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ORIGINAL CONTRIBUTION

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The effect of milk fermented by yogurt cultures plus *Lactobacillus casei* DN-114001 on the immune response of subjects under academic examination stress

Summary *Background* A suppressed immune response has been documented in students under examination stress. *Aim of the study* The current study aimed to evaluate the effect of milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 (Actimel®) on the immune system of subjects under academic examination stress. *Methods* University students were allocated to one of two groups, receiving during 6 weeks (3 weeks prior to, as well as the 3-week duration of the examination period) either: a) a glass of semi-skimmed milk each day (control group, $n = 63$) or b) two 100 mL portions per day of fermented milk (treatment group, $n = 73$). Anxiety and immunological measurements were monitored at baseline (Phase 0) and study end (Phase 1). *Results* The results were expressed as the differences between the data obtained from Phase 0 and Phase 1. This was calculated by subtracting Phase 1 results from the Phase 0 and it is denominated "Treatment effect". Mean (± SE) anxiety increased significantly ($P < 0.05$) over the 6-week study in all students, from 40.74 ± 2.50 to 61.19 ± 2.64 (in percentiles). There was no significant treatment effect since this increase was similar in the control and the treatment groups (21.65 ± 5.09 vs 19.14 ± 3.67, respectively). However, there was a significant treatment effect ($P < 0.05$) on the mean change in absolute number of lymphocytes during the 6-week study, which decreased in the control group (–0.04 ± 0.12 cells x $10^3/mm^3$) and increased in the treatment group (0.37 ± 0.11 cells x $10^3/mm^3$). There was also a significant treatment effect ($P < 0.05$) on the change in absolute numbers of CD56 cells during the 6-week study. Mean absolute CD56 cells significantly decreased ($P < 0.05$) in the control group (–51.97 ± 21.33 cells/mm^3), while remaining similar in the treatment group (17.29 ± 17.27 cells/mm^3). During the study, mean serum cortisol increased 4.30 ± 0.98 µg/dL in the control group, and 1.75 ± 1.05 µg/dL in the treatment group and no significant differences were found between both values ($P = 0.062$). *Conclusions* Milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 was able to modulate the number of lymphocytes and CD56 cells in subjects under academic examination stress.

Key words fermented milk – *Lactobacillus casei* DN114001 – examination stress – immunomodulation – cortisol

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Introduction

The body is under constant stress due to stressors that may be physical, such as trauma, chemicals, intense exercise, or may originate cognitively. Most of the time, these stressors are dealt with adequately by coping mechanisms, but if the coping mechanisms are overwhelmed illness can result. Examples of psychological stressors include bereavement, care-giving, divorce and

house-moving, and these life events may have profound effects on health and the immune system, reducing natural defences and increasing susceptibility to infection and cancer [1, 2]. A well-studied model of psychological stress is the one associated with academic examinations, since diverse changes in immunocompetence, such as cytokine dysregulation, altered immunoglobulin levels, or lymphocyte function in students have been well documented [3–9].

Three major pathways underlie the effects of psychological stress on the immune system; the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic-adrenal-medullary (SAM) axis, and the endogenous opioid system [3, 10]. The central nervous system (CNS), endocrine system and immune system are intimately linked and the effects are bi-directional so that changes in either can affect the others [11]. The CNS can modulate the immune system since immune cells have receptors for neuroendocrine hormones, neuropeptides and neurotransmitters, and neuro-effector junctions exist between lymphoid organs and sympathetic neurons [3]. In addition, the immune system can influence the CNS due to the presence of receptors for cytokines in the CNS [10].

Lactic acid bacteria (LAB) are examples of probiotics, living micro-organisms that when ingested in sufficient numbers have a beneficial effect on the health of the host. The effects vary with particular strains or possible strain mixture, and milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 has been proven to be highly effective in protecting mice against *Salmonella* infection [12], reducing diarrhea in children [13, 14], and protecting rats against rotavirus infection [15]. These beneficial effects may be exerted in a variety of ways, for example by altering the profile of the gastrointestinal microflora, enhancing the barrier function of the gastrointestinal mucosa and enhancing immunity. The combination of yogurt cultures plus *Lactobacillus casei* DN-114001 has previously been reported to have an immunomodulatory role, by reducing TNF-α production from gastrointestinal biopsies from patients with Crohn's disease [16]. In addition, consumption of milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 reduced the depression of peripheral blood NK cell numbers associated with strenuous exercise in healthy adults [17]. These effects may be due to enhancement of mucosal as well as systemic immunity and further studies are required to elucidate the mechanism of action of LAB on the immune system of healthy subjects.

The present study was designed to find out whether milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 could modulate the immunocompetence of university students under academic examination stress.

Subjects and methods

Healthy students ($n = 155$) aged 18–23 years were recruited from the Universidad Alfonso X El Sabio (Madrid, Spain), all belonging to Sciences careers, mostly related to Nutrition. The subjects had no history of milk allergy or intolerance, and no other atopic symptoms during the study. Subjects were also excluded if they had suffered any infection during the previous month to the study or during the whole period of the study or if they had participated in other nutritional intervention studies during the previous 3 months.

The students provided written informed consent prior to the start, and the study was conducted in accordance with the ethical rules of the Helsinki Declaration (Hong Kong revision, September 1989), following the EEC Good Clinical Practice guidelines (document 111/3976/88 of July 1990) and current Spanish law which regulates clinical research in humans (Royal Decree 561/1993 regarding clinical trials). A number of students ($n = 19$) did not complete the study, mostly due to examination rescheduling, and one subject was excluded due to illness. Of the 136 subjects completing the study, 96 were female and 40 male, and all with normal BMI.

Study design

The study was prospective, randomized, controlled and parallel, powered to determine differences in absolute lymphocyte counts. The subjects were allocated to one of two groups, receiving either a glass (200 mL) of semi-skimmed milk each day (control group, $n = 63$) or two 100 mL portions per day (treatment group, $n = 73$) of fermented milk (Actimel®, Danone, France) containing yogurt cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* (10^7/mL) and *Streptococcus salivarius* subsp. *thermophilus* (10^8/mL) *plus Lactobacillus casei* DN-114001 (10^8/mL). The students were suggested to consume either milk or the fermented milk early in the morning, as a product included into their breakfast. This intervention was conducted for the 3 weeks prior to, as well as the 3-week duration of the student's examination period. The students were told to continue with their normal diet, although they were not permitted to consume any other fermented milk products during the study.

Anxiety levels and hematological, biochemical and immunological measurements were recorded at the start of the study (baseline) and 6 weeks later at the end of the examination period (study end). On these days students arrived at the laboratory in the morning and provided fasted venous blood samples and underwent anthropometric measurements (body weight, height). Dietary compliance was assessed using both a 7-day diet diary and an eating frequency questionnaire.

Anxiety

Levels of stress were assessed using the Spielberger state-trait anxiety inventory (STAI). This instrument is comprised of two 20-item self-report scales to assess the current level of anxiety (anxiety-state, i. e. how respondents feel "right now, at this very moment") and anxiety-proneness (anxiety-trait, i. e. how people "generally feel"). Feelings of apprehension, tension, nervousness, and worry are evaluated on both scales.

Hematology and biochemistry

Routine blood hematology (blood cell differential, hemoglobin, hematocrit, hematic indexes, platelets) using whole blood collected into EDTA was assessed using an automated analyzer (Technicon H1, Bayer, Tarrytown, NY). Routine biochemistry (glucose, lipid profile, iron, ferritin, transferrin, total protein, albumin, pre-albumin, liver enzymes, uric acid and urea) was assessed in serum from clotted samples using an automated analyzer (Olympus AU 650). Serum cortisol was measured by nephelometry (Behring nephelometer analyzer, Dade-Behring).

Immunology

Lymphocyte subsets were measured by flow cytometry (FACScan, BD, Sunnyvale, CA). Heparinized whole blood was incubated with monoclonal antibodies (BD Biosciences): CD2 [total T cells + natural killer (NK) cells], CD3 (mature T cells), CD4 (helper cells), CD8 (cytotoxic/suppressor cells), CD19 (B cells), CD56 (NK + subpopulation of cytotoxic T cells) for 45 minutes at room temperature, and processed using the Immunoprep system (Q-prep, Coulter Corporation, Hialeah, Florida). Lymphocytes were gated by forward and side scatter and pan-leucocyte marker expression (CD45, BD Biosciences). Triple staining procedure (CD3/CD4/CD8, CD19/CD2/CD56) was used.

Cytokine production was assessed in cultured mitogen-stimulated peripheral blood mononuclear cells (PBMC). Mononuclear cells were isolated from heparinized peripheral blood on Ficoll-Hypaque (Lymphoprep, Hyegaard, Oslo, Norway) and washed twice in RPMI-1640 medium (BioWhittaker, Verviers, Belgium). The PBMC were resuspended in RPMI-1640 containing 10 % fetal bovine serum and 1 % penicillin/streptomycin. The concentration was adjusted to 10^6 viable cells/mL and 1 mL of cell suspension was incubated per well with phytohemagglutinin (3.5 µL/mL) and lipopolysaccharide (1.5 µL/mL) in 24-well plates for 48 hours, at 37 °C and 5 % CO_2. Following incubation the cells were removed by centrifugation and supernatant stored at –80 °C prior to analysis. Cytokine (IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ) content of the supernatant was assessed using a Human Th1/Th2 cytokine CBA kit (BD Biosciences Pharmingen, San Diego, CA), and analyzed by flow cytometry. Phagocyte function was quantitatively assessed using Phagotest kit (BD Biosciences) and flow cytometry. A quantitative determination of the leukocyte oxidative burst was achieved using Bursttest kit (BD Biosciences) and flow cytometry. Serum immunoglobulins (IgG, IgM, IgA) were measured by nephelometry (Behring nephelometer analyzer, Dade-Behring).

Statistics

Data were assessed for normality and homogeneity of variance, and are expressed as mean ± standard error (SE). Due to the fact that there were some significant differences between control and treatment groups in baseline values especially for cytokine production by mitogen-stimulated PBMC and for some lymphocyte subsets (data not shown), the results were expressed as the differences between the data obtained from Phase 0 and Phase 1. This was calculated by subtracting the study end results from the baseline and it is termed "Treatment effect". Differences from baseline were assessed for each measurement using paired Students *t* test or Wilcoxon as necessary. Comparisons between groups were performed using ANOVA (with treatment as a factor), or where data infringed the assumptions for parametric analysis they were converted into ranges prior to analysis by ANOVA. Analysis was performed using SPSS software, and $P < 0.05$ considered significant.

Results

Anxiety

Overall, the level of state-anxiety (expressed in percentiles) increased significantly ($P < 0.05$) during the 6-week study, from 40.74 ± 2.50 to 61.19 ± 2.64 with little change in trait-anxiety (38.84 ± 2.45 to 39.53 ± 2.34). Both the state-anxiety and the trait-anxiety mean values are shown in Table 1. There were no significant differences between the control and treatment groups for state-anxiety levels which were similar at baseline (43.59 ± 4.00 and 38.31 ± 3.12, respectively) and study end (65.12 ± 3.71 and 57.77 ± 3.73, respectively), and increased significantly and similarly in both groups during the study (21.65 ± 5.09 and 19.14 ± 3.67, respectively). There were no significant differences between the control and treatment groups for trait-anxiety levels which were similar at baseline (40.55 ± 3.71 and 37.36 ± 3.28, respectively) and study end (40.00 ± 3.47

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Table 1 Anxiety test percentiles at baseline and study end

	Control group			Treatment group		
	Baseline	Study end	P-value[a]	Baseline	Study end	P-value[a]
State-anxiety	43.59 ± 4.00	65.12 ± 3.71	< 0.05	38.31 ± 3.12	57.77 ± 3.73	< 0.05
Trait-anxiety	40.55 ± 3.71	40.00 ± 3.47	> 0.05	37.36 ± 3.28	39.13 ± 3.20	> 0.05

[a] Significance of the difference between baseline and study end. (Paired t test)
Values presented are mean ± SE

and 39.13 ± 3.20, respectively), and changed similarly during the study (–0.95 ± 2.80 and 1.34 ± 2.55, respectively).

Cortisol

There was no significant difference between control and treatment groups in serum cortisol measured at baseline (19.98 ± 1.21 and 19.63 ± 1.02 μg/dL, respectively). During the study, mean serum cortisol increased 4.30 ± 0.98 μg/dL in the control group, and 1.75 ± 1.05 μg/dL in the treatment group, and no significant differences were found between both values (P = 0.062) (Fig. 1).

Phagocytic activity and oxidative burst

No significant differences were shown between the treatment and the control groups in the changes, over the 6-week period, in phagocytic activity (23.58 ± 3.77 and 30.16 ± 4.18 %, respectively) and oxidative burst capacities (8.42 ± 2.71 and 6.44 ± 3.22 %, respectively).

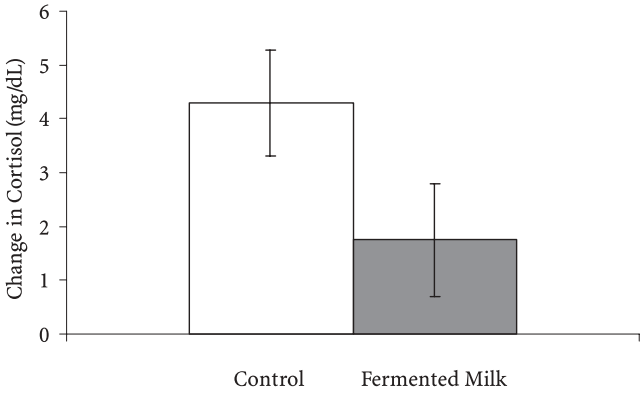

Fig. 1 The change in mean (± SE) serum cortisol during the 6-week study, calculated as study end minus baseline values, for control and treatment groups (P > 0.05)

Differential white blood cells

There was a significant treatment effect on the change in absolute lymphocyte numbers over the 6-week study, being –0.04 ± 0.12 x 10^3 cells/mm^3 for the control group and 0.37 ± 0.11 x 10^3 cells/mm^3 for the treatment group (P < 0.05). However, there was no significant treatment effect on the changes in total leukocyte, monocyte and granulocyte counts over the 6-week study (Table 2).

Lymphocyte subsets

Absolute numbers of CD56 cells (NK + cytotoxic T cells) at study end were 302.66 ± 27.32 and 301.31 ± 17.86 cells/mm^3 for the control and treatment groups, respectively; when comparing those levels to baseline levels (354.63 ± 28.88 and 284.02 ± 16.12 cells/mm^3, for the control and treatment groups, respectively) the absolute CD56 cell counts in the control group had significantly decreased at study end, whereas the absolute CD56 cell counts in the treatment group remained similar at study

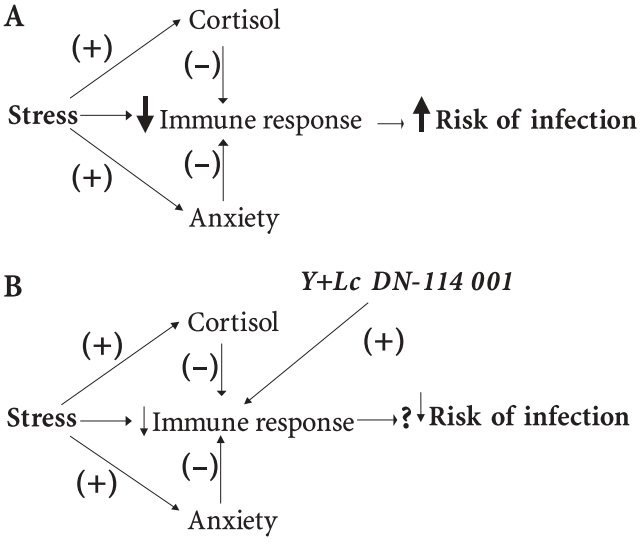

Fig. 2 **A** Consequences due to stress conditions. **B** Fermented milk with yogurt cultures *plus L. casei* DN-114001 after being consumed for 6 weeks produce a modulation of the immune response leading to lesser consequences due to stress conditions

Table 2 Change in differential white blood cell counts during the 6-week study

	Change over study[a]						
	Control group			Treatment group			Significance of difference between control and treatment groups
	Mean	N	SE	Mean	n	SE	
Leucocytes	154.74	57	271.49	554.14	70	263.30	> 0.05
Lymphocytes	–38.42	57	116.11	369.57	70	112.17	< 0.05
Monocytes	18.42	57	20.35	16.71	70	23.51	> 0.05
Granulocytes	174.56	57	228.99	162.71	70	198.09	> 0.05

[a] Change was calculated as study end minus baseline

end (Table 3). The treatment effect on change in CD56 cells over the 6-week study was statistically significant between both groups (–51.97 ± 21.33 cells/mm^3 and 17.29 ± 17.27 cells/mm^3 for control and treatment groups, respectively, $P < 0.05$). There was no significant treatment effect on the changes over the 6-week period, in CD2, CD3, CD4, CD8 and CD19 subsets (Table 3).

Cytokines

No significant differences were found between the treatment and the control groups in the changes, over the 6-week period, in IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ (Table 4).

Immunoglobulins

No significant differences between the treatment and the control group were shown in the changes in plasma immunoglobulin levels over the 6-week period (Table 5).

Table 3 Changes in absolute numbers of lymphocyte subsets during the 6-week study

Cells/mm^3	Change over study[a]				
	Control group, n = 53		Treatment group, n = 69		Significance of difference between control and treatment groups
	Mean	SE	Mean	SE	
CD56	–51.97	21.33	17.29	17.27	< 0.05
CD2	89.43	91.94	271.46	76.46	> 0.05
CD3	130.78	83.33	196.66	66.47	> 0.05
CD4	146.70	57.52	158.67	42.01	> 0.05
CD8	–60.28	34.90	9.83	27.65	> 0.05
CD19	29.76	17.14	67.31	14.90	> 0.05

[a] Change was calculated as study end minus baseline

Table 4 Change in cytokine concentrations during the 6-week study

Pg/mL	Change over study						
	Control group			Treatment group			Significance of difference between control and treatment groups
	Mean	N	SE	Mean	N	SE	
IL-2	108.22	52	93.06	274.69	62	166.12	> 0.05
IFN-γ	10,263.29	52	4,587.60	13,274.14	62	5,520.00	> 0.05
TNF-α	–141.24	52	377.24	–38.59	63	414.44	> 0.05
IL-4	103.92	52	27.26	133.59	63	63.45	> 0.05
IL-5	110.67	52	34.60	149.47	63	95.75	> 0.05
IL-10	99.28	52	246.53	236.07	62	144.23	> 0.05

Change was calculated as study end minus baseline

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Table 5 Change in immunoglobulin concentrations during the 6-week study

mg/dL	Change over study[a]						
	Control group			Treatment group			Significance of difference between control and treatment groups
	Mean	N	SE	Mean	n	SE	
IgG	−72.90	58	27.58	−49.91	69	24.93	> 0.05
IgA	−2.59	58	7.01	−11.93	69	4.79	> 0.05
IgM	0.29	58	5.69	1.49	69	3.73	> 0.05

[a] Change was calculated as study end minus baseline

Red blood cells and biochemistry

Red blood cell counts and corpuscular indices and serum biochemistry measurements remained within normal ranges during the course of the study, confirming the absence of disease (data not shown).

Background diet

There were no significant differences between groups in terms of overall dietary intake (data not shown).

Discussion

Psychological stress has long been associated with immunosuppression, which increases susceptibility to infectious disease [1] and may be implicated in cancer progression [2]. The present study found that in students under academic examination stress, dietary supplementation with a milk product fermented with yogurt cultures *plus Lactobacillus casei* DN-114001 modulates some immune parameters such as lymphocyte and CD56 cell number. This effect was seen in free-living healthy subjects consuming their usual diet, using practically achievable doses, and demonstrates a medium term (6 weeks) efficacy for milk fermented with yogurt cultures *plus Lactobacillus casei* DN-114001.

Models of psychological stress include medical students undergoing examinations, and study of these subjects has revealed that this stress is associated with dysregulation of the immune system and changes in many immune measurements. These include decreased IFN-γ, decreased lymphocyte proliferative response [4], decreased NK cell activity and numbers [6] and reduced CD4/CD8 T cell ratio [5]. Humoral immunity may also be affected, with changes in plasma immunoglobulin concentrations [6]. Overall, the suppression of cell-mediated immunity results in a reduction in the body's capacity to defend itself against pathogens and cancer, and increased respiratory tract infections, decreased response to vaccination and reactivation of latent viruses have been recorded as a result of examination stress [3]. Similar results have been obtained from other models of psychological stress including high school students [18] and carers of relatives with dementia [19]. The present study supports these findings with significant decreases in circulating CD56 cell numbers in students undergoing examination stress.

LAB in yogurt and fermented milks have long been known for their health promoting properties, since they beneficially alter the profile of the gastrointestinal microflora and may protect the host against pathogenic bacteria, protect against cancer and reduce allergy and atopy [20–23].

LAB also have an immunomodulatory function, binding to the luminal surface of gastrointestinal M cells and stimulating gut-associated lymphoid tissue. This outcome results in modulation of local and systemic immunity, strengthening the innate immune response and providing an adjuvant for the specific immune response [24].

There was a significant treatment effect in the students consuming milk fermented with yogurt cultures *plus Lactobacillus casei* DN-114001 in the present study, who showed significantly higher lymphocyte counts after 6 weeks and maintained their pre-examination CD56+ cell concentrations, compared to control subjects who showed unchanged lymphocyte counts and decreased NK cell concentrations at the end of the study. Although the CD56+ cell numbers measured in the current study would have contained a subset of T cells that are CD56+ CD8+, no significant treatment effect on CD8+ cells was shown after the 6-week period; thus, it could be assumed that CD56+ changes are due to NK cell changes. However, a putative contribution of decreasing CD8+ cell numbers to the overall CD56+ cells change can not be dismissed since this cell subset showed a decrease during the 6 week period which had a similar magnitude as the change in CD56+ cells.

These findings of increased NK cells support a previous study, where 30-day dietary supplementation with milk fermented with yogurt cultures *plus Lactobacillus casei* DN-114001 reduced the decline of NK cells associated with strenuous activity [17]. Studies with other *Lactobacillus* strains also support these findings, with increased NK cell responses in humans after 3

week-supplementation of fermented milk containing *Lactobacillus casei* shirota [25]. These outcomes could lead to a lower incidence of infections (Fig. 2). In this sense, an increased lymphocyte response and resistance to infection and cancer in animal models following supplementation with a variety of LAB have been documented [26–28]. Therefore, further research is required to find out to what extent this type of fermented milk can act as a probiotic, by reducing the risk of infection under and after stress situations.

Whereas some studies have reported increases in B lymphocytes, but not T lymphocytes following LAB administration [28], the present study found no significant difference in lymphocyte subsets compared to baseline, although there was a trend towards increases in both T cells (CD3+) and B cells (CD19+). Other measurements, such as cytokine production, also exhibited this trend, and support findings of increases in a range of mucosal and systemic immune measurements following LAB administration [23, 28–30]. However, other studies report no effect of LAB on immune measurements, or even decreased immune function [31, 32].

These contradictory findings may be due to differences in methodology (e. g. length of supplementation, dose, subjects, species, prevailing diet) or LAB strain [33]. In addition, the complex nature of the immune system should be considered during interpretation, since some studies report downregulation of the immune response associated with inflammation, allergy and autoimmunity following LAB supplementation [34, 35]. Downregulation of the inflammatory response was also observed following culture of mucosal explants from patients with Crohn's disease with *Lactobacillus casei* DN-114001, resulting in decreased production of TNF-α.

Stress ultimately results in cortisol release, although a number of previous studies have failed to find an association between stress and cortisol [4], or find increased cortisol only in a subset of subjects [36]. Serum cortisol was increased, although not significantly, by examination stress in the present study. However, the extent of the increase was almost significantly different between both groups. The control group showed a trend to a higher increase than the treatment group. Since cortisol and immunosuppression have been many times related, the findings in our study support that the link between these two deserves further attention.

The relationship between stress, glucocorticoids and immunosuppression is probably not straightforward [37], and there are many anomalies. For example, although administering exogenous corticosteroids reduces resistance to infection, corticosteroids are used to treat inflammatory diseases, and while chronic stress is known to exacerbate inflammatory disease, acute stress has the opposite effect [38]. Thus, although IL-6 production by peripheral blood mononuclear cells is reduced by stress, IL-6 production by the neuro-endocrine system is increased, which is pro-inflammatory [39]. In addition, cortisol and ACTH (adrenocorticotropic hormone) may be not necessarily correlated in models of stress [36]. It is likely that although pharmacological doses of corticosteroids suppress the immune response, physiological doses may have a range of effects from enhancement to suppression [40], and while acute stress enhances immune reactivity, chronic stress results in immunosuppression [40, 41]. The relationship between stress and cortisol is therefore complex, although anxiety (state-anxiety) and cortisol both increased during the current study.

Subjects consuming milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001 tended towards a reduced cortisol increase and increased lymphocytes. It is possible that the immunomodulatory action of LAB on lymphocyte numbers influenced the CNS through alterations in the balance of cytokines. Decreased release of cytokines such as IL-1 may reduce corticotrophin-releasing factor (CRF), ACTH and cortisol release [10], although IL-1 was not analyzed in the present study. However, this view is probably too simplistic since lymphocyte distribution is affected by the balance of corticosterone, epinephrine and norepinephrine, and increases and decreases in lymphocyte numbers are mediated through different pathways [40]. The interaction of the hypothalamic-pituitary-adrenocortical (HPA) and the sympathetic adrenal medullary (SAM) systems and opioids in orchestrating the response to stress is probably responsible for the variety of relationships between cortisol and immune measurements reported [42].

Alternatively, it is feasible that the LAB or gastrointestinal microflora had a direct effect on cortisol. Stress affects the gastrointestinal tract through a variety of mechanisms, including disruption of microcirculation [43] and decreased mucosal prostaglandin concentrations [44], and disrupts the balance of gastrointestinal microflora with a decrease in LAB [45]. However, these gastrointestinal changes were not correlated with cortisol secretion [43–45], and administration of a probiotic had no effect on serum cortisol in deer [46]. The link between the change in cortisol and lymphocyte numbers in the present study is therefore difficult to explain and requires further work.

Conclusion

This study is the first to demonstrate modulation of the altered immune response caused by psychological stress through consumption of milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114001. Therefore, further research is required to find out to what extent this type of fermented milk can act as a probiotic, by re-

ducing the risk of infection under and after stress situations. According to the design of this study, two daily servings of fermented milk with yogurt cultures *plus L. casei* DN-114001 for 6 weeks may be capable to modulate the immune system of university students going through academic examination stress by significantly increasing circulating lymphocyte numbers and preventing a decline in NK cell numbers. The effects shown on other immune parameters such as lymphocyte subsets, and cytokine response are not significant under the particular conditions of stress and treatment protocol (strain and dose of LAB contained in the fermented milk) used in this study. Finally, the observation of a nearly significant trend ($P < 0.06$) to a prevention of cortisol increase under academic examination stress with the consumption of the fermented milk could lead to a lower incidence of infections, although this outcome merits further investigation.

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