EE, and even the small energy gap induced by the increased EE may be effective for prevention of weight gain (7). Furthermore, there were no moderate- or vigorous-intensity activities in Wii Fit Plus yoga and balance exercise. However, we should emphasize that yoga and balance exercise are effective in improving flexibility and in fall prevention, respectively (11). In addition, active computer games stimulated positive activity behaviors: the players were on their feet, and they moved in all directions while performing basic motor control and fundamental movement skills that were not evident during seated gaming. Given the current prevalence of overweight and obesity, such positive behaviors should be encouraged.

The strength of the present study is that the metabolic chamber method could replicate the conditions under which the subjects play the games in their home because subjects were free from apparatus used to measure EE when playing the game. In fact, the MET values of Wii Sports activities in our study were slightly higher than those in previous reports using the IDEEA system (3) or indirect calorimeter (4,8). On the other hand, the limitations of this study were that the sample size was small and the results were applicable only to healthy adults and to the Wii Fit Plus and Wii Sports computer games, which are more active than other Wii games.

CONCLUSIONS

We determined the MET values of Wii Sports and Wii Fit Plus game activities under free-living conditions using an open-circuit indirect metabolic chamber in healthy adults. Time spent playing one-third of the activities supplied by Wii Sports and Wii Fit Plus can count toward the daily amount of exercise required according to the guidelines provided by the ACSM and the AHA, which focus on 30 min of moderate-intensity daily physical activity 5 $d \cdot wk^{-1}$. Further research is needed to investigate the efficacy of the games on health promotion.

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The results of the present study do not constitute endorsement by the authors and the American College of Sports Medicine.

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REVIEW

Dietary intake and depressive symptoms: A systematic review of observational studies

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The importance of research into the possible role of dietary intake in depressive symptoms is emphasized by the fact that diet is modifiable. We systematically reviewed observational studies investigating the association between dietary intake and depressive symptoms published in English as of December 2008. Using the PubMed database, 34 publications (23 cross-sectional, 10 prospective cohort, and 1 case-control studies) were identified. The number of subjects (n = 80–27 111), age of subjects (15–97 years), dietary assessment method (dietary record, diet history interview, and validated and non-validated dietary questionnaire), depressive symptom assessment (discharge diagnosis, established scale, and self-reported information) varied among studies. Dietary variables most frequently investigated included long chain n-3 PUFA, fish, folate, and other B vitamins. Most studies found no association between dietary variables and depressive symptoms. However, most studies included at least one important methodological limitation, such as no inference for causality, unreliable or rough assessment of diet or depressive symptoms, inadequate treatment of potential confounding factors, and ignorance of the possible mediating or confounding influence of other dietary variables. Further evidence from well-designed observational studies is required to confirm or refute the association between dietary intake and depressive symptoms in free-living settings.

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1 Introduction

The importance of research into the possible role of dietary factors in depressive symptoms, a common problem in community medicine [1], is emphasized by the fact that diet is modifiable, although the literature indicates that the relationship between eating and mood is complex, situation specific, heavily influenced by individual history and psychological state, and measurement dependent [2]. For example, an association between depressive symptoms and low levels of several dietary B vitamins has been suggested, including for folate, riboflavin (vitamin B-2), pyridoxine (vitamin B-6), and cobalamin (vitamin B-12), possibly mediated by homocysteine or the synthesis of monoamines in the brain [3, 4]. Further, n-3 PUFA, of which alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid are the most important representatives, may play various broad roles in brain function and activity and have been suggested to play a role in depression [5, 6]. Additionally, because food consumption pattern reflects complex interrelations and interactions among the individual, the culture, and society in which people live, social and psychological mechanisms in addition to biological mechanisms may be also possible [2].

From a prevention perspective, findings on associations between diet and depressive symptoms in observational human studies conducted in free-living settings are much more important than the extrapolation of results from animal studies or the results of human intervention trials, which are usually conducted in patients with clinically diagnosed depression. Despite the importance of the systematic collection, evaluation and application of previous findings to the evidence-based primary care of depressive

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symptoms, however, to our knowledge no systematic review of observational studies of the association between dietary intake and depressive symptoms has appeared.

Here, we systematically reviewed published observational studies examining the association between dietary variables such as nutrient and food intake and depressive symptoms. The purpose of this systematic review was to throw light on dietary strategies that may be helpful in the prevention of depression.

2 Materials and methods

We searched the PubMed database (National Library of Medicine, Bethesda, MD) for observational studies on the relation of dietary variables, including nutrient and food intake, with depression or depressive symptoms using the following search strategy: ("diet" or "food" or "dietary" or "consumption" or "intake") and ("depression" or "depressive" or "depressed"). The search was limited to English-language reports published up to the end of December 2008. Studies included in this review were observational studies that used at least one quantitatively assessed dietary variable in the analysis, and had either depression or depressive symptoms as the outcome. Studies including only analyses of a purely descriptive nature (*e.g.* comparison of dietary intake between subjects with and without depressive symptoms without consideration of potential confounding factors or without the use of statistical testing) were excluded, because these studies provide no information on independent association between diet and depressive symptoms. Bibliographies of retrieved articles were also reviewed. A total of 34 articles were identified [7–40].

For each article, the following information was tabulated: authors and year of publication; dataset analyzed, settings, and study design; subject characteristics (such as age, sex, and number); dietary assessment instrument used (as well as information on validity for questionnaire-based assessment); depressive symptom assessment used; variables used for adjustment; and main findings (from the fully adjusted models), including the dietary variables examined and information on statistical testing. When men and women were analyzed separately, we retrieved these separate analyses. A meta-analysis (for each dietary variable) was attempted, but the degree of heterogeneity among study designs was prohibitive, particularly with respect to the age groups of participants and to dietary and depressive symptom assessment, and therefore a more qualitative assessment is presented. All reported p-values are two-tailed, with $p<0.05$ considered statistically significant.

3 Results

A total of 34 articles on dietary intake and depressive symptoms were included. Major characteristics are shown in Table 1. The first study that met the inclusion criteria appeared in 2001, and more than two-thirds (24 out of 34) of the articles were published since 2005.

Studies were conducted in various countries: seven articles were reported from Finland; five each from Japan and France; three from the US; two each from New Zealand, The Netherlands, Spain, and China; one each from Australia, the UK, Canada, Norway; and one each from several European collaborating countries, namely France and Northern Ireland, and Finland, Italy, and the Netherlands. The majority of articles (23 out of 34) were cross-sectional in nature, while ten of the remainder used prospective and one used case-control analysis. Subject numbers ranged from 80 to 27 111, and age from 15 to 97 years. Seven articles were restricted to male subjects and 7 others to females; the remaining 20 examined both sexes, with 8 examining men and women separately and 12 in combination.

Dietary variables were measured using a variety of instruments. Five articles used dietary records, 3 used diet history interview, 15 used validated food frequency or diet history questionnaires, and 11 used non-validated questionnaire techniques. Twenty-one studies used crude (non-energy-adjusted) dietary variables while the other 13 used energy-adjusted variables. A total of 55 dietary variables were examined, with long-chain n-3 PUFAs, fish, folate, and other B vitamins being the most frequent.

Depressive symptoms were also measured using a variety of techniques. One paper used discharge diagnosis of depressive disorders. About four-fifths (27 out of 34) of the studies relied on validated scales (either full or modified) for depressive symptoms, such as the Center for Epidemiologic Studies Depression Scale; the Edinburgh Postnatal Depression Scale; the Beck Depression Inventory; the Hospital Anxiety and Depression scale; the Zung Self-rating Depression Scale; the mental health scale of the Short Form Health Survey 36; the Human Population Laboratory Depression Scale; a depressive mood scale; the Hopkins Symptom Checklist depression subscale; the brief-Patient Health Questionnaire; the Geriatric Depression Scale; the Depression, Anxiety and Stress Scales; and the Welsh Pure Depression sub-scale. The remaining six articles relied on information on depressive symptoms self-reported by each participant.

All studies adjusted for a wide range of potential confounding factors. Many but not all adjusted for well-known factors associated with depression such as age, sex, marital status, socioeconomic factors such as education and income, smoking, alcohol drinking, physical activity, and body mass index. Conversely, only a few studies adjusted for other dietary variables.

A summary of the results of the associations of dietary intake with depressive symptoms is shown in Table 2. Although several studies ($n = 1$–4) investigated the relationship of energy, macronutrients, and related dietary variables with depressive symptoms, with particular focus

Table 1. Findings from observational studies of the relations between dietary intake and depressive symptom

References	Study	Subjects	Dietary assessment	Depressive symptom assessment[a)]	Adjustment for potential confounders	Main findings
Cross-sectional design						
Tanskanen *et al.* [7]	Questionnaire survey, Finland, 1999, cross-sectional design	Random sample of community residents aged 25–64 years ($n = 1767$)	FFQ, 132 items, previously validated against DRs	BDI, 21 items, potential score of 0–63	Sex, age, marital status, education, employment status, work ability, area of living, financial status, general health, smoking, alcohol intake, coffee drinking, physical activity	Descriptive information of dietary intake and depressive symptoms not available. Significantly lower risk of being depressed (BDI $\geq$ 10) in frequent than more infrequent lake-fish consumers (OR (95% CI): 0.63 (0.43, 0.94))
Tanskanen *et al.* [8]	Survey of cardiovascular risk factors, Finland, 1992, cross-sectional design	Random sample of community residents aged 25–64 years ($n = 3204$)	FFQ, number of items not shown (only one item used), not validated	BDI, 21-items, potential score of 0–63	Age, marital status, employment status, current smoking, physical activity, sex, BMI, alcohol intake, coffee intake, education, serum cholesterol level	70% classified as frequent fish consumers (more than or equal to once a week) and 30% as infrequent fish consumers (less than once a week). Prevalence of depressive symptoms (BDI $\geq$ 10): 28%. Significantly higher risk of being depressed in infrequent than frequent fish consumers (OR (95% CI): 1.31 (1.10, 1.56))
Slivers and Scott, [9]	Combined data of 1996/1997 New Zealand Health Survey and 1997 Nutrition Survey, New Zealand, 1996–1997, cross-sectional design	Nationally representative sample of New Zealand adults aged $\geq$ 15 years ($n = 4644$)	FFQ, number of items not shown, not validated	Mental health scale of SF-36, nine items, potential score of 0–100 (higher score indicating better mental health)	Age, household income, smoking, alcohol consumption, eating pattern	2% of fish non-consumers. Significantly higher mental health score in fish consumers than non-consumers (mean: 78.8 *versus* 70.6, $p < 0.05$)
Tolmunen *et al.* [10]	Kuopio Ischemic Heart Disease Study, Finland, 1984–1989, cross-sectional design	2443 men aged 42–60 years	4-d DRs	HPLDS, 18 items, potential score of 0–18	Age, years of study, smoking habits, consumption of alcohol, appetite, BMI, living alone, education, adulthood SES, fat intake (energy-adjusted)	Mean dietary intake (energy-adjusted): 254 μg/day for folate; 9.5 μg/day for vitamin B-12; 1.9 mg/day for vitamin B-6; 2.2 mg/day for riboflavin. Prevalence of depressive symptoms: 10%. Significant inverse relation of folate intake (energy-adjusted) with the prevalence of depressive symptoms (OR (95% CI) for the lowest tertile compared with the highest: 1.46 (1.01, 2.12)). No relation of intake (energy-adjusted) of vitamin B-12, vitamin B-6, or riboflavin with the prevalence of depressive symptoms

Table 1. Continued

References	Study	Subjects	Dietary assessment	Depressive symptom assessment[a)]	Adjustment for potential confounders	Main findings
Fulkerson *et al.* [11]	Project EAT (Eating Among Teens), USA, 1998–1999, cross-sectional design	4734 adolescents with mean age of 15 years (2377 boys and 2357 girls)	YAQ, 149 items, previously validated 24-h dietary recalls	Depressive mood scale, six items, potential score of 10–30	Race, grade	Descriptive information of dietary intake not available. Prevalence of moderate (depressive scale score = 18–22) and severe (depressive scale score $\geq$23) depressive symptoms: 27 and 7% for boys and 36 and 17% for girls, respectively. Significant positive relation of intake (crude) of soft drinks ($p = 0.005$), but not vegetables, fruits, energy, total fat (energy-adjusted), calcium, iron, sucrose, vitamin D, folate, vitamin B-6, and vitamin B-12, with the severity of depressive mood in boys. No relation of intake (crude) of vegetables, fruits, soft drinks, energy, total fat (energy-adjusted), calcium, iron, sucrose, vitamin D, folate, vitamin B-6, or vitamin B-12 with the severity of depressive mood in girls
Suzuki *et al.* [12]	Lung Cancer Database Project, Japan, 1999–2001, cross-sectional design	771 patients with newly diagnosed primary lung cancer	FFQ, 138 items, previously validated against DRs	HAD depression scale, seven items, potential score of 0–21	Age, sex, performance status, clinical stage, histology, pain, breathlessness, employment, smoking, alcohol consumption, BMI	Mean dietary intake (energy-adjusted): 0.9% energy for ALA; 0.03% energy for OTA; 0.02% energy for ETA; 0.16% energy for EPA; 0.04% energy for DPA; 0.27% energy for DHA; 0.44% energy for DHA+EPA; 1.4% energy for total n-3 PUFA. Prevalence of depressive symptoms (HADS depression scale$\geq$5): 57%. Significant inverse relation of intake (energy-adjusted) of ALA (OR (95% CI) for the highest quartile compared with the lowest: 0.50 (0.31, 0.71); p for trend = 0.004) and total n-3 PUFA (OR (95% CI) for the highest quartile compared with the lowest: 0.55 (0.35, 0.88); p for trend = 0.02) with the prevalence of depressive symptoms. No relation of intake (energy-adjusted) of OTA, ETA, EPA, DPA, DHA, or DHA+EPA

Table 1. Continued

References	Study	Subjects	Dietary assessment	Depressive symptom assessment[a)]	Adjustment for potential confounders	Main findings
Timonen *et al.* [13]	Northern Finland 1966 Birth Cohort Study, Finland, 1997, cross-sectional design	1966 birth cohort (2721 men and 2968 women aged 31 years)	FFQ, number of items not shown (only one item used), not validated	HSCL depression subscale, 15 items, potential score of 0–3. Self-report of doctor-diagnosed lifetime depression	Alcohol intake, smoking, physical inactivity, marital status	Descriptive information of dietary intake not available. Prevalence of doctor-diagnosed and HSCL-based (HSCL depression scale > 2.0) depression: 3.6 and 4.3% for men and 5.3 and 7.1% for women, respectively. Significantly higher risk of HSCL-based, but not doctor-diagnosed, depression in female rare (monthly or more seldom) fish eaters than female regular (weekly or more often) fish eaters (OR (95% CI): 1.4 (1.1, 1.8)). No relation of consumption frequency of fish with the risk of doctor-diagnosed and HSCL-based depression in men
Barberger-Gateau *et al.* [14]	Three-City Study, France, 1999–2000, cross-sectional design	9280 community dwellers aged ≥65 years (3647 men and 5633 women)	FFQ, number of items not shown, not validated	CES-D, 20 items, potential score of 0–60	Age, sex, education, city	11.7% reported eating fish less than once a week, 38.4% once a week, 49.9% more than once a week. Information on the prevalence of depressive symptoms (CES-D ≥ 17 for men and ≥22 for women) not available. Significant inverse relation of consumption frequency of fish with the prevalence of depressive symptoms (OR (95% CI) for the highest category compared with the lowest: 0.63 (0.52, 0.75))
Samieri *et al.* [15]	Three-City Study, France, 2001–2002, cross-sectional design	1724 community dwellers aged ≥65 years (647 men and 1077 women)	FFQ, 43 items, previously validated against a 24-h dietary recall. Five dietary clusters identified in both men and women	CES-D, 20 items, potential score of 0–60	Age, education, income, marital status	Mean score (SD) of CES-D: 6.0 (6.4) for men; 9.2 (8.2) for women. Significantly higher CES-D score in the "pasta eaters" cluster in men (β: 0.26; 95% CI: 0.06, 0.46; the reference for the variable cluster is the average of cluster levels). No relation of dietary clusters with CES-D score in women
Bonnet *et al.* [16]	Survey, France, 1998–2000, cross-sectional design	840 hypertensive patients with the metabolic syndrome (532 men and 308 women)	7-d dietary recall. Unhealthy diet score calculated based on intake of energy,	HAD depression scale, seven items, potential score of 0–21	Age, SES, marital status, personal history, presence of diabetes, BMI	Descriptive information of dietary intake not available. Prevalence of mild depression (HADS depression scale = 8–10) and marked depression (HADS

Table 1. Continued

References	Study	Subjects	Dietary assessment	Depressive symptom assessment[a)]	Adjustment for potential confounders	Main findings
			cholesterol, alcohol, carbohydrate, total fat, SFA, MUFA, and PUFA (higher score indicating unhealthier diet)			depression scale ≥11): 13.7 and 7.3% for men and 26.0 and 13.0% for women, respectively. Significant positive relation of unhealthy diet score with the severity of depression in both men (p for trend = 0.01) and women (p for trend = 0.04)
Hintikka *et al.* [17]	Kuopio Depression Study, Finland, 1998, cross-sectional design	2011 adults aged 25–64 years (890 men and 1121 women)	FFQ, number of items not shown, not validated	BDI, 21-items, potential score of 0–63	Sex, age, marital status, basic education, vocational training, employment status, economic hardship, subjective health, smoking, alcohol drinking	22% reported daily drinking of tea. Prevalence of depression (BDI ≥15): 10%. Significant inverse relation of daily tea drinking with the prevalence of depression (OR (95% CI: 0.47 (0.27, 0.83)). No relation of consumption frequency of coffee, fresh vegetables, boiled vegetables, fruits, lake fish, or sea fish with the prevalence of depression
Kamphuis *et al.* [18]; Kamphuis *et al.* [19]	Zutphen Elderly Study, The Netherlands, 1990, cross-sectional design	332 men aged 70–90 years who were free from cardiovascular diseases and diabetes	DHI	ZSDS, 20 items, potential score of 25–100	Age, years of education, physical activity, living alone, energy intake. For EPA+DPA, also BMI, smoking, alcohol consumption, systolic blood pressure. For B vitamins, also self-reported health, disability in activities of daily living, cognitive functioning	Median (10th–90th percentiles) intake of EPA+DPA (energy-adjusted): 105 (8-463) mg/day in non-depressed men and 87 (6-355) mg/day in depressed men. Mean (SD) dietary intake (energy-adjusted): 194 (57) μg/day for folate; 1.65 (0.30) mg/day for vitamin B-6; 5.34 (3.14) μg/day for vitamin B-12. Prevalence of depressive symptoms (ZSDS ≥50): 22%. Significant inverse relation of EPA+DPA intake (energy-adjusted) with the prevalence of depressive symptoms (OR (95% CI) for the highest tertile compared with the lowest: 0.46 (0.22, 0.95); p for trend = 0.04). No relation of intake (energy-adjusted) of folate, vitamin B-6, or vitamin B-12 with the prevalence of depressive symptoms
Sanchez-Villegas *et al.* [20]	SUN (Seguimiento Universidad de Navarr[a)] cohort study, Spain, 1999–2004, cross-	9670 university graduates (4211 men and 5459 women)	FFQ, 136 items, baseline only, previously validated against DRs	Self-reported physician diagnosis of depression or use of regular	Age, marital status, employment status, weight, height, caffeine intake, alcohol intake, physical activity	Descriptive information on dietary intake and depression not available. No relation of intake (energy-adjusted) of folate, vitamin B-6, vitamin B-12, or

Table 1. Continued

References	Study	Subjects	Dietary assessment	Depressive symptom assessment[a)]	Adjustment for potential confounders	Main findings
	sectional design			antidepressant medication	during leisure time, personality traits, chronic diseases, medication use	n-3 PUFA with the prevalence of depression in either sex
Woo *et al.* [21]	Community survey, China, cross-sectional design	3313 adults aged ≥65 years	FFQ, 267 items, previously validated against urine and serum biomarkers	GDS, 15 items, potential score of 0–15	Community Screening Instrument for Dementia score, age, gender, education level, SES, number of medical diseases, cigarette smoking, alcohol drinking, Physical Activity Scale of the Elderly	Descriptive information on dietary intake not available. Prevalence of depressive symptoms (GDS ≥8): 8%. Significant inverse relation of intake (crude) of vitamin A (OR (95% CI) for the highest tertile compared with the lowest: 0.47 (0.32, 0.69)), riboflavin (0.67 (0.45, 1.00), fiber (0.66 (0.46, 0.96)), and vegetables (0.63 (0.44, 0.92)) with the prevalence of depressive symptoms. No relation of intake of total fat, SFA (energy-adjusted), MUFA (energy-adjusted), PUFA (energy-adjusted), thiamin, niacin, vitamin C, iodine, cholesterol, carbohydrate, iron, fish and shellfish, fruits, or total isoflavone with the prevalence of depressive symptoms
Appleton *et al.* [22]	Questionnaire survey, UK, 2003, cross-sectional design	2982 adults entering an intervention study (1027 men and 1955 women)	FFQ, number of items not shown (only two items used), not validated	DASS-21, 21 items, potential score of 0–63	Sex, age, Index of Multiple Deprivation score, date of questionnaire completion	Mean intake (range) of essential n-3 long -chain PUFA from fish: 0.6 (0–6) portions of fatty fish/wk (or equivalent). Mean score (range) of DASS depressed mood score: 7.8 (0-42). No relation of essential n-3 long-chain PUFA from fish with DASS depressed mood score
Appleton *et al.* [23]	Prospective Epidemiological Study of Myocardial Infarction, Northern Ireland and France, 1991–1994, cross-sectional design	10 602 men aged 50–59 years (2747 men in Northern Ireland and 7855 men in France)	FFQ, number of items not shown; not validated	Welsh Pure Depression sub-scale of the Minnesota Multiphasic Personality Inventory, ten items, potential score of 0–10	Age, housing type, number of toilets, number of baths, number of cars, work type, years at school, level of education, intake of cake, cheese, eggs, fruit, nuts, offal potatoes boiled/baked, potatoes fried, vegetables raw, vegetables cooked	Descriptive information on food consumption not available. Mean depression score (SD): 1.6 (1.8) for Northern Ireland samples and 1.6 (1.9) for French samples. Inverse relation of fish intake (crude) with the depression score in both Northern Ireland (β: -0.09; 95% CI: −2.25, −0.01)