Scientific Research Publishing

Open Journal of Endocrine and Metabolic Diseases, 2022, 12, 47-74
https://www.scirp.org/journal/ojemd
ISSN Online: 2165-7432
ISSN Print: 2165-7424

Soy-Enriched Bread, a Pilot Study to Determine Its Beneficial Effects in Menopause

Daniela Giustarini[1], Comasia Ricci[2], Ilaria Ceccarelli[3], Stefano Pieretti[4], Paolo Andre[3], Silvia Migliorini[3], Lauretta Massai[3], Paola Minosi[4], Ilenia Casini[3]*, Anna Maria Aloisi[3]

[1]Department of Biotechnology, Pharmacy and Biochemistry, University of Siena, Siena, Italy
[2]Department of Life Sciences, University of Siena, Siena, Italy
[3]Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy
[4]National Center for Drug Research and Evaluation, Italian National Institute of Health, Rome, Italy
Email: *ilenia.casini@student.unisi.it

How to cite this paper: Giustarini, D., Ricci, C., Ceccarelli, I., Pieretti, S., Andre, P., Migliorini, S., Massai, L., Minosi, P., Casini, I. and Aloisi, A.M. (2022) Soy-Enriched Bread, a Pilot Study to Determine Its Beneficial Effects in Menopause. *Open Journal of Endocrine and Metabolic Diseases*, **12**, 47-74.
https://doi.org/10.4236/ojemd.2022.122004

Received: December 7, 2021
Accepted: February 25, 2022
Published: February 28, 2022

Abstract

Menopause is the last step in the reproductive history of a woman. The ovaries stop producing hormones and the body reacts by lowering its functions, including the cognitive one. Phytoestrogens are plant products with the estrogen-like activity which are able to mimic many of estrogen's functions. The aim of the present experiment was to study the effects of 30 days of regular consumption of soy-enriched bread containing a known amount of phytoestrogens (genistein and daidzein) in climacteric or menopausal women. Thirty women at different stages of menopause (climacteric, within 5 years of menopause, more than 5 years of menopause) were asked to include 200 g/die of bread containing 40 mg of phytoestrogens in their diet. The effect of the regular consumption of this bread on common menopausal symptoms and cognitive parameters was determined before and after 30 days through questionnaires and experimental tests. Phytoestrogens were measured in the urine. Twenty-five women completed the study. Independence of the menopause stage, there was a significant increase of phytoestrogens in the urine and a decrease of the classical symptoms (*i.e.*, hot flushes). Moreover, the women showed a significant improvement in attentional performance tests, the quality of life index and pain intensity. Phytoestrogens would be an important supplement in aging women due to their ability to induce estrogen-like effects without the potential side effects of estrogens. Their presence in soy-enriched bread, a food commonly present in meals, avoids consideration of their consumption as a drug.

Keywords

Menopause, Women, Phytoestrogens, Bread, Soy

1. Introduction

Menopause is the physiological condition during which women experience an abrupt change in their body aspects and functions due to the rapid decline of gonadal hormones, in particular estrogens. Until a few years ago, these hormones were considered mainly related to the reproductive activity; they were not adequately considered with respect to functions such as cognition, circulation, digestion and many others not strictly related to reproduction but of crucial importance for health, particularly during aging [1]. The important involvement of gonadal hormones in these functions is confirmed by the observation that they are significantly affected by menopause [2]. For instance, after menopause, the decrease of estrogens impairs cognitive functions (with loss of memory and attentional capacity), triggers the uncomfortable symptoms of hot flushes, night sweats, sleep disturbances and vaginal dryness, and increases the incidence of cardiovascular and metabolic diseases [3] [4] [5]. Most of these conditions have been treated with drugs without consideration of the possible hormone dependence and subsequently with the use of hormone replacement therapy (HRT) [6]. The current lack of interest in HRT [7] [8] [9] could be due to the results of the Women's Health Initiative (WHI) trial indicating the possibility that long-term HRT would increase the risks of stroke and venous thromboembolism [10]. Therapies based on phytoestrogens are supposed to represent a promising alternative to HRT [11] [12], although there is still no definitive conclusion on this point [12]. Indeed, the role played by phytoestrogens to mimic many of the estrogen-related functions is now widely accepted [13] [14]. These molecules, present in numerous plants and structurally and functionally similar to estrogens, are known to modulate several body functions [15]. Some epidemiological studies suggest that dietary intake of phytoestrogens can contribute to the decreased incidence of postmenopausal cardiovascular disease [16] and that they are significantly more effective than placebo in reducing the frequency of hot flushes [17]. A recently published meta-analysis in non-Asian postmenopausal women suggested that soy isoflavone supplementation could reduce body weight and improve glucose metabolism [18].

Isoflavones, lignans and coumestans are the most extensively studied phytoestrogen groups. Isoflavones are present in various edible plants, being most abundant in soy [19] [20]. Their estrogenic activity is enhanced after metabolism to more active compounds such as genistein and daidzein by gut microbiota [21]. Once absorbed, genistein and daidzein undergo metabolic changes in the liver to be eliminated easily by the biliary tract and then reabsorbed, entering an enterohepatic cycle; significant quantities are then eliminated in the urine [22]. In people who consume soybean, blood levels of genistein and daidzein are higher than endogenous estrogens [23]. These molecules have a marked estrogenic activity, albeit 1000 times less than endogenous estrogen, and a maximum half-life of 24 hours, so daily intake is necessary to induce the positive effects and to ensure a constant level of phytoestrogens in the body [24].

Phytoestrogens bind to the Estrogen Receptor (ER) to carry out estrogenic and/or antiestrogenic activities [25] [26], with preferential affinity for ERβ [27]. Phytoestrogens have been studied for their possible involvement in the prevention and/or treatment of a variety of pathological conditions, such as cancer, metabolic and cardiovascular diseases, neurodegeneration, inflammation and osteoporosis [27] [28]. The biological activity of isoflavones on ERs seems to depend on the level of endogenous estrogens, since at high levels of endogenous estrogens the isoflavones exert antagonistic activity while at low levels they act as ER agonists [29]. Thus, in climacteric or menopausal women, their action as agonistic compounds would be beneficial.

The aim of the present study was to test the possibility that menopausal women consuming a correct amount of phytoestrogens (40 mg/die) with bread every day would show signs of phytoestrogen-related beneficial effects. This amount is based on important clinical trials suggesting that ≥40 - 90 mg isoflavones/die is necessary to produce physiological effects *in vivo*. The effect on hot flushes, mood, general health, cognitive abilities and antioxidant profile are reported.

2. Methods

2.1. Subjects

Thirty healthy women recruited in the general population were asked to participate in the study. The inclusion criteria were as follows: presence of climacteric or menopausal status, no metabolic disorders, signing of the informed consent form. The exclusion criteria were: hormone replacement therapy, professionally practicing sports, undergoing body mass reduction, any special diet (including regular use of soy products). Experimental procedures were carried out in agreement with the Code of Ethics of the World Medical Association (Helsinki Declaration). All participants gave their informed consent in writing before participation.

2.2. Experimental Procedure

The study was organized in two phases: TEST 1 (Baseline) and TEST 2 (30 days later). This duration was chosen on the basis of common diet periods in which 4 weeks are considered sufficient to detect possible effects.

During TEST 1, all subjects met a trained researcher to provide general data, to fill in questionnaires, to undergo tests and to give biological samples. After TEST 1, all participants were asked to include in their meals 200 g/die of soybean-enriched bread (Pariv Srl, Siena, Italy). No other dietary suggestions were given and the women had to continue their normal feeding and physical exercise habits. After 30 days, TEST 2 was carried out during which all the women were asked to repeat the measurement procedure of TEST 1.

The following experimental parameters were collected for each subject during TEST 1 and TEST 2:

- Anthropometric measures and Bioelectrical Impedance Analysis (BIA).
- Quality of life state by means of questionnaires:

- *Ad hoc* questionnaire created to assess menopause-related symptoms:
  - Number of hot flushes per day;
  - Intensity of hot flushes (0 - 10);
  - Decrease in sexual desire (0 - 10);
  - Quality of night sleep (0 - 10);
- Profile of Mood States, POMS;
- Short Form (36) Health Survey, SF-36.
- Pain questionnaires:
  - Visual Analogue Scale, VAS;
  - Italian Pain Questionnaire, QUID;
  - Present Pain Intensity, PPI.

➢ Zimmerman and Fimm's Test of Attentional Performance. It is a cognitive computer test assessing several aspects of attentional control: vigilance, sustained attention, working memory and response inhibition (go/no-go);

➢ Urine to measure daidzein, genistein and creatinine (as normalized factor);

➢ Blood to measure antioxidant parameters: thiols and disulfides:

- Red blood cells, RBC;
- Plasma;

➢ Saliva to measure cortisol and testosterone.

2.2.1. Anthropometric Measures and Bioelectrical Impedance Analysis (BIA)

Weight and height measurements were used to calculate the Body Mass Index (BMI). Bioelectrical Impedance Analysis (BIA) (Akern Srl, Firenze, Italy) is a commonly used method to estimate body composition [30]. The following parameters were considered: Total Body Water (TBW), Extracellular Water (ECW), Fat Mass (FM), Fat Free Mass (FFM) [31].

2.2.2. Quality of Life State Questionnaires

1) *Ad hoc* Questionnaire Created to Assess Menopause-Related Symptoms. The questionnaire consists in: a) one question about the mean number of hot flushes experienced per day in the last week; b) three scales consisting of three horizontal lines (10-point scale, 10 cm long) bounded at the ends by "0: no effect" and "10: maximum effect". These scales were used to estimate the intensity of hot flushes, the decrease in sexual desire and the quality of night sleep in the last week.

2) Profile of Mood States (POMS). POMS, consisting of 58 items rated on a 5-point scale, measures the current psychological state of the subject [32] [33]. It comprises six subscales: Tension-Anxiety (T-A), Depression-Dejection (D-D), Anger-Hostility (A-H), Vigor-Activity (V-A), Fatigue-Inertia (F-I) and Confusion-Bewilderment (C-B). In each subscale, values higher (T-A, D-D, A-H, F-I, C-B) or lower (V-A) than 55 were considered significantly altered with respect to the normal population [34].

3) Short Form (36) Health Survey (SF-36). The Italian version of the SF-36

questionnaire [35] is a generic multidimensional instrument for assessing quality of life. It consists of 36 items grouped into two components (PCS-36, MCS-36) and divided into eight scales: the first four scales, Physical Functioning (PF), Role Physical (RP), Bodily Pain (BP) and General Health (GH), are included in the Physical Component Summary (PCS-36); the other four, Vitality (V), Social Functioning (SF), Role Emotional (RE) and Mental Health (MH), are included in the Mental Component Summary (MCS-36). Individual items are scored on a 0 - 100 standardized Likert scale. For each scale, a *higher* score indicates a better quality of life and lower limitations.

4) Pain Questionnaires:

a) Visual Analogue Scale (VAS). VAS (0 - 10) was used to estimate the average pain intensity suffered during the *previous week* at three times of the day. VAS is a 10 cm horizontal line bounded at the ends by "no pain" (0) and "worst pain possible" (10) [36].

b) Italian Pain Questionnaire (QUID). QUID is a reconstructed Italian version of the McGill Pain Questionnaire used to determine the quality and intensity of the current pain experience [37]. It is a semantic interval scale consisting of 42 descriptors divided into four main classes: sensory (S), affective (A), evaluative (E), miscellaneous (M). All the ranks are added to obtain the Pain Rating Index Rank-Total (PRIr-T).

c) Present Pain Intensity (PPI) consists of a 6-point scale (0 - 5). The subject has to indicate her current pain: 0 (absent), 1 (mild), 2 (moderate), 3 (strong), 4 (very strong), 5 (terrible).

2.2.3. Zimmerman and Fimm's Test of Attentional Performance

This test evaluates the sustained attention of the subject [38]. The mean reaction time (mRT = time in milliseconds from stimulus to response) and the number of correct responses as percentage of total responses (% CR) during the test (accuracy) were determined to provide a combined estimate of the subject's performance. Each subject was seated in a comfortable reclining chair in front of the computer screen at a distance of about one meter, with the fingers of the dominant hand on a button on a modified computer keyboard (SuperLab Pro, Cedrus Corporation, USA). Figures were presented for 15 minutes on the computer screen that could be different or the same in shape, color (red, green, blue) and size (small, medium, large); the subject had to respond by pressing the button only if the figure that appeared was equal to the previous one in form or color or size. The test included 150 trials: each trial, lasting 6 seconds, consisted of a preparatory stimulus (a sound), followed after two seconds by the imperative stimulus, and then a 4-second latency period before the next preparatory stimulus.

2.2.4. Analysis of Genistein, Daidzein and Creatinine in the Urine

Genistein and daidzein were determined in the first-void urine together with createnine used to normalize values. Two hundred microliters (0.2 ml) of urine were incubated overnight with 0.55 ml of 0.17 M ammonium acetate buffer pH 4.6 and 50

µl glucuronidase (20,000 U/ml). Samples were then extracted twice with 0.5 ml diethyl ether and the pooled extracts were dried with a CentriVap centrifugal vacuum concentrator (Labconco), 60 min, 60˚C [39]. The resulting pellets were resuspended in 50 µl of 80% (v/v) methanol followed by acidification with 3.5 µl of 60% (w/v) trichloroacetic acid. Finally, samples were centrifuged at 10,000 xg for 2 min and the supernatants were analyzed by HPLC. HPLC separation was performed on a C18 column (Zorbax Eclipse XDB-C18) thermostated at 25˚C. Detection was performed at 247 nm wavelength for daidzein and 259 nm for genistein. An Agilent series 1100 HPLC (Agilent Technologies, Milan, Italy) equipped with diode array and a fluorimetric detector was used for all determinations [40].

Analysis of creatinine was carried out on urine samples according to the Jaffe reaction [41].

2.2.5 Measurement of Thiols and Disulfides in the Blood: Red Blood Cells (RBC) and Plasma

About 2 ml of blood were collected in the morning (10:00 hr to 11:00 hr) from the antecubital vein into tubes containing ethylenediaminetetraacetic acid and 1 ml was immediately transferred into microfuge tubes containing 100 µl of 310 mM N-ethylmaleimide (NEM) for analyses in RBC and for disulfide analyses in plasma as previously described [42]. Intra-erythrocytic glutathione (GSH) and glutathione disulfide (GSSG) levels were measured in the clear supernatant. The rest of the blood was centrifuged at 10,000 xg for 30 s to obtain plasma. Both low molecular mass thiols (LMM-SH) and protein thiols (P-SH) were measured in fresh plasma by HPLC and spectrophotometry, respectively. Conversely, low molecular mass disulfides (LMM-SS) and S-thiolated proteins (RSSP) were analyzed in plasma samples obtained from blood treated with NEM. The P-SH were quantified by colorimetric reaction with Ellman's reagent [43]. LMM-SH, LMM-SS and RSSP were measured by fluorometric HPLC [44]. The Protein Thiolation Index (PTI) was calculated as the ratio between *S*-thiolated proteins and P-SH groups in plasma [45].

2.2.6. Cortisol and Testosterone Determinations in Saliva

Saliva samples were collected using the Salivette collection device (Sarstedt Inc., Numbrecht, Germany) [46] as previously described [47]. For cortisol, the kit was based on competitive binding and the sensitivity was 0.049 ng/ml, the intra-assay variation was less than 8% and the inter-assay variation was less than 10%. For testosterone, the kit was based on the quantitative sandwich ELISA method and the sensitivity was 25 pmol/L, the intra-assay and inter-assay variations were less than 15% for both.

2.3. Soybean-Enriched Bread

A well-known local bakery (Pariv Srl, Sinalunga, Siena, Italy) agreed to produce the soy-enriched bread and to supply all experimental subjects with one piece (200 g) of the bread every day. This particular bread contains 20 mg/100 g of phytoestrogens derived from yellow soy as described by Ricci and Aloisi [48].

2.4. Statistical Analysis

Comparisons of data were carried out by analysis of variance (ANOVA) with the factors Group (3 levels: Group 1, Group 2, Group 3) and Test (2 levels: Test 1, Test 2) [49]. Data are presented as mean ± SEM. All analyses were performed with Statistica® software. A level of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Bread Composition

The bromatological composition of the bread, the energetic value and the content of phytoestrogens (genistein and daidzein) are reported in Table 1.

Table 1. Bromatological composition of the soybean-enriched bread per 100 g of the product. Phytoestrogens content (mean ± SEM) and energetic value. Abbreviations: DA (Daidzein), GI (Genistein).

Components (100 g)	
Proteins (%)	13.6%
Lipids (%)	4.9%
Carbohydrates (%)	34.9%
Fiber (%)	7.1%
Phytoestrogens (mean ± SEM)	
➢ **DA** (mg)	10.7 ± 1.17
➢ **GI** (mg)	14.3 ± 1.84
Energy (kCal)	251.68

3.2. Study Subjects

Out of the 30 women enrolled, 25 completed the study. They all reported having followed the indication to eat 200 g of bread per day. For different reasons, it was not possible to collect blood and saliva in 12 of them, as reported in Table 2.

For the measures in which all subject data were available, three groups were formed based on reproductive status, but independent of age:

- Group 1: Women with menses alteration due to the climacteric phase (N = 8);
- Group 2: Women with absence of menses for 1 to 5 years (N = 9);
- Group 3: Women with absence of menses for >5 years (N = 8).

Analysis applied to BMI and BIA data (Table S1) showed no differences among groups or between tests.

Table 2. Summary of data. Experimental groups: Group 1 (n = 8): Climacteric (C, climacteric phase); Group 2 (n = 9): Menopause 0-5 (M 0 - 5, absence of menses for 1 to 5 years); Group 3 (n = 8): Menopause > 5 (M > 5, absence of menses for >5 years). Abbreviations: BMI (Body Mass Index), N° HF (Number of hot flushes/day), DA (Daidzein), GI (Genistein), mRT (mean Reaction Time), CR (correct responses), PRIr-T (Pain Rating Index rank-Total), T1 (Test 1), T2 (Test 2), S (Subject), Yrs (years), na (not applicable). #: Subjects without blood and saliva collection.

Subjects	Age (Yrs)	Groups	BMI (Kg/m²)		N° HF		Phytoestrogens (nmol/ml)		mRT (msec)		% CR		PRIr-T	
			T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
S1	45	C	23.3	23.8	0	0	DA: 0 GI: 0.21	DA: 9.88 GI: 5.93	833.94	980.35	83	84	5	3

Continued

S2	49	C	30	30.6	0	0	DA: 0.11 GI: 0.54	DA: 4.21 GI: 4.21	705.21	596.73	82	88	10	0
S3	55	C	25.2	25.4	0	0	DA: 1.25 GI: 0.94	DA: 23.10 GI: 15.50	841.66	762.69	88	90	0	2
S4	50	C	21.6	21.8	9	8	DA: 0.31 GI: 1.79	DA: 1.30 GI: 1.37	1054.87	985.91	63	72	6	4
S5[#]	49	C	18	17.9	5	2	DA: 1.91 GI: 5.08	DA: na GI: na	966.89	841.75	86	78	0	0
S6[#]	51	C	20.1	20.6	1	1	DA: 32.50 GI: 34.00	DA: 82.30 GI: 47.40	922.07	923.91	85	78	5	4
S7[#]	51	C	33.8	33.7	3	0	DA: 1.50 GI: 4.15	DA: 21.70 GI: 22.80	1061.25	972.73	83	84	8	6
S8[#]	54	C	19.4	19.4	0	1	DA: 5.11 GI: 5.18	DA: 6.24 GI: 6.55	872.02	746.56	83	87	0	0
S9	56	M 0 - 5	21.5	21.6	1	2	DA: 0.48 GI: 1.24	DA: 3.45 GI: 5.36	912.57	990.08	58	72	18	7
S10	53	M 0 - 5	25.7	25.2	1	0	DA: 0.51 GI: 0.84	DA: 15.40 GI: 4.88	755.82	729.24	86	90	0	5
S11[#]	55	M 0 - 5	24.2	24.4	6	4	DA: 0 GI: 1.04	DA: 20.60 GI: 14.60	729.43	621.14	85	87	26	9
S12	58	M 0 - 5	19.8	20	6	4	DA: 0 GI: 0.34	DA: 6.88 GI: 7.60	705.61	624.96	89	90	10	8
S13[#]	51	M 0 - 5	23.5	23.7	2	2	DA: 35.90 GI: 12.70	DA: 55.90 GI: 58.60	926.78	887.13	79	84	0	0
S14[#]	56	M 0 - 5	21.8	21.6	3	3	DA: 6.99 GI: 2.85	DA:106.00 GI: 111.00	738.6	600.86	89	89	0	0
S15[#]	59	M 0 - 5	27.5	27.4	2	1	DA: 0 GI: 0.84	DA: 23.50 GI: 24.60	724.44	716.67	89	89	30	0
S16[#]	55	M 0 - 5	32.7	31.8	1	1	DA: 0.84 GI: 1.21	DA: 1.66 GI: 1.74	804.95	659.38	77	77	23	36
S17	53	M 0 - 5	20.6	22.1	2	na	DA: 2.68 GI: 0.66	DA: 7.77 GI: 0.98	1044.72	1043.55	77	88	13	15
S18	58	M > 5	25.2	25.3	6	4	DA: 2.29 GI: 0.29	DA: 7.81 GI: 5.33	996.56	911.42	73	84	29	7
S19	66	M > 5	22.9	23.2	0	0	DA: 0 GI: 0	DA: 41.60 GI: 3.31	1036.17	1020.18	84	89	0	3
S20	64	M > 5	24.4	24.4	0	0	DA: 0.22 GI: 0.61	DA: 0.14 GI: 0.68	1148.98	1073.94	83	85	9	0
S21	63	M > 5	27.5	25.6	3	3	DA: 1.48 GI: 0	DA: 3.43 GI: 0.45	na	na	na	na	4	8
S22[#]	54	M > 5	30	31	2	0	DA: 1.32 GI: 1.44	DA: 62.00 GI: 65.10	na	na	na	na	0	0
S23[#]	59	M > 5	22	22.2	4	4	DA: 0.68 GI: 0.96	DA:18.00 GI: 18.90	709.9	659.5	88	89	0	0
S24[#]	67	M > 5	24.7	25.8	3	1	DA: 1.88 GI: 3.07	DA: 26.60 GI: 27.90	1174.68	1070.57	81	79	6	0
S25	54	M > 5	27.1	27.4	6	6	DA: 0.95 GI: 1.86	DA: 4.39 GI: 3.94	1182.57	1174.82	81	89	20	6

3.3. Menopause-Related Questionnaires

As reported in Table 2 and Figure 1, a significant decrease was recorded in the number of Hot Flushes (HF) from Test 1 to Test 2 independently of the group (Test: $F(1, 21) = 8.19$, $p < 0.01$). No significant changes were found in the other measures.

The number of HF was correlated with the other quality of life measures (HF intensity: $R = 0.83$, decrease in sexual desire: $R = 0.52$; sleep quality: $R = -0.45$, all $p < 0.01$) and body weight ($R = -0.30$, $p = 0.05$).

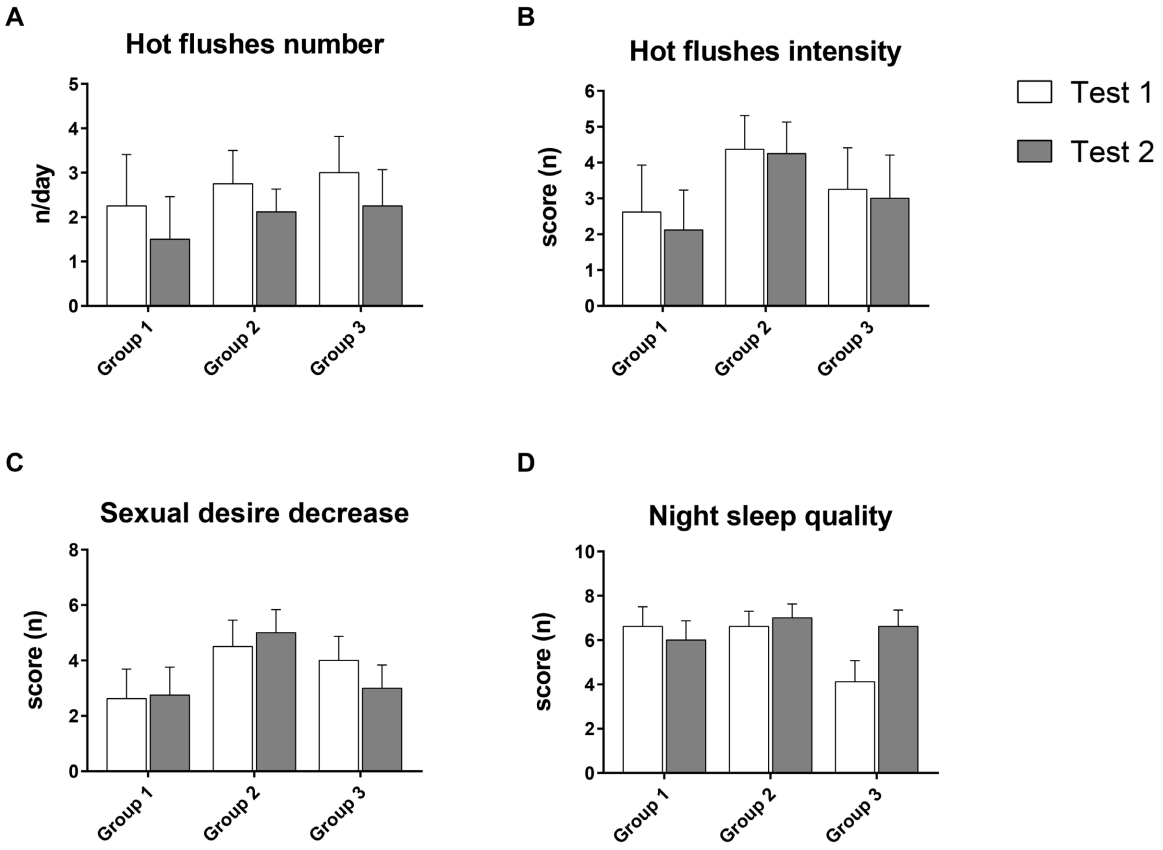

Figure 1. Number of hot flushes (A), intensity of hot flushes (B), decrease in sexual desire (C) and night sleep quality (D) in the three groups (Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years). Two phases: TEST 1 (Baseline) and TEST 2 (30 days later).

3.4. POMS

Most of the POMS values obtained during Test 1 were outside the normal range (lower or higher than 55, as shown in Table 3) suggesting some forms of discomfort in most of the tested women. ANOVA carried out with the factors Test and Group showed a significant *decrease* (improvement) of the following subscales independent of the group: Tension-Anxiety ($F(1, 22) = 6.9$, $p < 0.05$), Depression-Dejection ($F(1, 22) = 8.45$, $p < 0.01$), Fatigue-Inertia ($F(1, 22) = 9.01$, $p < 0.01$), Confusion-Bewilderment ($F(1, 22) = 18.33$, $p < 0.001$).

Depression-Dejection was correlated with BMI ($R = 0.31$, $p = 0.04$). Moreover,

as reported in Table S2, many POMS subscales were correlated with pain measures; all these correlations were positive except Vigor-Activity, suggesting a strong interaction between pain and mood factors.

Table 3. Profile of Mood States (POMS) determined in the three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later). Abbreviations: T-A (Tension-Anxiety), D-D (Depression-Dejection), A-H (Anger-Hostility), V-A (Vigor-Activity), F-I (Fatigue-Inertia), C-B (Confusion-Bewilderment). ANOVA significance of the factor Test. *p < 0.05, **p < 0.01, ***p < 0.001 Test 2 vs Test 1. Values are reported as Mean ± SEM.

		POMS T-A*	POMS D-D**	POMS A-H	POMS V-A	POMS F-I**	POMS C-B***
Group 1	*Test 1*	54.12 ± 3.34	58.12 ± 4.68	54.25 ± 4.68	48.50 ± 2.87	62.50 ± 5.75	60.00 ± 4.05
	Test 2	50.12 ± 3.27	49.12 ± 3.67	51.50 ± 4.77	53.75 ± 3.34	56.50 ± 4.27	50.37 ± 2.84
Group 2	*Test 1*	57.22 ± 3.63	58.55 ± 4.71	60.67 ± 4.52	52.44 ± 2.52	62.22 ± 4.75	58.22 ± 4.02
	Test 2	53.44 ± 4.09	55.44 ± 4.79	56.89 ± 4.95	51.11 ± 2.25	57.89 ± 5.66	53.33 ± 4.52
Group 3	*Test 1*	57.62 ± 5.81	58.00 ± 5.81	59.37 ± 5.14	51.75 ± 4.46	63.87 ± 5.67	60.75 ± 4.77
	Test 2	51.12 ± 3.58	49.87 ± 3.03	53.00 ± 2.42	54.50 ± 3.72	56.75 ± 3.98	48.37 ± 3.34

3.5. SF-36

For the cumulative PCS-36 value (Figure 2 and Figure S1), treatment induced a significant increase (improvement) from Test 1 to Test 2 ($F(1, 22) = 8.58$, $p < 0.01$): for the single components, significance was found in physical functioning (PF, $F(1, 22) = 6.3$, $p < 0.05$), role physical (RP, $F(1, 22) = 4.6$, $p < 0.05$) and bodily pain (BP, $F(1, 22) = 4.54$, $p < 0.05$); there was no significant difference in General Health (GH). For the cumulative MCS-36 (Figure 2 and Figure S1), treatment induced a significance increase from Test 1 to Test 2 ($F(1, 22) = 7.59$, $p < 0.01$): for the single components, significance was found in social functioning (SF, $F(1, 22) = 9.45$, $p < 0.01$) and role emotional (RE, $F(1, 22) = 5.48$, $p < 0.05$).

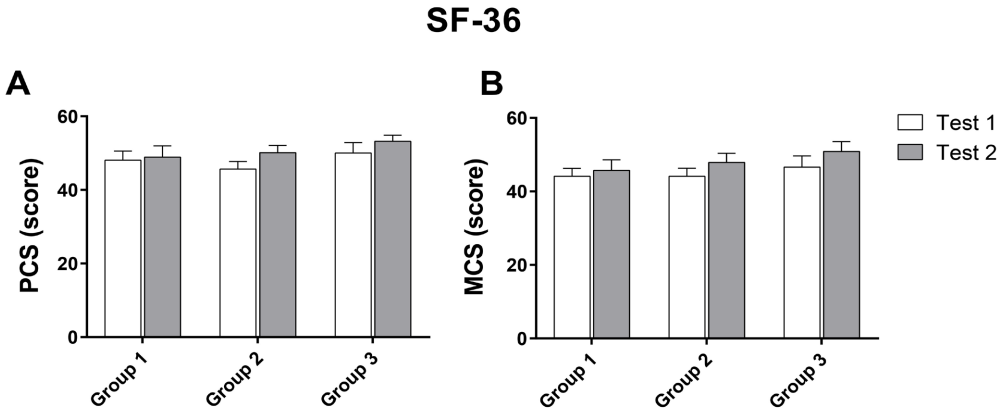

Figure 2. SF-36. Cumulative values of the Physical Component Summary (PCS-36) (A) and Mental Component Summary (MCS-36) (B). Three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years; n = 25. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later).

Bodily pain was found to be negatively correlated with HF number and intensity (n = 42, R = −0.51, R = −0.39, $p < 0.001$ and $p < 0.01$ respectively); it must be considered that a higher BP score means a better condition. Moreover, as reported in Table S3 PCS and its single components were correlated with PRTI-T, PPI and all three VAS scales (morning, afternoon, night), while MCS and its subscales were correlated with PRI-T and PPI.

3.6. Pain Evaluation. VAS and QUID

Pain was present in most of the subjects, as reported in Table 2 (PRi-T), and in some of them it was quite high (>5). VAS was considered separately in the morning, afternoon and night. The morning VAS decreased from Test 1 to Test 2, as shown by significance of the factor Test ($F(1, 22) = 8.4$, $p < 0.01$). No changes were found in the other determinations (Table 4).

VAS at night was found to be positively correlated with HF number and intensity (n = 42, R = 0.53, R = 0.38, $p < 0.001$ and $p < 0.01$ respectively).

ANOVA applied to the QUID sensorial (s) component revealed a significant effect of the factor Test ($F(1, 22) = 7.14$, $p < 0.01$) due to the decrease from Test 1 to Test 2 (Table 4). For the PRIr-T, there was significance of Test ($F(1, 22) = 4.25$, $p < 0.05$) due to the higher levels in Test 1 than Test 2. No significant changes were recorded in the other QUID components and PPI.

PRI-T was negatively correlated with daidzein and genistein urinary levels (n = 42, R = −0.31, R = −0.29, $p < 0.04$ and $p < 0.05$ respectively). Thus the increase in the urine of phytoestrogens induced an improvement in the QUID, the main effect being attributed to the sensorial component.

Table 4. Pain parameters determined in the three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later). Visual Analogue Scale (VAS) and Italian Pain Questionnaire (QUID), abbreviations: mo (morning), a (afternoon), n (night), s (sensorial), a (affective), e (emotional), m (miscellaneous), PRIr-T (Pain Rating Index rank-Total), PPI (Present Pain Intensity, 0 - 5). ANOVA significance of the factor Test *p < 0.05, **p < 0.01 Test 2 vs Test 1. Values are reported as Mean ± SEM.

VAS		VAS mo**	VAS a	VAS n			
Group 1	*Test 1*	3.63 ± 1.19	2.88 ± 0.97	2.63 ± 1.07			
	Test 2	2.25 ± 0.96	1.25 ± 0.65	1.75 ± 0.90			
Group 2	*Test 1*	3.89 ± 1.11	2.67 ± 0.98	1.67 ± 0.80			
	Test 2	3.00 ± 0.82	2.56 ± 0.75	2.22 ± 0.80			
Group 3	*Test 1*	3.75 ± 1.22	3.25 ± 1.31	1.75 ± 1.16			
	Test 2	1.00 ± 0.47	1.00 ± 0.50	0.88 ± 0.44			
QUID		**QUIDs****	**QUIDa**	**QUIDe**	**QUIDm**	**PRIr-T***	**PPI**
Group 1	*Test 1*	2.50 ± 0.78	0.00 ± 0.00	1.63 ± 0.53	0.13 ± 0.13	4.25 ± 1.37	1.00 ± 0.27
	Test 2	1.00 ± 0.33	0.13 ± 0.13	1.25 ± 0.56	0.00 ± 0.00	2.38 ± 0.80	1.00 ± 0.38
Group 2	*Test 1*	5.89 ± 1.59	2.55 ± 1.08	2.89 ± 1.05	2.00 ± 1.01	13.33 ± 3.91	1.56 ± 0.44
	Test 2	4.33 ± 1.53	1.67 ± 1.20	1.67 ± 0.60	1.22 ± 1.10	8.89 ± 0.78	1.11 ± 0.31
Group 3	*Test 1*	3.38 ± 1.38	2.50 ± 1.51	1.88 ± 0.55	0.75 ± 0.53	8.50 ± 3.77	1.13 ± 0.35
	Test 2	1.38 ± 0.60	0.63 ± 0.46	0.75 ± 0.62	0.25 ± 0.40	3.00 ± 1.24	0.88 ± 0.19

3.7. Test of Attentional Performance

ANOVA applied to **mean reaction time** values (mRT, Figure 3(A)) revealed significance of the factor Test ($F(1, 20) = 12.43$, $p < 0.01$) due to the shorter reaction time in Test 2 than in Test 1 present in all groups. The significance of Group ($F(2, 20) = 4.24$, $p < 0.02$) was due to Group 3 showing longer mRT than Group 2. Moreover, when the test was divided into 3 parts (Figure S2): first 5 min, second 5 min, third 5 min, Group 3 showed longer mRT than Groups 1 and 2 ($p < 0.05$ for all) in the first and third parts. Groups 1 and 2 never differed.

mRT was found to be correlated with age ($n = 42$, $R = 0.41$, $p = 0.006$) and quality of night sleep ($R = -0.39$, $p < 0.01$).

For the **number of correct responses** expressed as percentage of total responses **(% CR)** (Figure 3(B)), the significance of Test ($F(1, 20) = 7.5$, $p < 0.01$) indicates that the performance was significantly improved from Test 1 to Test 2 independently of the group of women considered.

The number of correct responses was correlated with the quality of night sleep, SF-36 subscales (physical activity, general health and vitality), POMS T and D (Table S4 and Table S5 in **Supplementary Files**). This underlines the importance of sleep quality, exercise and mood state in the execution of the trial.

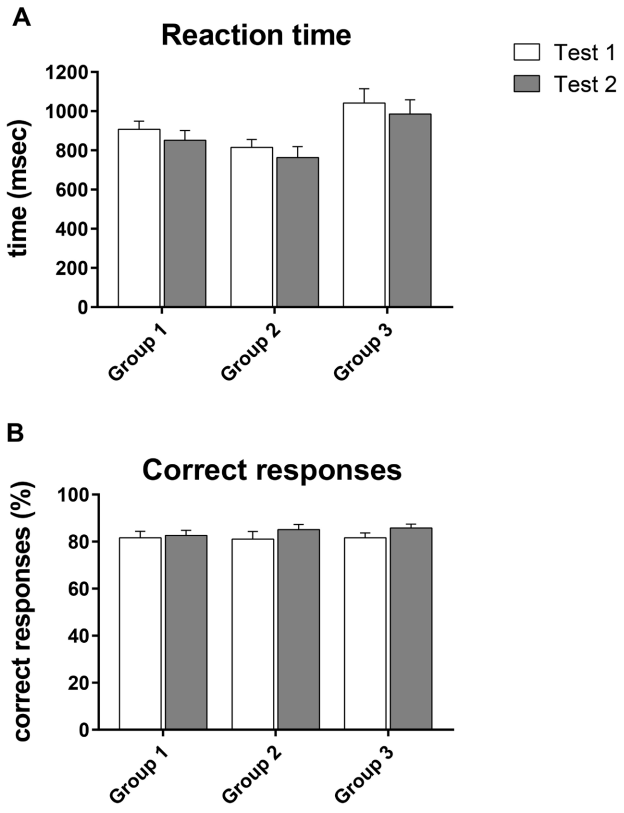

Figure 3. Test of attentional performance. Mean value of the reaction time in milliseconds (msec) (A) and number of correct responses as a percentage (%) (B). Three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years. Group 3 (n = 8): absence of menses for >5 years; n = 25. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later).

3.8. Daidzein and Genistein Levels in Urine

Daidzein and genistein were measured in urine before (Test 1) and after the consumption of soy-enriched bread for 30 days (Test 2). The measured values normalized for creatinine content are reported in Figure 4. For both daidzein and genistein, ANOVA revealed a significant effect of Test ($F(1, 21) = 14.08$, $p = 0.001$, $F(1, 21) = 8.65$, $p < 0.01$ respectively) due to the increase in urinary levels of both compounds from Test 1 to Test 2 in all groups. Daidzein as well as genistein were negatively correlated with pain measures (Table S6 in **Supplementary files**).

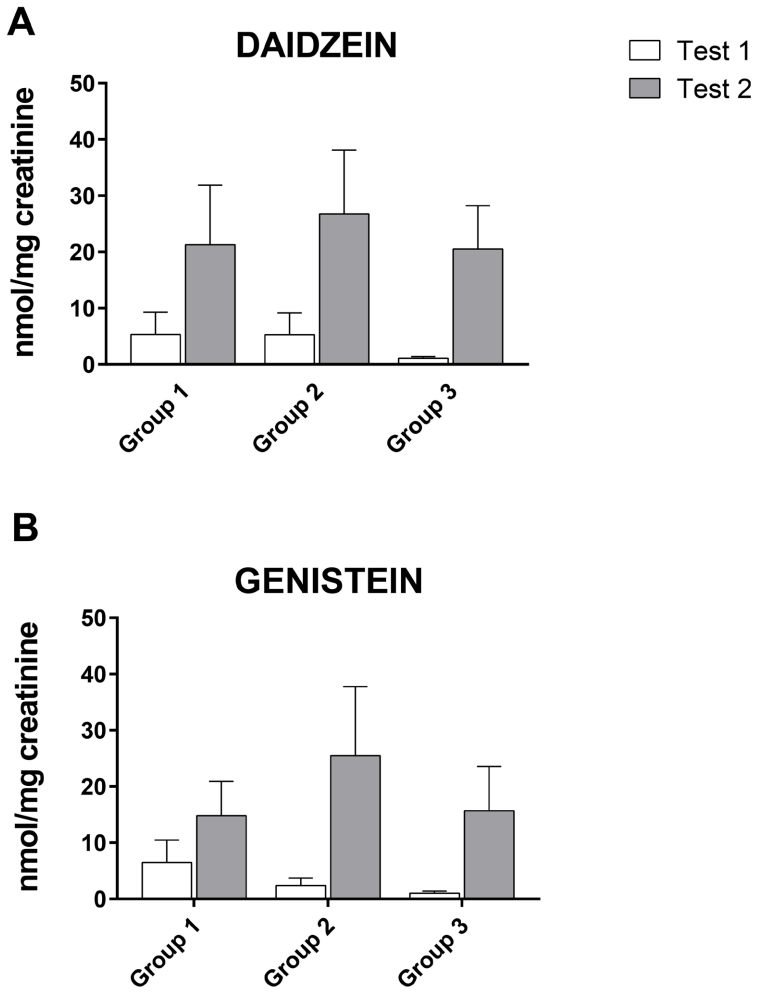

Figure 4. Urinary levels of daidzein (nmol/mg) (A) and genistein (nmol/mg) (B). The first-void urine of the women enrolled in the study was analyzed for the contents of glucuronidated daidzein and genistein by means of HPLC; values were normalized with creatinine. Three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years. Group 3 (n = 8): absence of menses for >5 years; n = 25. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later).

3.9. Thiols and Disulfides in Red Blood Cells and Plasma

The potential effects of the soy-enriched bread on the antioxidant pattern were

evaluated by measuring the levels of thiols and their ratio with disulfides in both Red Blood Cells (RBC) and plasma. Since not all women were tested for these parameters, the analysis did not consider the factor Group. The RBC content of GSH and GSSG was in the normal range and was not influenced by consumption of the functional bread (Table 5). Consequently, the GSH/GSSG ratio was also unvaried.

Table 5. Glutathione (GSH) and glutathione disulfide (GSSG) levels in red blood cells (RBC). Two phases: TEST 1 (Baseline) and TEST 2 (30 days later). Women Test 1, n = 12; Test 2 =, n = 11. Values are reported as Mean ± SEM.

	GSH (nmol/mg Hb)	**GSSG** (nmol/mg Hb)	**GSH/GSSG**
Test 1 (*N = 12*)	5.52 ± 0.26	0.011 ± 0.001	586.67 ± 68.74
Test 2 (*N = 11*)	6.08 ± 0.34	0.012 ± 0.001	546.45 ± 59.86

In plasma there was a significant increase of thiols and LMM-SH, as reported in detail in Table 6.

Table 6. Plasma thiols and disulfides determination. Abbreviations: Cys (cysteine), CysGly (cysteinylglycine), Hcys (homocysteine), γ-GluCys (γ-glutamylcysteine), GSH (glutathione), P-SH (protein sulfhydryl groups), LMM-SS (low molecular weight disulfide), CySS (cystine), CySSGly (cystinylglycine), HcySS (homocysteine), γ-GluCySS (γ-glutamylcystine), GSSG (glutathione disulfide), RSSP (S-thiolated proteins), CySSP (protein mixed disulfides with cysteine), CyGlySSP (protein mixed disulfides with cysteinylglycine), HcySSP (protein mixed disulfides with homocysteine), γ-GluCySSP (protein mixed disulfides with γ-glutamylcysteine), GSSP (protein mixed disulfides with glutathione), PTI (Protein Thiolation Index). Two phases: TEST 1 (Baseline) and TEST 2 (30 days later). Women Test 1 n = 12, Test 2 n = 11. ANOVA significance of the factor Test *p < 0.05 and **p < 0.01, Test 2 vs Test 1. Values are reported as Mean ± SEM.

Reduced Thiols	**P-SH**	**Cys**	**CysGly**	**Hcys**	**γ-GluCys**	**GSH**
Test 1	401.17 ± 8.13	9.59 ± 0.60	1.24 ± 0.11	0.11 ± 0.01	0.04 ± 0.003	1.66 ± 0.24
Test 2	437.73 ± 8.30*	11.3 ± 0.58*	1.89 ± 0.23*	0.17 ± 0.01*	0.05 ± 0.004	2.00 ± 0.16
LMM-SS	**CySS**	**CySSGly**	**HcySS**	**γ-GluCySS**	**GSSG**	
Test 1	61.40 ± 2.84	5.77 ± 0.27	0.99 ± 0.11	0.67 ± 0.03	0.84 ± 0.10	
Test 2	90.27 ± 6.63**	6.48 ± 0.30*	1.13 ± 0.14	0.90 ± 0.06**	0.89 ± 0.07	
RSSP	**CySSP**	**CyGlySSP**	**HcySSP**	**γ-GluCySSP**	**GSSP**	**PTI**
Test 1	127.52 ± 7.19	12.01 ± 0.9	4.92 ± 0.60	1.41 ± 0.12	2.11 ± 0.18	0.38 ± 0.02
Test 2	138.04 ± 8.91	11.57 ± 0.55	4.99 ± 0.45	1.51 ± 0.11	2.45 ± 0.14	0.36 ± 0.02

3.10. Hormones in Saliva

Due to the low number of samples (Table 2), the factor Group was not considered for these parameters. ANOVA applied to cortisol values revealed a significant increase from Test 1 to Test 2 ($F(1, 9) = 6.34$, $p < 0.03$); there were no significant changes for testosterone (Figure 5).

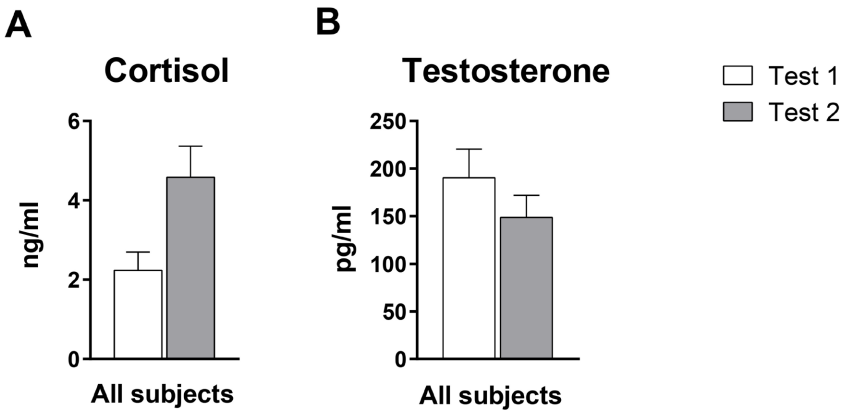

Figure 5. Salivary levels of cortisol (ng/ml) (A) and testosterone (pg/ml) (B). The salivary samples were collected in two phases: TEST 1 (Baseline) and TEST 2 (30 days later). n = 12.

4. Discussion

The main result of the present experiment was the positive change in most of the parameters considered in climacteric/menopausal women after 30 days of consumption of the newly prepared soy-enriched bread, *i.e.*, a very common food but with determined phytoestrogens content. Behavioral, psychological and attentional tests all showed significant improvement in the various parameters such as hot flushes, mood, pain, quality of life and reaction time performance. The involvement of the soy-enriched bread in these changes is strongly suggested by the significant increase in the urinary levels of genistein and daidzein in the tested women.

Soy is an important product commonly used in human and animal feeding because of its high nutrient values. Indeed it is often used to extract proteins or phytoestrogens to be commercialized as supplements [22] [50]-[55]. Phytoestrogens have been proposed to help menopausal women, in which estrogens levels are very low. Thus the study of functional foods with known amounts of phytoestrogens must be considered a serious effort to provide a solution to estrogen depletion.

With the preparation of this functional bread, we considered the possibility to provide phytoestrogens not as an extract, as commonly available in many commercial products, but as whole beans in order to maintain the "natural context" of the compounds essential to their functions. The soy-enriched bread used in the present experiment [48] was prepared to supply 40 mg of phytoestrogens, 28 mg of proteins and low levels of lipids and carbohydrates every day. As expected the presence of isoflavones in the urine clearly increased after 30 days of treatment, suggesting their presence in the circulation and distribution among tissues [56] [57]. Similar phytoestrogens levels have been effective in postmenopausal women, for instance 40 - 60 mg/die were found to improve the cardiovascular system [58], to reduce oxidative DNA damage [59] and to reduce LDL-cholesterol oxidation [60].

Phytoestrogens mimic several actions mediated by ERα and ERβ estrogen receptors [61], albeit with a different ability to induce their activation [62]; indeed,

phytoestrogens display a substantially higher affinity for ERβ [63] which appears to be associated with antiproliferative and anticarcinogenic effects [64] [65], unlike ERα [66]. Daidzein, in particular, can cross the blood-brain barrier and a detectable concentration has been reported in the brain within the first hour of its administration [57] including the hippocampus, striatum, cortex, cerebellum, brainstem and hypothalamus.

We suggest that the changes recorded in the present study, *i.e.*, the decrease in reaction time and the higher percentage of correct responses present in all groups at the second Test of Attentional Performance, are due to the regular consumption of the soy-enriched bread for 30 days. This type of test analyzes the subject's ability to suppress an inadequate response (no-go) and to react in the presence of stimuli activating the paradigm of a go/no-go complex [34]. This ability requires significant interventions by the central nervous system. These changes were not induced by repetition of the test, since 30 days of delay from the first to the second test are enough to cancel the memory of the event [67].

Hot flushes are very common in menopausal women and can impair a woman's quality of life until 7 - 8 years after the climacteric period. They are not present in all women and can be modulated by phytoestrogens [50]. In the present study, the consumption of soy-enriched bread decreased the daily frequency of hot flushes in all groups. This decrease can be attributed to specific effects on central thermoregulatory systems but also to non-specific effects related to common life aspects, as shown by the changes in the physical and general health status. In fact, most of the POMS and SF-36 parameters, related to mood state and quality of life, improved significantly; the correlation of these parameters with pain measures should also be noted.

Pain, and particularly chronic pain, is very common in the general population, affecting 30% of women [68] [69]. At menopausal age, pain can easily affect a woman's behavior, lowering the time spent walking and moving in general. Thus the fact that pain was reduced in the present study, particularly in the morning (VAS score and QUID sensorial component), is of particular importance. Pain in these women is not of high intensity, only rarely reaching the VAS score of 5. However, it can be present in different parts of the body and can be difficult to treat with analgesics. This kind of pain can be the result of general inflammation often present at the subclinical level. Hence, phytoestrogens are suitable to play a positive role in pain control since several studies have shown their anti-inflammatory action [70] [71]. Sakamoto *et al.* examined the effect of daidzein on the markers of pro-inflammatory cytokines in co-cultures of 3T3L1 adipocytes and RAW264 macrophages [72]. Daidzein (25 μM) treatment significantly inhibited the mRNA expression of the pro-inflammatory cytokines CCL2 and IL6 in adipocytes induced by co-culture. The anti-inflammatory effect of daidzein has also been examined by using TNFα-treated (20 ng/ml) murine MLE-12 epithelial cells [73]. In the present study, daidzein and genistein urinary levels were negatively correlated with pain measures.

Isoflavones are supposed to exert some beneficial effects by virtue of their an-

tioxidant properties [54]. In order to investigate this possibility, the thiol to disulfide ratio was measured in blood of the enrolled women at the beginning of the soy-enriched bread consumption and after 30 days. There was no significant variation in the GSH/GSSG ratio (a widely accepted biomarker of oxidative stress) in RBC or in the thiol composition in plasma. Indeed, the thiol to disulfide ratio for all the physiological molecules occurring in plasma indicates that the measured parameters in menopausal women are within the range of the same values measured in the rest of the population [74] [75]; moreover, after one month of soy-enriched diet, there was a slight but significant increase in both the reduced and oxidized forms (namely LMM-SS) of some thiols. Thus, it can be inferred that this kind of diet did not have a strong impact on the extracellular thiol/disulfide balance.

We tested the soy-enriched bread in three groups of women with different menopausal condition: women in the climacteric period with "irregular" menstrual cycles; women in menopause, *i.e.*, with the absence of menses for 1 to 5 years; women with the absence of menses for more than 5 years. Interestingly, although at least 5 years separated one group from the others, they differed only in a few parameters, *i.e.*, longer reaction time in the Test of Attentional Performance in the older group and higher pain measures and hot flashes in the second group.

This is interesting since it suggests that the second group, those with 1 - 5 years of menopause, is experiencing a condition during which the CNS, and in particular the hypothalamus, is fighting against the forced estrogen-mediated decrease of CNS functions. Hot flushes are episodes of hyper-sweating during which the body loses heat and becomes colder. It has been shown that cold followed by rewarming (as during hibernation) forces synaptogenesis [76] [77] [78]. Hence we hypothesize that hot flushes help women to perform better in the attentional test in these years before the slow decrease in cognitive functions.

5. Conclusions

Our multifactorial approach to the study of menopause showed that 30 days of phytoestrogens intake was associated with improvement in the physical and mental components of the life of menopausal women. The presence of phytoestrogens in a "common" food such as bread allows their consumption without any pharmacological approaches.

The limitations of the study are the low number of subjects. Although we consider the duration of 30 days as appropriate to show possible effects, the positive results indicate that this topic warrants further research. Indeed, we expect an even greater and longer-lasting effect by supplying phytoestrogens (also from plant sources other than soybean) for a longer period.

6. Potential Clinical Value

Aging is accompanied by several changes in the body. The brain is particularly

affected by the estrogen decline in women. Herein we describe the improvement of mental and physical parameters in women of different menopausal ages after 30 days of regular consumption of soy-enriched bread. The present data can be used to convince physicians to suggest the inclusion of products containing soy in the diet of women in menopause.

Acknowledgements

The authors thank the University of Siena for funding this research, Pariv Srl for the collaboration in preparing and producing the special bread, Dr Valeria Bachiocco for the critical reading of the manuscript and Dr Peter Christie for the English language revision.

Conflicts of Interest

All authors declare no conflict of interest.

References

[1] Lauretta, R., Sansone, M., Sansone, A., Romanelli, F. and Appetecchia, M. (2018) Gender in Endocrine Diseases: Role of Sex Gonadal Hormones. *International Journal of Endocrinology*, **2018**, Article ID: 4847376. https://doi.org/10.1155/2018/4847376

[2] Takahashi, T.A. and Johnson, K.M. (2015) Menopause. *Medical Clinics of North America*, **99**, 521-534. https://doi.org/10.1016/j.mcna.2015.01.006

[3] Tufano, A., Marzo, P., Enrini, R., Morricone, L., Caviezel, F. and Ambrosi, B. (2004) Anthropometric, Hormonal and Biochemical Differences in Lean and Obese Women before and after Menopause. *Journal of Endocrinological Investigation*, **27**, 648-653. https://doi.org/10.1007/BF03347497

[4] Lobo, R.A., Davis, S.R., De Villiers, T.J., Gompel, A., Henderson, V.W., Hodis, H.N., Lumsden, M.A., Mack, W.J., Shapiro, S. and Baber, R.J. (2014) Prevention of Diseases after Menopause. *Climacteric*, **17**, 540-556. https://doi.org/10.3109/13697137.2014.933411

[5] Guarnieri, B. (2019) Sleep Disorders and Cognitive Alterations in Women. *Maturitas*, **126**, 25-27. https://doi.org/10.1016/j.maturitas.2019.04.214

[6] Santoro, N., Epperson, C.N. and Mathews, S.B. (2015) Menopausal Symptoms and Their Management. *Endocrinology and Metabolism Clinics of North America*, **44**, 497-515. https://doi.org/10.1016/j.ecl.2015.05.001

[7] Newson, L. (2018) Menopause and Cardiovascular Disease. *Post Reproductive Health*, **24**, 44-49. https://doi.org/10.1177/2053369117749675

[8] Sumino, H., Ichikawa, S., Yoshida, A., Murakami, M., Kanda, T., Mizunuma, H., Sakamaki, T. and Kurabayashi, M. (2003) Effects of Hormone Replacement Therapy on Weight, Abdominal Fat Distribution, and Lipid Levels in Japanese Postmenopausal Women. *International Journal of Obesity*, **27**, 1044-1051. https://doi.org/10.1038/sj.ijo.0802371

[9] Stuenkel, C.A. (2018) Vasomotor and Related Menopause Symptoms. *Clinical Obstetrics and Gynecology*, **61**, 433-446. https://doi.org/10.1097/GRF.0000000000000385

[10] Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M.,

Ockene, J. and Writing Group for the Women's Health Initiative Investigators (2002) Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women: Principal Results from the Women's Health Initiative Randomized Controlled Trial. *JAMA*, **288**, 321-333. https://doi.org/10.1001/jama.288.3.321

[11] Kutlesic, R.M., Popovic, J., Stefanovic, M., Vukomanovic, P., Lukic, B. and Lilic, G. (2016) Alternatives of Menopausal Hormone Therapy. *Medicinski Pregled*, **69**, 177-182. https://doi.org/10.2298/MPNS1606177K

[12] Franco, O.H., Chowdhury, R., Troup, J., Voortman, T., Kunutsor, S., Kavousi, M., Oliver-Williams, C. and Muka, T. (2016) Use of Plant-Based Therapies and Menopausal Symptoms: A Systematic Review and Meta-Analysis. *JAMA*, **315**, 2554-2563. https://doi.org/10.1001/jama.2016.8012

[13] Rao, A.V. and Agarwal, S. (2000) Role of Antioxidant Lycopene in Cancer and Heart Disease. *Journal of the American College of Nutrition*, **19**, 563-569. https://doi.org/10.1080/07315724.2000.10718953

[14] Lambert, M.R. and Edwards, T.M. (2017) Hormonally Active Phytochemicals and Vertebrate Evolution. *Evolutionary Applications*, **10**, 419-432. https://doi.org/10.1111/eva.12469

[15] Howes, M.J.R., Perry, N.S.L., Vásquez-Londoño, C. and Perry, E.K. (2020) Role of Phytochemicals as Nutraceuticals for Cognitive Functions Affected in Ageing. *British Journal of Pharmacology*, **177**, 1294-1315. https://doi.org/10.1111/bph.14898

[16] Gencel, B.V., Benjamin, M., Bahou, N.S. and Khalil, A.R. (2012) Vascular Effects of Phytoestrogens and Alternative Menopausal Hormone Therapy in Cardiovascular Disease. *Mini-Reviews in Medicinal Chemistry*, **12**, 149-174. https://doi.org/10.2174/138955712798995020

[17] Taku, K., Melby, M.K., Kronenberg, F., Kurzer, M.S. and Messina, M. (2012) Extracted or Synthesized Soybean Isoflavones Reduce Menopausal Hot Flash Frequency and Severity: Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Menopause*, **19**, 776-790. https://doi.org/10.1097/gme.0b013e3182410159

[18] Zhang, Y.B., Chen, W.H., Guo, J.J., Fu, Z.H., Yi, C., Zhang, M. and Na, X.L. (2013) Soy Isoflavone Supplementation Could Reduce Body Weight and Improve Glucose Metabolism in Non-Asian Postmenopausal Women—A Meta-Analysis. *Nutrition*, **29**, 8-14. https://doi.org/10.1016/j.nut.2012.03.019

[19] Desmawati, D. and Sulastri, D. (2019) Phytoestrogens and Their Health Effect. *Open Access Macedonian Journal of Medical Sciences*, **7**, 495-499.

[20] Barnes, S. (2010) The Biochemistry, Chemistry and Physiology of the Isoflavones in Soybeans and Their Food Products. *Lymphatic Research and Biology*, **8**, 89-98. https://doi.org/10.1089/lrb.2009.0030

[21] Han, Z.K., Wang, G., Yao, W. and Zhu, W.Y. (2006) Isoflavonic Phytoestrogens—New Prebiotics for Farm Animals: A Review on Research in China. *Current Issues in Intestinal Microbiology*, **7**, 53-60.

[22] Zhou, T., Meng, C. and He, P. (2018) Soy Isoflavones and Their Effects on Xenobiotic Metabolism. *Current Drug Metabolism*, **20**, 46-53. https://doi.org/10.2174/1389200219666180427170213

[23] Křížová, L., Dadáková, K., Kašparovská, J. and Kašparovský, T. (2019) Isoflavones. *Molecules*, **24**, Article No. 1076. https://doi.org/10.3390/molecules24061076

[24] Anupongsanugool, E., Teekachunhatean, S., Rojanasthien, N., Pongsatha, S. and Sangdee, C. (2005) Pharmacokinetics of Isoflavones, Daidzein and Genistein, after Ingestion of Soy Beverage Compared with Soy Extract Capsules in Postmenopausal Thai Women. *BMC Clinical Pharmacology*, **5**, Article No. 2.

https://doi.org/10.1186/1472-6904-5-2

[25] Basu, P., Sunny, S. and Maier, C. (2016) Estrogenic and Antiestrogenic Activities of Commercial Dietary Supplements Containing Herbal Ingredients and Isoflavones. *International Journal of Pharmacy and Pharmaceutical Sciences*, **8**, 307-312. https://doi.org/10.22159/ijpps.2016v8i11.14687

[26] Sirotkin, A.V. and Harrath, A.H. (2014) Phytoestrogens and Their Effects. *European Journal of Pharmacology*, **741**, 230-236. https://doi.org/10.1016/j.ejphar.2014.07.057

[27] Paterni, I., Granchi, C., Katzenellenbogen, J.A. and Minutolo, F. (2014) Estrogen Receptors Alpha (ERα) and Beta (ERβ): Subtype-Selective Ligands and Clinical Potential. *Steroids*, **90**, 13-29. https://doi.org/10.1016/j.steroids.2014.06.012

[28] Lecomte, S., Demay, F., Ferrière, F. and Pakdel, F. (2017) Phytochemicals Targeting Estrogen Receptors: Beneficial Rather than Adverse Effects? *International Journal of Molecular Sciences*, **18**, Article No. 1381. https://doi.org/10.3390/ijms18071381

[29] Hsieh, C.J., Hsu, Y.L., Huang, Y.F. and Tsai, E.M. (2018) Molecular Mechanisms of Anticancer Effects of Phytoestrogens in Breast Cancer. *Current Protein & Peptide Science*, **19**, 323-332. https://doi.org/10.2174/1389203718666170111121255

[30] Haverkort, E.B., Reijven, P.L.M., Binnekade, J.M., De Van Der Schueren, M.A.E., Earthman, C.P., Gouma, D.J. and De Haan, R.J. (2015) Bioelectrical Impedance Analysis to Estimate Body Composition in Surgical and Oncological Patients: A Systematic Review. *European Journal of Clinical Nutrition*, **69**, 3-13. https://doi.org/10.1038/ejcn.2014.203

[31] Kotler, D.P., Burastero, S., Wang, J. and Pierson, R.N. (1996) Prediction of Body Cell Mass, Fat-Free Mass, and Total Body Water with Bioelectrical Impedance Analysis: Effects of Race, Sex, and Disease. *The American Journal of Clinical Nutrition*, **64**, 489S-497S. https://doi.org/10.1093/ajcn/64.3.489S

[32] McNair, D.M., Lorr, M. and Droppelman, L.F. (1971) Manual for the Profile of Mood States. Education and Industrial Testing Service, San Diego, CA.

[33] Morfeld, M., Petersen, C., Krüger-Bödeker, A. and Von Mackensen, S.M.B. (2007) The Assessment of Mood at Workplace—Psychometric Analyses of the Revised Profile of Mood States (POMS) Questionnaire. *Psycho-Social-Medicine*, **4**, Doc06.

[34] Fontani, G., Corradeschi, F., Felici, A., Alfatti, F., Migliorini, S. and Lodi, L. (2005) Cognitive and Physiological Effects of Omega-3 Polyunsaturated Fatty Acid Supplementation in Healthy Subjects. *European Journal of Clinical Investigation*, **35**, 691-699. https://doi.org/10.1111/j.1365-2362.2005.01570.x

[35] Apolone, G. and Mosconi, P. (1998) The Italian SF-36 Health Survey: Translation, Validation and Norming. *Journal of Clinical Epidemiology*, **51**, 1025-1036.

[36] Huskisson, E.C., Jones, J. and Scott, P.J. (1976) Application of Visual-Analogue Scales to the Measurement of Functional Capacity. *Rheumatology*, **15**, 185-187. https://doi.org/10.1093/rheumatology/15.3.185

[37] De Benedittis, G., Massel, R., Nobili, R. and Pieri, A. (1988) The Italian Pain Questionnaire. *Pain*, **33**, 53-62. https://doi.org/10.1016/0304-3959(88)90203-5

[38] Zimmermann, P. and Fimm, B. (1994) Test d'Évaluation de l'Attention (TEA). Version 1.02. P. Zimmermann, Psychologisches Institut der Universität Freiburg, Freiburg.

[39] Xu, X., Wang, H.J., Murphy, P.A., Cook, L. and Hendrich, S. (1994) Daidzein Is a More Bioavailable Soymilk Isoflavone than Is Genistein in Adult Women. *The Journal of Nutrition*, **124**, 825-832. https://doi.org/10.1093/jn/124.6.825

[40] Montero, G., Günther, G., Valdés, K., Arriagada, F. and Morales, J. (2018) An HPLC Method for the Determination of Isoflavones and the Evaluation of Their Antioxidant Capacity in Both Homogeneous and Microheterogeneous Systems. *Journal of AOAC INTERNATIONAL*, **101**, 235-241. https://doi.org/10.5740/jaoacint.17-0104

[41] Max, J. (1941) Earthquake in Japan. *Nature*, **148**, 110. https://doi.org/10.1038/148110b0

[42] Giustarini, D., Dalle-Donne, I., Milzani, A., Fanti, P. and Rossi, R. (2013) Analysis of GSH and GSSG after Derivatization with N-Ethylmaleimide. *Nature Protocols*, **8**, 1660-1669. https://doi.org/10.1038/nprot.2013.095

[43] Giustarini, D., Lorenzini, S., Rossi, R., Chindamo, D. and Di Simplicio, P. (2005) Altered Thiol Pattern in Plasma of Subjects Affected by Rheumatoid Arthritis. *Clinical and Experimental Rheumatology*, **23**, 205-212.

[44] Fanti, P., Giustarini, D., Rossi, R., Cunningham, S.E.D., Folli, F., Khazim, K., Cornell, J., Matteucci, E. and Bansal, S. (2015) Dietary Intake of Proteins and Calories Is Inversely Associated with The Oxidation State of Plasma Thiols in End-Stage Renal Disease Patients. *Journal of Renal Nutrition*, **25**, 494-503. https://doi.org/10.1053/j.jrn.2015.06.003

[45] Giustarini, D., Dalle-Donne, I., Lorenzini, S., Selvi, E., Colombo, G., Milzani, A., Fanti, P. and Rossi, R. (2012) Protein Thiolation Index (PTI) as a Biomarker of Oxidative Stress. *Free Radical Biology and Medicine*, **53**, 907-915. https://doi.org/10.1016/j.freeradbiomed.2012.06.022

[46] Katsumata, M., Hirata, K., Inagaki, H., Hirata, Y. and Kawada, T. (2009) [Evaluation of New Saliva Collection Device for Determination of Salivary Cotinine, Cortisol, Dehydroepiandrosterone and Testosterone Concentrations]. *Nihon Eiseigaku Zasshi*, **64**, 811-816. https://doi.org/10.1265/jjh.64.811

[47] Gröschl, M., Köhler, H., Topf, H.G., Rupprecht, T. and Rauh, M. (2008) Evaluation of Saliva Collection Devices for the Analysis of Steroids, Peptides and Therapeutic Drugs. *Journal of Pharmaceutical and Biomedical Analysis*, **47**, 478-486. https://doi.org/10.1016/j.jpba.2008.01.033

[48] Ricci, C. and Aloisi A.M. (2016) Il Pane delle Donne. *Journal of the Siena Academy of Sciences*, **8**, 50.

[49] McHugh, M.L. (2011) Multiple Comparison Analysis Testing in ANOVA. *Biochemia Medica*, **21**, 203-209. https://doi.org/10.11613/BM.2011.029

[50] Avis, N.E., Crawford, S.L. and Green, R. (2018) Vasomotor Symptoms across the Menopause Transition: Differences among Women. *Obstetrics and Gynecology Clinics of North America*, **45**, 629-640. https://doi.org/10.1016/j.ogc.2018.07.005

[51] Scalbert, A. and Williamson, G. (2000) Dietary Intake and Bioavailability of Polyphenols. *The Journal of Nutrition*, **130**, 2073S-2085S.

[52] Szeja, W., Grynkiewicz, G. and Rusin, A. (2016) Isoflavones, Their Glycosides and Glycoconjugates. Synthesis and Biological Activity. *Current Organic Chemistry*, **21**, 218-235. https://doi.org/10.2174/1385272820666160928120822

[53] Piao, Y.Z. and Eun, J.B. (2020) Physicochemical Characteristics and Isoflavones Content during Manufacture of Short-Time Fermented Soybean Product (*Cheonggukjang*). *Journal of Food Science and Technology*, **57**, 2190-2197. https://doi.org/10.1007/s13197-020-04255-2

[54] Kumari, S. and Chang, S.K.C. (2016) Effect of Cooking on Isoflavones, Phenolic Acids, and Antioxidant Activity in Sprouts of Prosoy Soybean (*Glycine max*). *Journal of Food Science*, **81**, C1679-C1691. https://doi.org/10.1111/1750-3841.13351

[55] Dey, P. (2019) Gut Microbiota in Phytopharmacology: A Comprehensive Overview of Concepts, Reciprocal Interactions, Biotransformations and Mode of Actions. *Pharmacological Research*, **147**, Article ID: 104367. https://doi.org/10.1016/j.phrs.2019.104367

[56] Prasain, J.K., Arabshahi, A., Moore, D.R., Greendale, G.A., Wyss, J.M. and Barnes, S. (2010) Simultaneous Determination of 11 Phytoestrogens in Human Serum Using a 2 Min Liquid Chromatography/Tandem Mass Spectrometry Method. *Journal of Chromatography B*, **878**, 994-1002. https://doi.org/10.1016/j.jchromb.2010.02.032

[57] Nabavi, S., Nabavi, S., Daglia, M., D'Onofrio, G., Budzyńska, B., Tariq, A., Javed, S. and Ahmed, T. (2017) Daidzein and Its Effects on Brain. *Current Medicinal Chemistry*, **24**, 365-375. https://doi.org/10.2174/0929867323666161101140214

[58] Nestel, P.J., Yamashita, T., Sasahara, T., Pomeroy, S., Dart, A., Komesaroff, P., Owen, A. and Abbey, M. (1997) Soy Isoflavones Improve Systemic Arterial Compliance but Not Plasma Lipids in Menopausal and Perimenopausal Women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **17**, 3392-3398. https://doi.org/10.1161/01.ATV.17.12.3392

[59] Djuric, Z., Chen, G., Doerge, D.R., Heilbrun, L.K. and Kucuk, O. (2001) Effect of Soy Isoflavone Supplementation on Markers of Oxidative Stress in Men and Women. *Cancer Letters*, **172**, 1-6. https://doi.org/10.1016/S0304-3835(01)00627-9

[60] Wiseman, H., O'Reilly, J.D., Adlercreutz, H., Mallet, A.I., Bowey, E.A., Rowland, I.R and Sanders, T.A.B. (2000) Isoflavone Phytoestrogens Consumed in Soy Decrease F2-Isoprostane Concentrations and Increase Resistance of Low-Density Lipoprotein to Oxidation in Humans. *The American Journal of Clinical Nutrition*, **72**, 395-400. https://doi.org/10.1093/ajcn/72.2.395

[61] Cederroth, C.R., Zimmermann, C. and Nef, S. (2012) Soy, Phytoestrogens and Their Impact on Reproductive Health. *Molecular and Cellular Endocrinology*, **355**, 192-200. https://doi.org/10.1016/j.mce.2011.05.049

[62] Harris, D.M., Besselink, E., Henning, S.M., Go, V.L.W. and Heber, D. (2005) Phytoestrogens Induce Differential Estrogen Receptor Alpha- or Beta-Mediated Responses in Transfected Breast Cancer Cells. *Experimental Biology and Medicine*, **230**, 558-568.

[63] Fuentes, N. and Silveyra, P. (2019) Estrogen Receptor Signaling Mechanisms. *Advances in Protein Chemistry and Structural Biology*, **116**, 135-170. https://doi.org/10.1016/bs.apcsb.2019.01.001

[64] Qiu, S.M. and Jiang, C.M. (2019) Soy and Isoflavones Consumption and Breast Cancer Survival and Recurrence: A Systematic Review and Meta-Analysis. *European Journal of Nutrition*, **58**, 3079-3090. https://doi.org/10.1007/s00394-018-1853-4

[65] Fritz, H., Seely, D., Flower, G., Skidmore, B., Fernandes, R., Vadeboncoeur, S., Kennedy, D., Cooley, K., Wong, R., Sagar, S., Sabri, E., and Fergusson, D. (2013) Soy, Red Clover, and Isoflavones and Breast Cancer: A Systematic Review. *PLoS ONE*, **8**, e81968. https://doi.org/10.1371/journal.pone.0081968

[66] Girgert, R., Emons, G. and Gründker, C. (2019) Estrogen Signaling in ERα-Negative Breast Cancer: ERβ and GPER. *Frontiers in Endocrinology*, **10**, Article No. 781. https://doi.org/10.3389/fendo.2018.00781

[67] Laurin, R. and Finez, L. (2020) Working Memory Capacity Does Not always Promote Dual-Task Motor Performance: The Case of Juggling in Soccer. *Scandinavian Journal of Psychology*, **61**, 168-176. https://doi.org/10.1111/sjop.12589

[68] Meisler, J.G., Pinn, V., Kitt, C., LeResche, L., Stohler, C. and Levine, J. (1999) Chronic Pain Conditions in Women. *Journal of Women*'s *Health*, **8**, 313-320.

https://doi.org/10.1089/jwh.1999.8.313

[69] Dias, R.C.A., Junior, J.K., da Costa, E.H.F. and Nisihara, R.M. (2019) Fibromyalgia, Sleep Disturbance and Menopause: Is There a Relationship? A Literature Review. *International Journal of Rheumatic Diseases*, **22**, 1961-1971. https://doi.org/10.1111/1756-185X.13713

[70] Rietjens, I.M.C.M., Louisse, J. and Beekmann, K. (2017) The Potential Health Effects of Dietary Phytoestrogens. *British Journal of Pharmacology*, **174**, 1263-1280. https://doi.org/10.1111/bph.13622

[71] Wei, J., Chen, J.R., Pais, E., Wang, T.Y., Miao, L., Li, L., Li, L.Y., Qiu, F., Hu, L.M., Gao, X.M. and Fan, G.W. (2017) Oxyresveratrol Is a Phytoestrogen Exerting Anti-Inflammatory Effects through NF-κB and Estrogen Receptor Signaling. *Inflammation*, **40**, 1285-1296. https://doi.org/10.1007/s10753-017-0572-y

[72] Sakamoto, Y., Kanatsu, J., Toh, M., Naka, A., Kondo, K. and Iida, K. (2016) The Dietary Isoflavone Daidzein Reduces Expression of Pro-Inflammatory Genes through PPARα/γ and JNK Pathways in Adipocyte and Macrophage Co-Cultures. *PLoS ONE*, **11**, e0149676. https://doi.org/10.1371/journal.pone.0149676

[73] Li, H.Y., Pan, L., Ke, Y.S., Batnasan, E., Jin, X.Q., Liu, Z.Y. and Ba, X.Q. (2014) Daidzein Suppresses Pro-Inflammatory Chemokine Cxcl2 Transcription in TNF-α-Stimulated Murine Lung Epithelial Cells via Depressing PARP-1 Activity. *Acta Pharmaceutica Sinica*, **35**, 496-503.

[74] Giustarini, D., Dalle-Donne, I., Tsikas, D. and Rossi, R. (2009) Oxidative Stress and Human Diseases: Origin, Link, Measurement, Mechanisms, and Biomarkers. *Critical Reviews in Clinical Laboratory Sciences*, **46**, 241-281. https://doi.org/10.3109/10408360903142326

[75] Grintzalis, K., Papapostolou, I., Zisimopoulos, D., Stamatiou, I. and Georgiou, C.D. Multiparametric Protocol for the Determination of Thiol Redox State in Living Matter. *Free Radical Biology and Medicine*, **74**, 85-98. https://doi.org/10.1016/j.freeradbiomed.2014.06.024

[76] Kirov, S.A., Petrak, L.J., Fiala, J.C. and Harris, K.M. (2004) Dendritic Spines Disappear with Chilling but Proliferate Excessively upon Rewarming of Mature Hippocampus. *Neuroscience*, **127**, 69-80. https://doi.org/10.1016/j.neuroscience.2004.04.053

[77] Cheng, H.H., Huang, Z.H., Lin, W.H., Chow, W.Y. and Chang, Y.C. (2009) Cold-Induced Exodus of Postsynaptic Proteins from Dendritic Spines. *Journal of Neuroscience Research*, **87**, 460-469. https://doi.org/10.1002/jnr.21852

[78] Luppi, M., Cerri, M., Di Cristoforo, A., Hitrec, T., Dentico, D., Del Vecchio, F., Martelli, D., Perez, E., Tupone, D., Zamboni, G., and Amici, R. (2019) C-Fos Expression in the Limbic Thalamus Following Thermoregulatory and Wake-Sleep Changes in the Rat. *Experimental Brain Research*, **237**, 1397-1407. https://doi.org/10.1007/s00221-019-05521-2

Supplementary Tables "Soy-Enriched Bread, a Pilot Study to Determine Its Beneficial Effects in Menopause"

Table S1. Bioelectrical Impedance Analysis (BIA) determined in the three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later). Abbreviations: FFM (fat free mass), (FM) fat mass, TBW (total body water), ECW (extracellular water), BMI (Body Mass Index). Values are reported as Mean ± SEM.

		FFM	FM	TBW	ECW	BMI
Group 1	*Test 1*	29.85 ± 1.39	14.59 ± 3.77	30.71 ± 2.25	15.95 ± 0.47	23.93 ± 1.95
	Test 2	30.11 ± 1.37	14.69 ± 3.70	28.13 ± 2.68	16.11 ± 0.55	24.15 ± 1.96
Group 2	*Test 1*	30.43 ± 2.11	14.51 ± 2.87	27.20 ± 3.16	15.49 ± 0.74	24.14 ± 1.35
	Test 2	30.44 ± 2.03	14.31 ± 2.56	27.17 ± 3.09	15.70 ± 0.54	24.20 ± 1.21
Group 3	*Test 1*	29.45 ± 1.45	15.50 ± 1.92	26.44 ± 3.02	16.25 ± 0.40	25.48 ± 0.92
	Test 2	29.00 ± 1.51	16.08 ± 2.15	26.33 ± 3.20	16.13 ± 0.50	25.61 ± 0.96

Table S2. Correlations between Profile of Mood States (POMS) values and pain measures. Abbreviations: PRIr-T (Pain Rating Index rank-Total), PPI (Present Pain Intensity, 0 - 5), Visual Analogue Scale (VAS), mo (morning), a (afternoon), n (night), N.S. (no significance), R (linear correlation coefficient), p (statistical significance, p-value). Women, n = 42. Correlations are significant at $p < 0.05$.

POMS	PRIr-T	PPI	VAS mo	VAS a	VAS n
Tension-Anxiety	R: 0.39 $p < 0.01$	R: 0.44 $p < 0.01$	R: 0.35 $p < 0.05$	N.S.	N.S.
Depression-Dejection	R: 0.39 $p < 0.01$	R: 0.35 $p < 0.05$	N.S.	N.S.	N.S.
Anger-Hostility	R: 0.34 $p < 0.05$	R: 0.36 $p < 0.05$	N.S.	N.S.	N.S.
Vigor-Activity	N.S.	R: −0.41 $p < 0.01$	R: −0.33 $p < 0.05$	N.S.	R: −0.37 $p < 0.01$
Fatigue-Inertia	R: 0.32 $p < 0.05$	R: 0.44 $p < 0.01$	R: 0.32 $p < 0.05$	N.S.	N.S.
Confusion-Bewilderment	R: 0.31 $p < 0.05$	N.S.	R: 0.38 $p < 0.01$	N.S.	N.S.

Table S3. Correlations between SF-36 values and pain measures. Abbreviations: PRIr-T (Pain Rating Index rank-Total), PPI (Present Pain Intensity, 0 - 5), Visual Analogue Scale (VAS), mo (morning), a (afternoon), n (night), PF (physical functioning), RP (role physical), BP (bodily pain), GH (general health), PCS (Physical Component Summary), V (vitality), SF (social functioning), RE (role emotional), MH (mental health), MCS (Mental Component Summary), N.S. (no significance), R (linear correlation coefficient), p (statistical significance, p-value). Women, n = 42. Correlations are significant at $p < 0.05$.

SF-36	PRIr-T	PPI	VAS mo	VAS a	VAS n
PF	N.S.	R: −0.47 $p < 0.01$	R: −0.44 $p < 0.01$	R: −0.43 $p < 0.01$	R: −0.49 $p < 0.001$
RP	R: −0.49 $p < 0.001$	R: −0.49 $p < 0.001$	N.S.	N.S.	R: −0.41 $p < 0.01$
BP	R: −0.56 $p < 0.001$	R: −0.64 $p < 0.001$	R: −0.46 $p < 0.01$	R: −0.36 $p < 0.05$	R: −0.58 $p < 0.001$
GH	R: −0.42 $p < 0.01$	R: −0.45 $p < 0.01$	N.S.	N.S.	N.S.
PCS	R: −0.54 $p < 0.001$	R: −0.44 $p < 0.01$	R: −0.62 $p < 0.001$	R: −0.31 $p < 0.05$	R: −0.51 $p < 0.001$
V	R: −0.45 $p < 0.01$	R: −0.60 $p < 0.001$	N.S.	N.S.	N.S.
SF	R: −0.32 $p < 0.05$	R: −0.30 $p < 0.05$	N.S.	N.S.	N.S.
RE	N.S.	R: −0.30 $p < 0.05$	N.S.	N.S.	N.S.
MH	N.S.	N.S.	N.S.	N.S.	N.S.
MCS	R: −0.37 $p < 0.05$	R: −0.43 $p < 0.01$	N.S.	N.S.	N.S.

Table S4. Correlations between Profile of Mood States (POMS) values and number of correct responses (CR). Abbreviations: T-A (Tension-Anxiety), D-D (Depression-Dejection), A-H (Anger-Hostility), V-A (Vigor-Activity), F-I (Fatigue-Inertia), C-B (Confusion-Bewilderment), N.S. (no significance), R (linear correlation coefficient), p (statistical significance, p-value). Women, n = 42. Correlations are significant at $p < 0.05$.

	POMS T-A	POMS D-D	POMS A-H	POMS V-A	POMS F-I	POMS C-B
% CR	R: − 0.42 $p < 0.01$	R: − 0.31 $p < 0.05$	N.S.	N.S.	R: − 0.35 $p < 0.05$	N.S.

Table S5. Correlations between Short Form (36) Health Survey (SF-36) values and number of correct responses (CR). Abbreviations: PF (physical functioning), RP (role physical), BP (bodily pain), GH (general health), V (vitality), DF (social functioning), RE (role emotional), MH (mental health), N.S. (no significance), R (linear correlation coefficient), p (statistical significance, p-value). Women, n = 42. Correlations are significant at $p < 0.05$.

	PF	RP	BP	GH	V	SF	RE	MH
% CR	R: 0.50 $p < 0.001$	N.S.	R: 0.49 $p < 0.001$	R: 0.33 $p < 0.05$	R: 0.33 $p < 0.05$	N.S.	R: 0.47 $p < 0.001$	N.S.

Table S6. Correlations between DA (Daidzein) and GI (Genistein) levels in urine and pain measures. Abbreviations: PRIr-T (Pain Rating Index rank-Total), PPI (Present Pain Intensity, 0-5), Visual Analogue Scale (VAS), mo (morning), a (afternoon), n (night), N.S. (no significance), R (linear correlation coefficient), p (statistical significance, p-value). Women, n = 42. Correlations are significant at $p < 0.05$.

	PRIr-T	PPI	VAS mo	VAS a	VAS n
DA	R: −0.32 $p < 0.05$	N.S.	R: −0.28 $p < 0.07$	R: −0.33 $p < 0.05$	N.S.
GI	R: −0.29 $p < 0.05$	R: −0.27 $p < 0.08$	N.S.	R: −0.32 $p < 0.05$	N.S.

Supplementary Figures "Soy-Enriched Bread, a Pilot Study to Determine Its Beneficial Effects in Menopause"

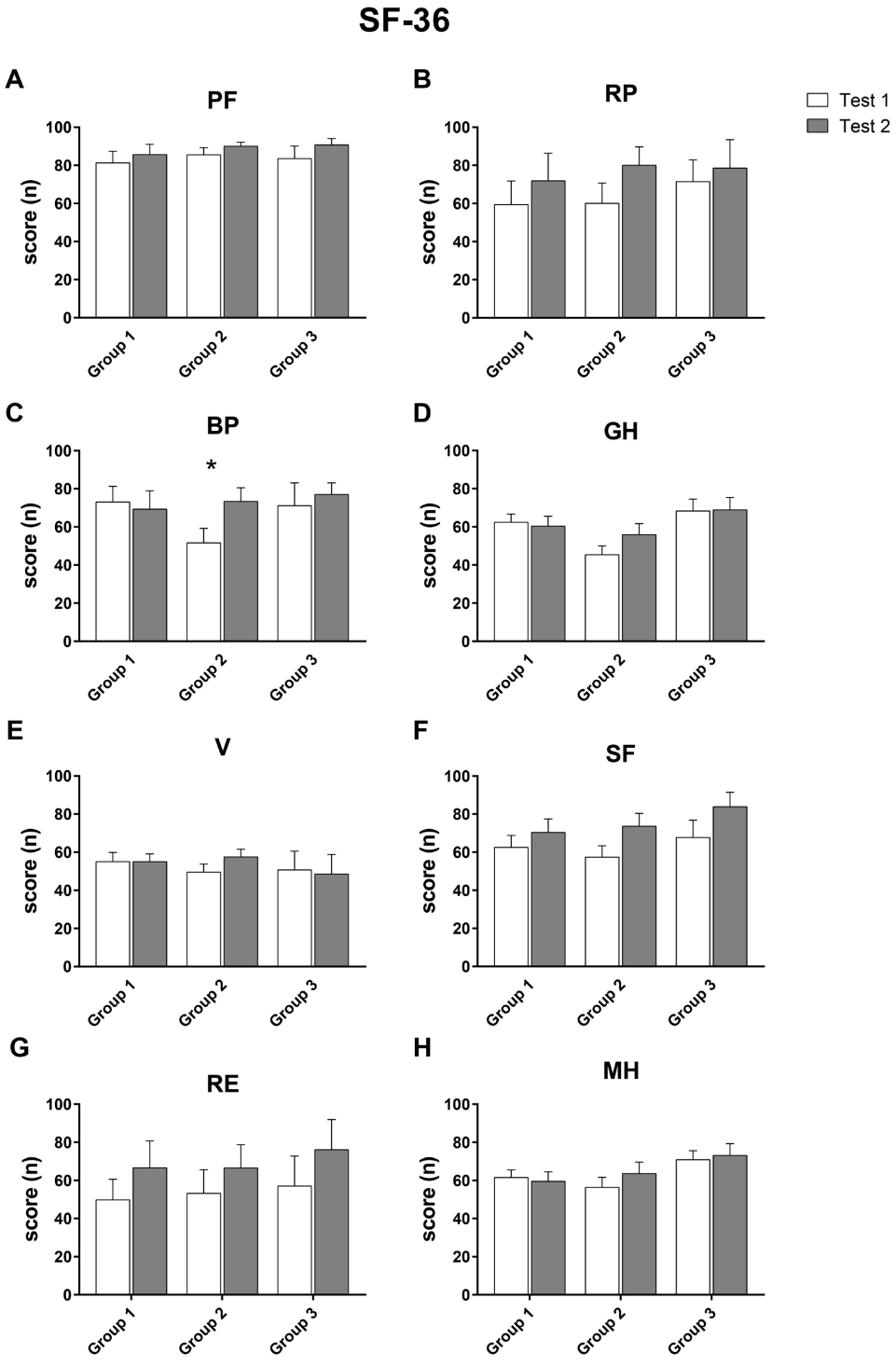

Figure S1. SF-36: scales referred to the quality of life PF (physical functioning) (A), RP (role physical) (B), BP (bodily pain) (C), GH (general health) (D), V (vitality) (E), SF (social functioning) (F), RE (role emotional) (G), MH (mental health) (H). Experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years; Group 3 (n = 8): absence of menses for >5 years; n = 25. Two phases: TEST 1 (Baseline) and TEST 2 (30 days later).

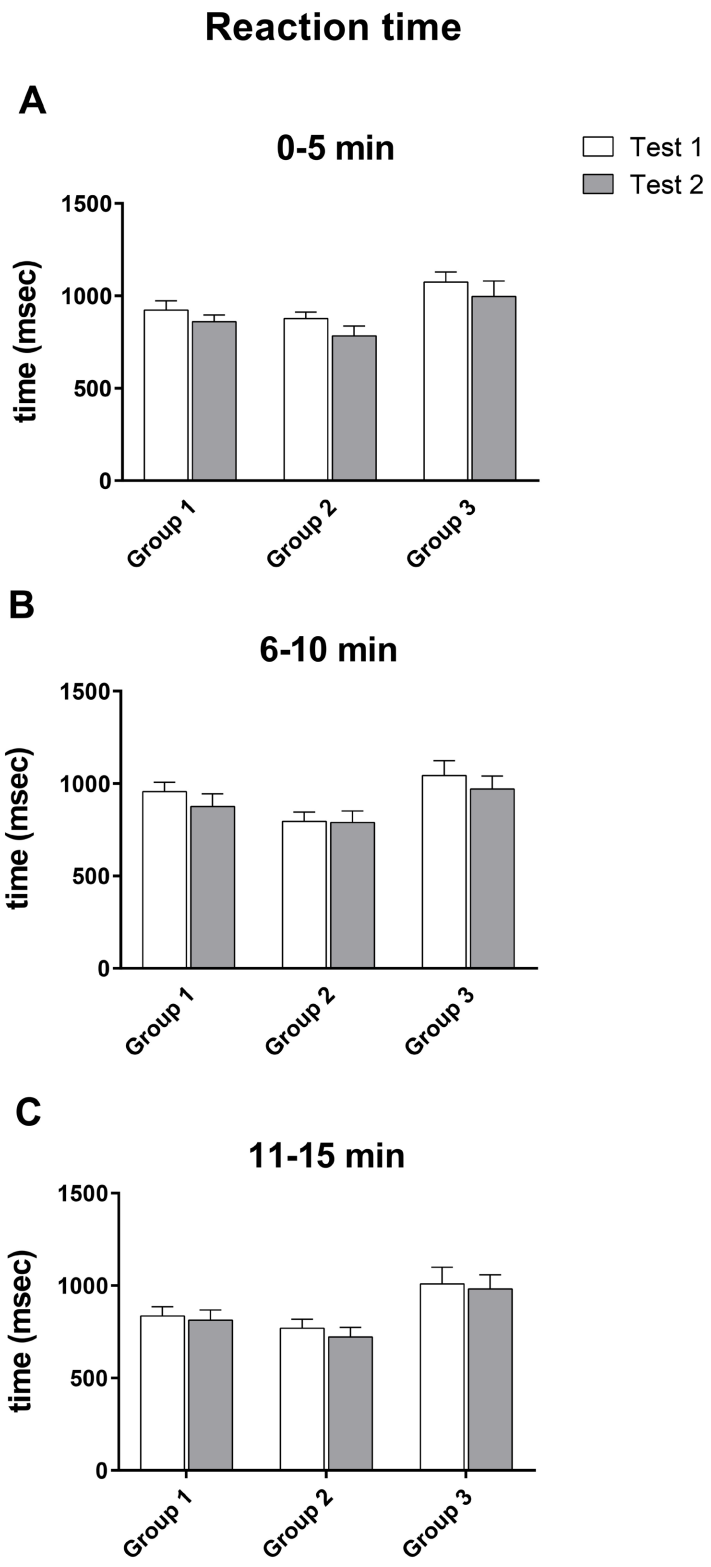

Figure S2. Test of Attentional Performance. Mean value of the reaction time in milliseconds (msec) before (Test 1) and after (Test 2) 30 days. The tests were divided into three sessions of 5 min (0 - 5 min, A; 6 - 10 min, B; 11 - 15 min, C). Three experimental groups: Group 1 (n = 8): climacteric phase; Group 2 (n = 9): absence of menses for 1 to 5 years. Group 3 (n = 8): absence of menses for >5 years; n = 25.

Highlights

- Women spend many years of their life with low levels of circulating estrogens;
- Intake of phytoestrogens can counteract the ovary's inability to secrete estrogens;
- Regular consumption of soybean in the diet can improve menopause-related symptoms;
- Thirty days of soy consumption is associated with improvement in some cognitive and psychophysical parameters.