The effect of a randomized 12-week soy drink intervention on everyday mood in postmenopausal women.

Running title: Dietary soy and mood in postmenopause

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Conflict of interest and Source of Funding: This research was supported by funding from the Alpro Foundation

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Abstract

Objective*:** Dietary soy may improve menopausal symptoms, and subsequently mediate mood. This novel study examines various does of dietary soy drink on everyday mood stability and variability in postmenopausal women. ***Methods*:** Community dwelling women (n=101), within 7 years post menopause consumed daily either a low (10mg, n=35), medium (35mg, n=37) or high (60mg, n=29) dose of isoflavones, for twelve weeks. Menopausal symptoms and repeated measures of everyday Mood (Positive (PA) and Negative (NA) affect) (assessed at four time points per day for four consecutive days, using PANAS) were completed at baseline and follow up. ***Results*:** The dietary soy intervention had no effect on everyday mood stability (for PA ($F(2,70) = .95$, $p = .390$) and NA ($F(2,70) = 0.72$, $p = .489$) or variability (for PA ($F(2,70) = .21$, $p = .807$) and for NA ($F(2,70) = .15$, $p=.864$) or on menopausal symptoms (for vasomotor ($F (2,89) = 2.83$, $p = .064$), psychological ($F (2,88) = 0.63$, $p = .535$), somatic ($F (2,89) = 0.32$, $p =.729$) and total menopausal symptoms ($F (2,86) = 0.79$, $p = .458$)). There were between group differences with the medium dose reporting higher PA (low, mean: 24.2, SD: 6 and medium, mean: 29.7, SD: 6) and the low dose reporting higher NA (P = 0. 048) (Low, mean: 11.6, SD: 2 and high, mean: 10.6, SD: 1) in mood scores. Psychological (baseline M = 18 and follow up M = 16.5) and vasomotor (baseline M = 4.2 and follow up M = 3.6) scores declined from baseline to follow up for the overall sample. ***Conclusions: Soy isoflavones had no effect on mood at any of the doses tested. Future research should focus on the menopausal transition from peri to post menopause as there may be a window of vulnerability, with fluctuating hormones and increased symptoms which may affect mood.

Keywords: isoflavones, menopause, hot flush, hot flash

INTRODUCTION

The menopausal transition (MT) may impact on mood due to increased vasomotor symptoms[1,2] and psychosocial changes at MT[3-5], leading to a decline in well-being[6]. Fluctuations in mood at MT have been associated with hormonal changes in some studies[7] but contested in others (for a review see Hengartner, 2017[8]), suggesting the underlying processes are not well understood[9,10]. There are no specific mood disorders of the menopause[4]. Treatment of mood disorders occurring during MT with hormone therapy (HT) has produced conflicting results[11,12].

The few studies looking at mood and MT have suggested differential effects on positive (PA) and negative (NA) mood[13]. PA was not influenced by MT in one study[14], but NA was related to the severity of menopausal symptoms and stage of MT, the closer to MT the greater the impact of symptoms on NA[15], further supported by longitudinal research[16]. One other study reported elevated menopausal symptoms was detrimental to PA[17], while another reported increased NA and a decline in PA[18]. The conflicting findings may be due to methodological issues around how and when mood was measured, samples vary in relation to age and menopausal status. Moods fluctuate and change throughout the day and need to be assessed using repeated measures[19]. In the current study mood or affect was regarded as a subjective state conceptualized as two independent continua, positive affect (PA) and negative affect (NA)[20,21]. PA reflects states such as joy, alertness, and enthusiasm, while NA measures the amount of unpleasantness or dissatisfaction the person is experiencing[22].

Vasomotor symptoms respond well to HT, however, due to increased fears around its use[23], the number of women using HT and doctors prescribing it has declined. This has led to women seeking out alternatives to HT[24], of which soy is one.

Soy contains a high concentration of isoflavones, regarded as plant-based phytoestrogens, including genistein, daidzein and glycitein, which have a similar chemical structure to 17-β estradiol[25], binding to estrogen receptors and mimicking their effects[26]. They are recommended for management of vasomotor symptoms[27]. Isoflavones have several health benefits for MT such as promoting heart health, bone mass and alleviation of vasomotor symptoms[28,29]. Some soy isoflavones, such as glyceollins, have been found to produce insulinotropic, antiestrogenic, antiproliferation, antioxidant, anti-inflammatory and cholesterol lowering effects in women at midlife[30]. Their impact on psychological factors at MT are less clear.

Dietary soy products were found to reduce psychological symptoms and improve well-being in menopausal women. In a 6-week intervention participants consumed 200ml soy drink per day, with improvements in menopausal symptoms and quality of life[31]. Positive effects were noted for a soy drink (12.5 g of soy protein with genistein, 13 mg and daidzein, 4.13 mg day) and an exercise intervention[32] improving menopausal symptoms and psychological well-being. These findings were further supported by reviews of soy products[13,33]. However, several studies reported no beneficial effects after a six-month dietary soy intervention (100mg isoflavones in flour) for depressive symptoms[34], or a three month intervention for menopausal symptoms (118 mg of isoflavones in supplement)[35] or 8 months consumption of 500ml of soy drink per day (containing 28.86 mg/dl of genistein and 8.25 mg/dl of daidzein) on quality of life[36] in menopausal women. Mood tends to be assessed using symptom scales or visual analogue scales, in the few studies that have looked at mood during menopause, rather than by repeated measures employing scales designed to capture mood change.

To our knowledge this is the first study to look at the effect of a 12-week dietary soy intervention on everyday PA and NA stability and variability in postmenopausal women. The first objective was to examine if PA and NA were influenced pre to post intervention. The second objective was to determine the effect on mood with different concentrations of isoflavones consumed over time.

METHODS

Study design & Participants

This study was a 12-week prospective randomized trial, conducted between October 2015 and May 2018, looking at the effect of a soy drink intervention on cognitive function (results will be reported elsewhere), menopausal symptoms and everyday PA and NA in postmenopausal women. Following ethical approval from the University Research Ethics Committee, women were recruited from community groups and organizations throughout Ulster, an overview of this process is given in the flow chart in Figure 1. All the participants were required to be within seven years postmenopausal, defined using the WHO definition, as cessation of menses for at least 12 months at hormone levels >30 mIU/ml for follicle stimulating hormone. They were excluded if taking HT, had an abnormal blood profile (assessed by Sysmex KX21-N, Sysmex UK Ltd, Milton Keynes, UK) and liver and kidney function tests (assessed photometrically by Cobas 6000 analyzer [Cobas C501 module], Roche Diagnostics at Causeway Hospital, Coleraine, UK), had a hysterectomy and ovariectomy, were taking antibiotics or psychoactive medication, and if they had a history of cardiovascular disease, cancer, diabetes, thyroid, renal or kidney disease, alcohol or drug abuse. Self-reported consumption of soy-based products beyond two portions per week was also an exclusion criterion.

Participants were also screened for cognitive decline and psychological distress as determined by the Mini Mental State Examination (scores less than 24 were excluded) and General Health Questionnaire-28 (adopting 26 as the threshold for the presence of distress), respectively.

Those eligible, were invited for baseline measures on mood (PANAS to be completed over 4 days prior to beginning the soy drink intervention) and menopausal symptoms. They were then randomly allocated to one of three conditions a low (10mg = 100ml), medium (35mg = 350ml) or high (60mg = 600ml) dose of isoflavones contained within a soy drink, by an independent clinical trials manager using MINIM software[37], a placebo with no isoflavones was not available, a low dose was used that has not been previously shown to have a beneficial effect on menopausal symptoms[50,51,57]. Participants were recommended to consume a specific volume of their soy drinks throughout each day for 12 weeks. This approach provided the opportunity to look at doses that are commercially available and can be incorporated into the daily diet and is comparable to previous studies[32]. Mood and menopausal symptoms were re-assessed post-intervention. Researchers contacted participants throughout the study and monitored their progress intermittently.

Compliance was monitored by measuring plasma concentrations of soy isoflavones, using LC-MS/MS by LGC Limited (Cambridgeshire, UK). Participants were instructed to consume the amounts across each day, rather than at once, as previous research suggests it is more effective at reducing menopausal symptoms[38]. The study was registered at https://www.clinicaltrials.gov (NCT03561662).

Variables

All questionnaires used in this study, except for the MMSE which was administered by the researcher, were self-report.

Sociodemographic variables (age, education, BMI and menopause history), health, lifestyle and menopausal status were assessed using a questionnaire employed in previous studies based on the EPIC Health and Lifestyle Questionnaire, and adapted by Simpson et al.[40].

An indication of *cognitive decline* was assessed using the Mini Mental State Examination[41]. Scores are classified as 24-30 no cognitive impairment, 18-23 mild cognitive impairment, and 0-17 severe cognitive impairment. The current study participant inclusion criteria was set at no impairment.

Screening for *psychological distress* was assessed using The General Health Questionnaire-28 (GHQ-28)[42], a 28-item scale with four subscales assessing somatic symptoms, anxiety/insomnia, social dysfunction and severe depression. For inclusion in the current study, a total score of 26 or less was required (the GHQ-28 cut-off for distress).

Mood was assessed using The Positive and Negative Affect Schedule (PANAS)[20] a 20-item scale comprised of 10 items assessing PA (e.g. happy, alert) and 10 items measuring NA (e.g. nervous, irritable). PA is associated with feelings of alertness, enthusiasm and happiness and NA with displeasure and dissatisfaction. These are regarded as higher-level mood states, accounting for the majority of the variance in discrete moods[21]. Momentary mood was measured four times a day for four consecutive days: upon rising; at 14.30; after dinner (17.00-18.00) and at 22.30, at baseline and follow up. The scales have high internal consistency, with Cronbach's alpha ranging from .84 to .90 for PA scale and .84 to .87 for NA scale[43] and proven

validity[20]. The dependent trait measures of affect were based on overall intra-individual means and SDs for PA and NA for the four days.

Menopausal symptoms were assessed by the 21 item Greene Symptom Checklist[44] measuring psychological, somatic, and vasomotor symptoms. Each item was rated on a 4 point Likert scale (0 = not at all, 1 = a little, 2 = quite a bit, 3 = extremely), a higher score being indicative of greater severity of symptoms. It has proven reliability for each subscale (test retest reliabilities of: r = .87 for psychological; r = .84 for somatic and r = .83 for vasomotor) and content and construct validity[45].

Data analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 24. Prior to data analyses, the psychological data were checked for normality by first examining statistics for skewness and kurtosis which were within an acceptable range[46]. Plasma isoflavones were analyzed using Kruskal-Wallis with post-hoc Mann-Whitney U Tests. A series of 2 (time: baseline and follow-up) * 3 (Dose: low medium and high isoflavones) ANOVA with repeated measures were conducted to establish the effect of the intervention on mood and menopausal symptoms.

RESULTS

Sample description

A total of 101 postmenopausal women completed the study. As the original power calculation was based on cognition, a retrospective one was carried out to confirm the sample size required for mood, based on a small effect size of .2, found in previous study on mood and dietary intervention[39], with a power of 0.8 and a 0.05 level

of significance, 66 participants were required. An overview of the age range across the groups and educational level in each group is given in Table 1. Participants were within an expected age range for menopause, with just over half of the sample achieved tertiary level education.

Compliance with soy intervention

Compliance was assessed by plasma isoflavone concentrations and is given in (Table 2). Blood samples were available for isoflavones analysis for 95 participants at baseline and 87 post-intervention. There were no differences found for Isoflavones concentrations between the groups at baseline. At follow up, genistein concentration was higher in the medium (P = 0.007) and high (P = 0.013) dose groups compared to the low dose group. Post-intervention daidzein was higher in the medium (P = 0.006) and high (P = 0.029) dose groups compared to the low dose group. No group differences were noted post-intervention for isoflavone concentrations. Feedback from participants following the intervention showed the majority adhered to the dose given and were creative in adding it to their diet, in coffee, tea, porridge and puddings. Those that had experienced problems with consumption of the specified volume were more likely to be from the high dose, with seven women failing to complete the intervention for this reason.

The effect of dietary soy on mood

In order to determine the dietary soy intervention effect on mood and menopausal symptoms, repeated measures ANOVAs were carried out. These aimed to determine the change in mood (stability and variability) and menopausal symptoms over time, group differences between the three soy isoflavone doses and interaction

effects to detect the impact of the soy intervention. An overview of the results is given in Table 3 and will now be discussed in turn.

Mood stability and dietary soy

Stability of mood was established by calculating the means of the 16 measures of PA and the 16 measures of NA recorded separately for baseline and follow-up. There were no changes in mood from baseline to post intervention for PA ($F^{(1, 70)}$ = 1.10, P = 0.296) or NA ($F^{(1,70)}$ = 0.42, P = 0.521).There was a difference between the groups for dose for PA ($F^{(2,70)}$ = 6.6, P = 0.002), Bonferroni post hoc tests showed that low dose (M = 24.2) differed significantly from the medium dose (M = 29.7), with the latter reporting higher PA scores (P = 0.002). There was a difference between the groups for NA ($F^{(2,70)}$ = 3.12, P = 0.05), Bonferroni post hoc tests showed that low dose (M = 11.6) differed significantly from the high dose (M = 10.6), with the latter reporting lower NA scores (P = 0.048). There were no interaction effects for time and dose for PA ($F^{(2,70)}$ = .95, P = 0.390) and NA ($F^{(2,70)}$ = 0.72, P = 0.489).

Mood variability and dietary soy

Mood variability is established by looking at the SD of the 16 PA and 16 NA measures recorded separately at baseline and follow up. There were no differences in mood variability from baseline to follow up for PA variability ($F^{(1, 70)}$ = .35, P = 0.555) and NA variability ($F^{(1, 70)}$ = .49, P = 0 .488). There were no between group differences for dose for either PA variability ($F^{(1, 70)}$ = 1.46, P = 0.240) or NA variability ($F^{(1, 70)}$ = 2.83, P = 0.066), nor were there interaction effects for time by dose for PA variability ($F^{(2, 70)}$ = .21, P = 0.807) or NA variability ($F^{(2,70)}$ = .15, P = 0.864).

Menopausal symptoms and dietary soy

There were reduced menopausal symptoms between baseline and follow up for vasomotor (baseline M = 4.2 and follow up M = 3.6) ($F^{(1, 89)} = 9.10$, $P = 0.003$), psychological (baseline M = 18 and follow up M = 16.5) ($F^{(1,88)} = 8.34$, $P = 0.005$) and total symptoms (baseline M = 34 and follow up M = 32) ($F^{(1,86)} = 9.02$, $P = 0.004$), and no change in somatic symptoms over time ($F^{(1,89)} = 1.59$, $P = 0.210$). There were no between group differences for dose for vasomotor ($F^{(2,89)} = 2.35$, $P = 0.102$), psychological ($F^{(2,88)} = 0.69$, $p = 0.502$), somatic ($F^{(2,89)} = 0.16$, $P = 0.848$) or total ($F^{(2,86)} = 0.53$, $p = 0.590$) menopausal symptoms. Nor were there any interaction effects for vasomotor ($F^{(2,89)} = 2.83$, $P = 0.064$), psychological ($F^{(2,88)} = 0.63$, $P = 0.535$), somatic ($F^{(2,89)} = 0.32$, $P = 0.729$) or total menopausal symptoms ($F^{(2,86)} = 0.79$, $P = 0.458$).

DISCUSSION

To our knowledge this is the first randomized trial looking at the effects of dietary soy on everyday mood stability and variability of PA and NA. In the current study, mood was assessed at baseline and post intervention, using 16 repeated measures of mood (four times a day for four consecutive days), employing a well-established method[47], and addressing a methodological weakness in other studies[8]. The findings suggest that doses of soy (isoflavones 10, 35 and 60mg/day) used in this study had no effect on stability (mean) or variability (SD) of everyday PA or NA, nor on menopausal symptoms.

Isoflavones had no effect on everyday mood in this study, in keeping with previous dietary soy studies looking at general measures of quality of life and psychological well-being at menopause[34-36]. Similarly, using a protocol comparable to the current study, supplementation with 60 mg isoflavones (capsules) per day for

either 6 or 12 weeks, had no effect on menopausal symptoms or mood assessed using visual analogue scales(VAS)[48,49]. However, our findings are contradictory to other soy interventions, (isoflavone consumption ranged from 60mg/d to 160mg/d) that reported improvements in quality of life and psychological symptoms in postmenopausal women[31,50,51]. The amount of soy in the current study is comparable to some studies[36,48,49,51], but lower than in others[34,50]. The amount consumed in the current study for the medium and high doses are within the recommended dose for management of menopausal symptoms by the EFSA guidelines[57], which recommends 35-150mg/day. However, the doses used in the current study may not have been taken for long enough as another intervention found 60mg/d of isoflavones taken for 6 months had a beneficial effect on VAS mood post intervention[51].

There was no change in menopausal symptoms post intervention. Most of the women in the current study were all post-menopausal for 3 years or more prior to taking part in the study. Menopausal symptoms were much lower than reported for a sample of recently menopausal women, just 12 months cessation of menses [58]. The means and SD for PA and NA are comparable to previous research looking at older women aged 55-70 years[47]. Previous research looking at MT suggests that women are more at risk of mood disturbances during the perimenopausal phase, where hormone fluctuations and increased menopausal symptoms[52] may result in a “window of vulnerability”, characterized by increases in NA and depressive symptoms[53]. Therefore, in the current study we may have missed this most turbulent time occurring closer to MT[15] (from peri into post menopause), this may be the time that dietary soy could benefit mood. As women progress through MT into postmenopause, hormone levels become more stable and symptoms begin to subside, with improvements in PA and reduced NA being observed[15]. There are a number of methodological issues that

make comparisons between the studies of mood and menopause difficult. Much of the research utilizes women from a range of ages, severity of menopausal symptoms and at different stages in MT[8]. There is no consistency in the assessment of mood, with many studies relying on one off measures, or using menopause symptom scales or quality of life measures as proxy mood assessments.

This study has several strengths, it is a randomized trial which is regarded as the gold standard when designing interventions and addressed a paucity of research looking at the effects of soy and psychological well-being in menopause[31]. The researchers believe this may be the first study to examine everyday mood assessed prospectively over four days at baseline and four days at post intervention, in postmenopausal women looking at the effect of soy on its stability and variability. We used self-report and well-established clinical hormone assessment to determine menopausal status. The use of well-established and validated repeated measures of mood is also a strong support for the study[20] and has been validated in previous dietary intervention trials on healthy older adults[39,47].

The study has limitations that need to be considered. Although the researchers adhered to very strict inclusion and exclusion criteria and stringent screening methods, this sample were healthy, with no major physical or mental health problems and may not be representative of all postmenopausal women. As a consequence, those from lower socio-economic groups may have been excluded, this may be supported by over half of the sample completing tertiary level education. The sample size is also small but is adequately powered to look at changes in mood following a dietary intervention. Future studies need to be carried out on women nearer to MT and on a more representative sample before any firm conclusions can be drawn. We have not considered other lifestyle factors and stress that have the potential to impact on mood

and quality of life in menopause[3]. Also, personality variables have been found to be related to mood and were not assessed in the current study[54]. The duration of the intervention may not have been long enough to see a change in mood[50]. Also, the current study did not have a placebo condition which may have impacted on our findings as all the groups received some isoflavones, a placebo was not available to the researchers. The low dose was selected as it had not been found previously to impact on menopausal symptoms or well-being and was well below that recommended for use in menopause symptom management[57]. Another factor that was not considered in this study, but has the potential to impact on our findings, is the possibility of individual differences in the ability to metabolize isoflavones e.g. approximately one third of the population produce equol, a metabolite of soy derived from daidzein[56] that alleviates vasomotor symptoms[59,60]. This may have contributed to some of the conflicting findings in studies looking at dietary soy intervention on quality of life[36,50]. Future studies need to consider this in relation to the impact of soy on psychological variables such as mood.

CONCLUSION

A commercially available soy drink, containing 10, 35 and 60 mg/day of isoflavones, and consumed for 12 weeks in a sample of postmenopausal women, did not produce an effect on mood or menopausal symptoms. Results from research examining MT, peri to postmenopause, with increased fluctuations in hormones and symptoms, may reflect a window of vulnerability in some women and identify a key time for soy intervention. Future studies need to consider this key MT phase and the differences in the effect of soy supplementation for equol and non-equol producers, on symptoms and psychological well-being.

Acknowledgments

The authors would like to thank Callan Dickey, Hannah Anglin, Emma Crawford, Rachael Ervine and Shannon Kennedy for their assistance in recruitment and data collection and all participants involved in the study.

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Table 1 *Sample characteristics for age and education level*

	Soy isoflavone treatment group		
Measure	Low *(n=35)*	Medium (*n=37)*	High (*n=29)*
Age in years Mean (SD)	53.69 ± 3.72	53.86 ± 3.28	53.72 ± 4.62
Education level (n)[a]			
Primary	0	1	0
Secondary	19	13	12
Tertiary	15	21	16

[a] Data on education was unavailable for 4 participants (n = 1, n = 2 and n= 1 in the low, medium and high dose groups respectively) as they failed to complete the health and lifestyle questionnaire

Table 2. *Plasma isoflavone concentrations for genistein and daidzein at baseline and 12 weeks of soy drink intervention to determine compliance*

Treatment group	Low (*n*=35)		Medium (n=37)		High (n=29)	
	Baseline	Week12	Baseline	Week 12	Baseline	Week 12
genistein (ng/ml)	10.01 (22.07)	82.75 (124.6)	9.83 (24.25)	168.71 (166.8)	6.40 (9.27)	216.71 (305.36)
daidzein (ng/ml)	5.03 (11.88)	20.44 (30.82)	2.29 (4.37)	39.20 (36.9)	2.50 (3.34)	49.82 (66.49)

mean (SD) given for all values. Ng/ml = Nanograms per millilitre.

Table 3. *Means (SD) for mood measures and menopausal symptoms at baseline and follow up, showing effects for time, dose and interactions*

	Baseline			12-week Follow up			*P* value		
	Isoflavone dose			Isoflavone dose					
	Low	Medium	High	Low	Medium	High	Time	Dose	Time X Dose
PA mood mean	24.2(6)	29.7(7)	25.3(5)	24.2(7)	29.7(6)	27.6(7)	0.296	0.002	0.390
PA mood SD	6.2(2)	7.5(5.3)	6.6(2)	6.3(3)	7.2(3)	5.9(3)	0.555	0.240	0.807
NA mood mean	11.7(2)	10.9(1)	10.6(1)	11.6(2)	11.3(1)	10.7(1)	0.521	0.050	0.489
NA mood SD	1.8(2)	1.4(2)	.95(1)	2(2)	4.6(1)	.96(.8)	0.488	0.066	0.864
Psychological symptoms	18.3(4)	18.5(5)	17.2(4)	17.3(4)	16.4(4)	16.2(3)	0.005	0.502	0.535
Somatic symptoms	9.8(2)	9.7(2)	9.8(3)	9.1(2)	9.4(2)	9.7(3)	0.210	0.848	0.729
Vasomotor symptoms	4.5(2)	4.5(2)	3.6(1)	4.0(2)	3.4(1)	3.5(2)	0.003	0.102	0.064
Total symptoms	34.6(6)	34.8(7)	32.9(7)	32.7(7)	31.1(6)	31.4(7)	0.004	0.590	0.458

Mean (SD) given for all values, except *P*. Differences for time: changes in the variables between baseline and follow up. Dose: between group difference for low (10mg) medium (35mg) and high (60mg) isoflavone and 2 (time) X 3 (dose) interactions. Significant *P* values are accepted as < 0.005. N values changed for mood and menopausal symptoms across the groups in the analysis as the datasets were not complete across the two time points, for mood the ANOVA are based on: Low (n = 29), Medium (n = 26) and High (n = 18) and for menopausal symptoms the ANOVA are based on: Low (n = 33), Medium (n = 34) and High (n=24). Mood mean = the mean of the 16 measures taken to represent respective mood types and represents the extent to which mood is stable over

Figure 1. *Flowchart showing recruitment of participants*