

ORIGINAL ARTICLE

Efficacy of α_{s1} -casein hydrolysate on stress-related symptoms in women

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Objective: To examine the effects of α_{s1} -casein hydrolysate on females with stress-related symptoms.

Design: Double-blind, randomized, crossover, placebo-controlled trial.

Setting: The α_{s1} -casein hydrolysate was manufactured by INGREDIA (Arras, France) and the placebo was manufactured by DIETAROMA (Bourg, France). Study was designed and performed at PROCLAIM (Rennes, France), and the statistical analyses were performed by D Desor (Nancy, France).

Subjects: A total of 63 female volunteers suffering from at least one disorder that may be related to stress such as anxiety, sleep problems and general fatigue.

Interventions: A total of 63 volunteers participated in a double-blind, randomized, crossover, placebo-controlled study. Subjects were randomly allocated to receive either tablets containing α_{s1} -casein hydrolysate or placebo at the dose of 150 mg/day for 30 days. After a 3 weeks washout period, they were crossed over for a new 30-day period of tablets intake. The outcome measure was a questionnaire including 44 items of symptoms that may be related stress in which the severity of each sign was evaluated using a 10-degree scale. These measures were studied repeatedly at the day of 0, 15 and 30 after the start of each interventional period.

Results: The 30-day treatment by α_{s1} -casein hydrolysate in females with stress-related symptoms reduced their symptoms, particularly in digestion ($P < 0.01$), cardiovascular ($P < 0.05$), intellectual ($P < 0.01$), emotional ($P < 0.05$) and social problems ($P < 0.05$).

Conclusion: This study showed that a 30-day ingestion of α_{s1} -casein hydrolysate decreased the stress-related symptoms in females suggesting that this product may be used as an effective functional ingredient alleviating such symptoms.

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Introduction

Stress refers to the response to any physical or psychological stimulus that disrupts ongoing homeostasis (Pacak and

Palkovits, 2001). The stress response comprises adaptive physiological processes activated in animals and humans in challenging situations (Carlson, 1994). In a stressful condition, the sympathetic branch of the autonomic nerve system is active, and the adrenal gland secretes epinephrine, norepinephrine and steroid stress hormones (Carlson, 1994).

Prolonged stress can be noxious in various areas: sleep (Buguet *et al.*, 1994), memory (de Quervain *et al.*, 1998) and feeding behavior (Hotta *et al.*, 1999). Subjects presenting long-term high-serum concentration of glucocorticoids may suffer from fatal lesions of the central nervous system

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(McEwen, 2005). Sympathetic stimulation by stress activates atherogenesis appearance, facilitates platelet aggregation, induces arrhythmia and can lead to myocardial ischemia onset by increasing the needs of the myocardium for oxygen and metabolites and by reducing its contribution (Engler and Engler, 1995). In addition, many studies have highlighted the role of the professional or psychosocial stress in some types of hypertension (Vrijkotte *et al.*, 2000). Evidence is now accumulating that psychological and social stress may contribute to the apparent excess mortality risk in women's coronary disease. Women are known to be more frequently diagnosed with depression, and to more frequently display depressive symptoms than men (Weissman and Klerman, 1992). Stress also modifies digestive secretion and motility, leads to gastro-duodenal ulcer (Murison, 2000) and affects the endocrine (Sapolsky, 1997) and immune systems (Cacioppo *et al.*, 1995).

As a consequence, stress can increase the risk of various diseases, exacerbate many medical disorders, and change healthy life-style behaviors. Therefore, effective management of stress is essential to decrease vulnerability to illnesses and to improve the quality-of-life.

Nowadays, milk proteins are among the most widely consumed human food proteins. Milk protein consists of soluble whey protein and insoluble colloidal casein. Caseins account for 76–86% of the total milk proteins and can be represented by major gene products: α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein (Odagiri, 1985). Many peptides stemming specifically from bovine α_{s1} -casein have already been identified as having biological activity (Maruyama *et al.*, 1987; Léonil *et al.*, 2001).

A milk α_{s1} -casein hydrolysate (lactium) and a bioactive decapeptide (α_{s1} -casein-(f91–100), α -casozepine), a fragment of this hydrolysate that has been spatially modeled (Lecouvey *et al.*, 1997a, b), have already shown an anxiolytic-like profile in the conditioned defensive burying (CDB) test and in the elevated plus maze in rats, two well-known models used to study anxiolytic agents in rodents (Miclo *et al.*, 2001). Moreover, the effect of lactium (the industrial α_{s1} -casein hydrolysate containing the bioactive α -casozepine) on hemodynamic responses was evaluated in healthy human volunteers facing successive mental and physical stress situations (Messaoudi *et al.*, 2004).

The purpose of this study was to investigate whether oral intake of α_{s1} -casein hydrolysate (α_{s1} -CH) in females with stress-related symptoms would modify various symptoms in physiological, psychological and social areas according to their responses to self-report questionnaires.

Methods

Subjects

Subjects were recruited by way of advertisements in public places (University Henri Poincaré, Vandoeuvre-lès-Nancy, France). A total of 63 volunteer women aged of more than 18

Table 1 Socio-professional characteristics of subjects

Socio-professional status	n	Age (mean \pm s.d.)	(min, max)
Active women	22 63%	37 \pm 10	(20, 59)
Inactive or jobless women	10 16%	34 \pm 8	(26, 50)
Student	23 36%	21 \pm 11	(18, 23)
Retired	8 13%	57 \pm 4	(50, 61)
Whole sample	63 100%	33 \pm 14	(18, 61)

years old who pretend to suffer from at least one symptom that may be stress-related: anxiety, sleep, general fatigue were recruited after announcements in public places such as a halls of public institutions. They participated after informed consent by filling the questionnaire. At the beginning of the study 69 subjects were selected, but six of them did not complete the trial for various reasons not in relation with the treatment. All the data concerning the subjects were in accordance with the rules of the Commission Nationale de l'Informatique et des Libertés (CNIL). Subjects were free of any known primary alimentary allergic problems or medication by anti-depressant, anxiolytic, vegetal sedative or hypnotic treatment in progress or in the 15 days before the beginning of the study. They had no history of any illness, no excessive consumption of alcohol or tobacco or no body mass index over 25. The descriptive socio-professional status of subjects was shown (Table 1).

Tested products

α_{s1} -CH (lactium), consisting of a tryptic hydrolysate of a α_{s1} -casein enriched protein fraction, was supplied by INGREDIA (Arras, France) and the placebo composed of only bovine skimmed milk powder was supplied by DIETAROMA (Bourg, France). Both groups were administered similar looking white tablets containing either 75 mg of α_{s1} -CH or 75 mg of placebo.

Experimental schedule

The study was based on a randomized, double-blind, cross-over schedule. Each subject ingested two tablets containing either α_{s1} -CH or placebo (150 mg/day) every evening during the treatment periods. One group received the product α_{s1} -CH for 30 days, and then had a wash-out period of 3 weeks after which they received the placebo product for a period of 30 days. The other group received the placebo product for 30 days, had a wash-out period of 3 weeks, and then they received the α_{s1} -CH for a period of 30 days.

Evaluation of the efficacy of α_{s1} -CH

The questionnaire covered three main areas potentially affected by stress: Physical and physiological area

(digestive tract, respiratory system, cardiovascular system, locomotor system, other physical symptoms of stress); psychological area (intellectual functions, emotional area); social life. The questionnaire was made up from items from the Hamilton Anxiety Scale (Hamilton, 1967; Maier *et al.*, 1988) and Ferreri Anxiety Rating Diagram (FARD) (Ferreri *et al.*, 1988) partly for assessing stress symptoms including anxiety, depression and internal tension. Totally, the questionnaire included 44 items of stress symptoms.

The efficacy of the products was investigated by the use of the above-mentioned questionnaire in which the severity of each sign was evaluated using a visual 10-degree scale (0=not at all, 9=excessively). We applied the format used in a classical perceived stress scale in French adapted from Lafleur and Béliveau (1994). The scoring method was changed, from a 4-point scale to a 10-point one (0–9). All subjects filled in the questionnaire repeatedly at day 0, for example, before any intake of the products, and at days 15 and 30 of each intake period after the start of this study.

Statistics

Statistical analysis of the data was performed using the SPSS statistical software program.

The effect of the α_{s1} -CH was investigated relatively to the symptoms of each area through the detection of the maximum variation of major symptom because someone who suffers from stress mainly hopes a decrease of his/her most unpleasant symptoms.

Given that the distribution of the behavioral scores and percentages are generally not Gaussian, and when the values are <30 or >70%, non parametric statistics were used. The Wilcoxon test was used to analyze the difference between treatment groups for major stress symptoms. Differences were considered to be significant when $P < 0.05$.

First of all, the stability of the baseline was investigated. When the response was stable, it was possible to perform direct comparisons using raw values of the scores. If the values of the scores were not stable from one period to the other, the variations of the scores (in terms of percentages relatively to day 0 of each period) were compared.

Further relationship between predictor valuables and major stress symptoms was analyzed using discriminant function analysis. The discriminant analysis was conducted to show if treatment by α_{s1} -CH could be discriminated from the placebo substance by taking into account the results in the eight studied areas, the dimensions of digestive, respiratory, cardiovascular, locomotion, physical, intellectual, emotional and social symptoms. For each dimension, the percentages of variation under α_{s1} -CH and placebo were used. When the initial value was equal to 0, the computation of the percentage of variation was impossible and the value was suppressed in this analysis.

Results

Stability of the baseline for period 1 and 2

Between the first day of the first period and the first day of the second period of treatment, and whatever the treatment received during period 1, the intensity of 30 symptoms out of 44 (= 68.2%) significantly decreased. It indicated a strong effect of time and/or participation to the study on the perceived symptoms of stress.

Comparison of the effect of treatment by α_{s1} -CH and placebo on the development of various aspects of stress between day 0 and 15

The results of the statistical comparisons of the magnitude variation of the major stress symptoms between day 0 and day 15 of treatment in the two groups (α_{s1} -CH and placebo) were shown (Table 2). The intensity of all the stress symptoms between day 0 and day 15 of treatment was decreased in both groups. Concerning the whole population, the only significant improvement was found for cardiovascular symptom. Improvement in this dimension was found in the subjects treated by α_{s1} -CH (49.0%) versus the subjects treated by placebo (24.3%).

The results of the statistical comparisons in subjects with the highest initial intensities (intensity > 4 on day 0) for the symptoms, between α_{s1} -CH and placebo groups, on days 0 and 15 of treatment, were shown (Table 3). The intensity was decreased in both groups. Significant improvements of symptoms were found for the product α_{s1} -CH for dimensions of digestive, cardiovascular, and physical symptoms was significantly greater in the subjects treated by α_{s1} -CH than in the subjects treated by placebo.

Comparison of the effect of treatment by α_{s1} -CH and placebo on the development of various aspects of stress between day 0 and 30

The results of the statistical comparisons of the magnitude variation of the symptoms between day 0 and day 30 of treatment in the two groups (α_{s1} -CH and placebo) were shown (Table 4). The intensity of the measured symptoms

Table 2 Percentage of improvement of the major symptoms evaluated on the whole samples at day 15 of the treatments

Symptom items	% Improvement		Difference	P-value
	α_{s1} -CH	Placebo		
Digestion	49.9	41.4	8.5	
Respiration	50.4	31.4	19.0	
Cardiovascular	49.0	24.3	24.7	< 0.05
Locomotion	53.6	54.5	-0.9	
Physical area	44.3	38.2	6.1	
Intellectual area	41.0	49.0	-8.0	
Emotional area	31.7	30.1	1.6	
Social life	40.9	48.9	-8.0	

Abbreviation: α_{s1} -CH: α_{s1} -casein hydrolysate.

between day 0 and day 30 of treatment was decreased in the α_{S1} -CH and placebo groups. The improvement was significant for digestive symptom and intellectual problems in the subjects treated by α_{S1} -CH versus in the subjects treated by placebo.

The results of statistical comparisons of the intensity of symptoms in the subjects with the highest symptom

Table 3 Percentage of improvement of the major symptoms evaluated on the subjects with highest intensities of the symptoms at day 15 of the treatments

Symptom items	% Improvement		Difference	P-value
	α_{S1} -CH	Placebo		
Digestion	58.0	38.6	19.4	<0.05
Respiration	53.2	51.0	2.2	
Cardiovascular	53.4	21.8	31.6	<0.001
Locomotion	61.8	59.5	2.3	
Physical area	61.5	43.5	18.0	<0.05
Intellectual area	50.8	42.8	8.0	
Emotional area	31.9	24.4	7.5	
Social life	41.2	44.6	-3.4	

Abbreviation: α_{S1} -CH: α_{S1} -casein hydrolysate.

Table 4 Percentage of improvement of the major symptoms evaluated on the whole samples at day 30 of the treatments

Symptom items	% Improvement		Difference	P-value
	α_{S1} -CH	Placebo		
Digestion	65.6	44.6	21	<0.01
Respiration	51.5	60.4	-8.9	
Cardiovascular	48.9	39.0	9.9	
Locomotion	61.1	64.7	-3.6	
Physical area	45.3	37.6	7.7	
Intellectual area	62.5	46.2	16.3	<0.05
Emotional area	39.7	34.1	5.6	
Social life	40.2	30.5	9.7	

Abbreviation: α_{S1} -CH: α_{S1} -casein hydrolysate.

Table 5 Percentage of improvement of the major symptoms evaluated on the subjects with highest intensities of the symptoms at day 30 of the treatments

Symptom items	Number of subjects	Number of subjects	% Improvement		Difference	P-value
	α_{S1} -CH	placebo	α_{S1} -CH	placebo		
Digestion	37	35	66.1	36.6	29.5	<0.01
Respiration	9	8	68.9	43.1	25.8	
Cardiovascular	34	33	48.0	35.5	12.5	<0.05
Locomotion	34	32	65.8	63.9	1.9	
Physical area	32	30	53.8	41.0	12.8	
Intellectual area	34	32	64.8	36.7	28.1	<0.01
Emotional area	38	37	43.8	23.5	20.3	<0.05
Social life	51	50	36.7	22.5	14.2	<0.05

Abbreviation: α_{S1} -CH: α_{S1} -casein hydrolysate.

intensities (intensity > 4 on day 0) in the α_{S1} -CH and placebo groups, on the day 0 and day 30 of treatment, were shown (Table 5). The intensity of the symptoms between day 0 and day 30 of treatment was significantly decreased in both groups. Concerning the subjects with the highest symptom intensities, significant improvements could be demonstrated in the α_{S1} -CH group after a 30-day long treatment for digestion (36.6% for placebo/66.1% for α_{S1} -CH), cardiovascular symptom (35.5% for placebo/48.0% for α_{S1} -CH), intellectual problems (36.7% for placebo/64.8% for α_{S1} -CH), problems in emotion (23.5% placebo/43.8% for α_{S1} -CH) and social life (22.5% for placebo/36.7% for α_{S1} -CH) (Figure 1). There were no significant differences for the subjects treated with α_{S1} -CH versus the subjects treated with placebo for respiratory symptom, locomotion or other physical symptoms.

Discriminant analysis

Discriminant function can be represented as a discriminant axis, gathered in s.d. units. In the present case, a gap was seen between the two groups on the discriminant axis

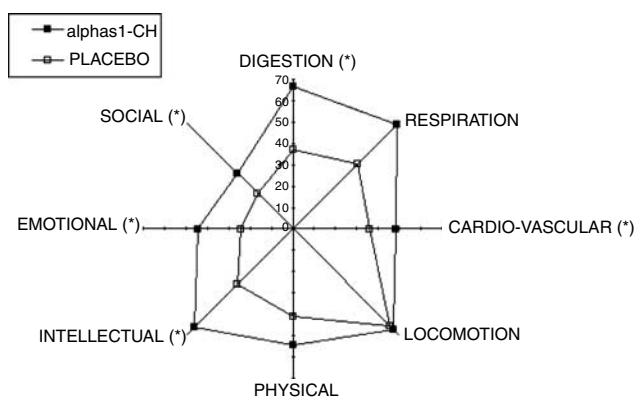


Figure 1 Graphical representation of percentage improvement of the major symptoms evaluated on the subjects with highest intensities of the symptoms at day 30 of the treatments.

according to treatments. The ordinates in standard deviation units of the barycenters of the groups treated by α_{s1} -CH and placebo on the discriminant axis was 0.481 and -0.435 , respectively. It indicated that the subjects treated with α_{s1} -CH had globally greater percentages of improvement of their symptoms than the placebo subjects. The quality of the classification can be evaluated through the final classification matrix computed from the discriminant equation for each subjects, the probability of being in one or the other group. The result was compared to the real group of the subject. In consequence, discriminant analysis correctly classified 67.8% of the subjects. The following Fisher's exact test showed that the null hypothesis is $P=0.0089$. It indicated that the treatments by α_{s1} -CH and placebo led to highly significant differences, with greater improvements in the α_{s1} -CH treatment group compared to the placebo group.

Discussion

The aim of the present study was to evaluate the effects of orally taken α_{s1} -CH on women who suffered from at least one symptom that may be stress-related according to their responses to a questionnaire. Various tools have been used to characterize the different components of stress reaction, questionnaires of psychology (Cohen questionnaire; Spielberger questionnaire; Thayer questionnaire), endocrine assay (Ilardo *et al.*, 2001) and physiological measurements (Boiten *et al.*, 1994; Larson *et al.*, 2001). In this study, the tool used is a questionnaire covering three main areas, physical and physiological, psychological area, and social life by reason that stress is linked to the concept of tension, which is itself related to the muscular and postural systems other physiological systems, and internal state with negatively affective intellectual and the relational functions.

The present study demonstrated that α_{s1} -CH reduced the stress-related symptoms, in various tension aspects. In addition, there was a considerable effect of the placebo treatment ranging from 15% to more than 40% of improvement of the major symptoms. It is interesting to point out that the intensity of some of the assessed symptoms was significantly decreased also in the subjects treated by placebo. Stress, anxiety, depression are well known for having an important psychological component and placebo response is often very high in clinical studies concerning them (Berk and Dodd, 2005). The 'HURIET-SERUSCLAT' law, which is concerned with trials on human subjects in France, implies that the subjects sign an 'informed consent' form before the beginning of the experiment. This means that when the study begins, they know that they will be treated by a substance (α_{s1} -CH), or by placebo, during the two periods, and that α_{s1} -CH can possibly reverse their symptoms of stress. During the study, the subjects' compliance was not monitored. Participation in this study might have conferred a placebo effect.

The effect of α_{s1} -CH could be detected on the 15th day of treatment in the cardiovascular system and on the 30th day of treatment in the digestive system and intellectual symptoms. The supplementation with α_{s1} -CH at the dose of 150 mg/day, was particularly efficient on the subjects who demonstrated the highest initial intensities (>4) for their major symptoms. The cardiovascular, the digestive system and the physical major symptoms were improved on the highest intensity of the symptom at day 15 of the treatment and the digestive, the cardiovascular, the intellectual, the emotional and the social symptoms/problems were improved on the highest intensity of the symptom at day 30 of the treatment.

Taken together, α_{s1} -CH could have a regulating effect in the field of stress-related symptoms, namely, the tension related physiological system (cardiovascular and digestive systems), internal state that negatively interacts with the affective emotional troubles, the intellectual functions and the relational functions (social behavior) of the subjects.

Cow's milk has long been considered a tranquilizing beverage with sleep-inducing capacity, but the molecular causes of this potential action are not known. Numerous bioactive peptides which are identified in milk protein may be released after enzymatic digestion (Meisel, 1997; Clare and Swaisgood, 2000). The functional peptides from milk showed many physiological effects, such as calcium bio-transfer activity (Lee *et al.*, 1979), opiate activity (Meisel and FitzGerald, 2000), immunomodulating activity (Pitt *et al* 1974; Totima *et al.*, 1994), anti-hypertensive activity (Maruyama *et al.*, 1987), and anti-thrombotic activity (Chabance *et al.*, 1995). The α_{s1} -CH has also been reported to exhibit a benzodiazepine (BDZ)-like activity of the GABA_A receptor without side effects (Miclo *et al.*, 2001). However, there are no data on clinical human studies.

The placebo (skimmed milk powder) contains casein and particularly α_{s1} -casein (entire proteins), but the bioactive peptide identified in the α_{s1} -CH, namely α_{s1} -casein (f91–100) cannot be significantly released (from it) because *in vivo* digestion can be assimilated as a multi-enzymatic process including first a pepsic hydrolysis in the stomach. Pepsin cleaves 91–92, 95–96, 98–99 and 99–100 bonds of α_{s1} -casein (Mercier *et al.*, 1970) preventing α_{s1} -casein (f91–100) release from the entire protein contained in placebo. The significant content of the bioactive peptide in the α_{s1} -CH and its relative small size may protect it from further digestion and may explain the significant effects after *in vivo* intake. In this study, the α_{s1} -CH results from a mono-enzymatic digestion of purified α_{s1} -casein.

The results of discriminant analysis showed that the percentages of variations of the magnitude of the symptoms in the eight areas contained enough information to discriminate the two groups according to the treatments. It could therefore be considered that the treatments by α_{s1} -CH and placebo lead to significant differences with greater improvements in the α_{s1} -CH treatment group compared to the placebo group.

In conclusion, this study provided evidence that a 30-day ingestion of α_{s1} -CH displayed positive effect in females with self-reported complaints similar to many clinical stress symptoms suggesting that α_{s1} -CH may be used as an effective functional ingredient alleviating stress. Further studies are needed to investigate if the conclusions drawn from this population can be generalized to males and to non-clinical populations with similar symptomatology, and if the effects can be observed on a long-term basis.

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