EFFECT OF SOUR MILK ENRICHED WITH CONJUGATED LINOLEIC ACID AND PROBIOTICS ON THE CONCENTRATIONS OF OXIDIZED LOW DENSITY LIPOPROTEINS AND GLUCOSE IN THE BLOOD PLASMA OF YOUNG PEOPLE

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Summary

Studies of conjugated linoleic acid (CLA) in certain animal models show that it tends to reduce body fat, has an antidiabetic effect, and retards the process of atherosclerosis. However, controversial evidence in some studies in humans has shown that CLA supplementation does not change overall body fat mass, increases the concentration of low density lipoproteins (LDL), decreases concentration of high density lipoproteins (HDL), and induces insulin resistance and oxidative stress. On the contrary, probiotics, if consumed by a immunocompetent individuals in adequate amounts, have almost exclusively beneficial effects. The aim of our study was to evaluate the effect of sour milk products enriched with CLA and probiotics on the concentrations of oxidized LDL and glucose in the blood plasma of young people. The study was performed on 59 randomly selected, healthy, non-obese young people divided in two groups: experimental and control. For 20 days, the experimental group (n=29) consumed 0.5 l/day of sour milk with CLA and probiotics. At the same time, the control group (n=30) consumed 0.5 l/day of sour milk with specific lactic acid *lactococci* strains and probiotics only. Before and after consumption of the product, body mass index (BMI) was calculated and blood samples were taken to investigate concentrations of oxidized LDL and glucose. The concentration of oxidized LDL in the experimental group increased from 34.19±14.14 to 47.63±13.57 U/l (P=0.0007), and the concentration of glucose decreased from 4.79±0.04 to 4.53±0.34 mmol/l (P=0.0001). The concentration of oxidized LDL in control group increased from 34.99±10.98 to 43.07±9.33 U/l (P=0.004), and the concentration of glucose decreased from 4.57±0.38 to 4.30±0.36 mmol/l (P=0.003). BMI did not change significantly in either group investigated. Sour milk enriched with CLA and probiotics increased the concentration of oxidized LDL in the blood plasma of young people more than sour milk without CLA. CLA possibly induced oxidative stress and lipid peroxidation. Sour milk enriched with CLA and probiotics has a positive effect on the metabolism of carbohydrates because it significantly decreased the concentration of glucose.

Key words: conjugated linoleic acid, oxidized low density lipoproteins, glucose, lipid peroxidation, oxidative stress, sour milk, atherosclerosis.

Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric linoleic acid isomers that have conjugated double bonds in their molecular structure. Since its discovery in 1987, CLA, based on certain animal models (1-7), has received much attention as a substance that possibly promotes antiobesity, has antidiabetic and antiatherogenic characteristics. Despite 30 years of intense, ongoing research, the effects of CLA on humans are still debated. It appears that CLA do contributes to antiobesity and muscle mass enlargement, but its antidiabetic and antiatherogenic role in human studies is much harder to prove. An increasing amount of evidence showing that CLA supplementation does not change overall body fat mass, increases LDL, decreases HDL, and induces insulin resistance and oxidative stress is accumulating (8-11).

On the contrary, if consumed by a immunocompetent individuals in adequate amounts, probiotics have almost exclusively beneficial effects that range from the stimulation of immune and digestive tract function to antioxidative and anticarcinogenic activity (12-14), depending on specific probiotic strains.

The aim of our study was to evaluate the effect of sour milk enriched with CLA and probiotics on the concentrations of oxidized LDL and glucose in the blood plasma of young people. To our knowledge this is the first attempt to use two substances beneficial to health – CLA and probiotics – into one product.

Methods

The prospective, randomized, double-blind, placebo-controlled study was performed in Vilnius University Hospital Santariskiu Klinikos in Lithuania. Sixty-five healthy, non-obese (body mass index, BMI=18.5-24.9) people aged 20-25 years were randomly selected and divided into an experimental group (n=29) and a control group (n=30). Healthy status was determined by absence of complaints or clinical signs and normal C-reactive protein levels before and after the study. Six people were eliminated from the trial because of acute infection (C-reactive proteins levels above normal at the end of the study) or intolerance to the product.

For 20 days, the experimental group consumed 0.5 l/day of sour milk with CLA (2.5 g/l) and probiotics. The sour milk contained approximately equal amounts of trans-10, cis-12 and cis-9, and trans-11 isomers of CLA. As probiotics *Bifidobacterium Bb-12* were used. At the same time, the control group received 0.5 l/day of sour milk with specific lactic acid *lactococci* strains and probiotics only. Subjects were asked to precede during the study with the same diet and other habits of their personal life. The products that were prepared did not contain any other added antioxidants.

Before and after consumption of the sour milk, BMI was calculated and blood samples were taken to investigate concentrations of oxidized LDL and glucose. Oxidized LDL and glucose concentrations were measured by enzyme linked immunosorbent assay (*StatFax 2100, Awareness Technology INC*) and GOD-PAP (*DIMENSION RxL System*) methods respectively. Data are expressed as mean ± standard deviation. The level of statistical significance was set at $p<0.05$. All calculations were made with the SPSS computer program (v. 13.0).

The study protocol was approved by the Lithuanian Bioethics Committee (Approval No. 29 of 24 July 2007).

Results

Our results show significantly higher concentrations of oxidized LDL and significantly lower concentrations of plasma glucose in both groups after consumption of sour milk (Fig. 1 and 2).

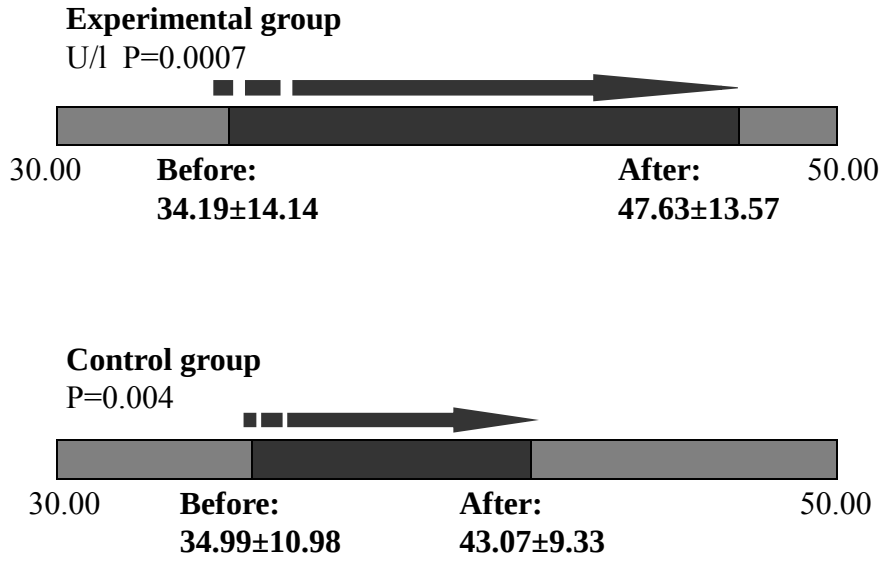

Figure 1. Oxidized low density lipoprotein concentrations before and after consumption of sour milk in experimental and control groups.

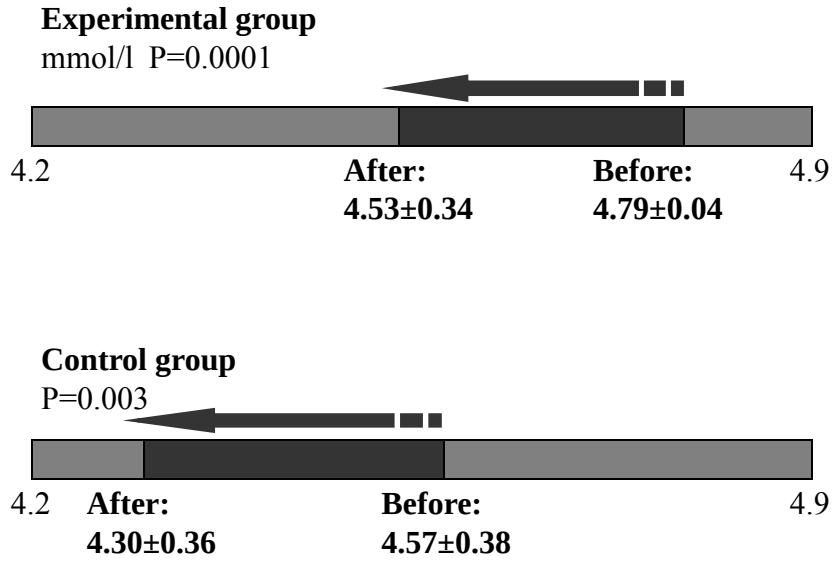

Figure 2. Glucose concentrations before and after consumption of sour milk in experimental and control groups.

We found that the concentration of oxidized LDL in the experimental group significantly increased from 34.19±14.14 U/l to 47.63±13.57 U/l (P=0.0007), and the concentration of oxidized LDL in the control group increased from 34.99±10.98 U/l to 43.07±9.33 U/l (P=0.004). The concentration of glucose in the experimental group decreased from 4.79±0.04 mmol/l to 4.53±0.34 mmol/l (P=0.0001), and the concentration of glucose in the control group decreased from 4.57±0.38 mmol/l to 4.30±0.36 mmol/l (P=0.003). BMI did not change significantly in either group investigated.

Discussion

The results of our study show that sour milk enriched with CLA and probiotics increased the concentration of oxidized LDL in the blood plasma of young people almost two-times more than sour milk with specific lactic acid *lactococci* strains and probiotics only (Fig.1). We anticipated that changes in oxidized LDL concentrations in both groups would be almost the same if probiotics could decrease the oxidative action of CLA. Possible explanation of the antioxidative action of probiotics could be that probiotics colonize the digestive tract and decrease pH. This more acidic environment inhibits the growth of pathogenic microorganisms in the digestive tract, which causes less inflammation. This leads to the release of fewer free radicals and diminished oxidative stress.

It is remarkable that our results show that sour milk with lactic acid *lactococci* strains and probiotics also increases concentrations of oxidized LDL, but we cannot adequately explain this increase. To our knowledge this is the first attempt to investigate the link between CLA and oxidized LDL. Most studies investigating the effects of CLA on oxidative stress were based on an analysis of antioxidative system components or markers of oxidative stress rather than oxidized LDL (9, 10).

Oxidized LDL is important because of its direct contribution to the process of atherosclerosis. Oxidized LDLs are engulfed through the mediation of scavenger receptors by macrophages and smooth muscle cells of progressing atherosclerotic streaks and plaques (15-19). Sigurdardoffir et al supported the hypothesis that circulating oxidized LDL could be a marker of unstable echolucent plaque phenotype in arteries (15), and Wallenfeldt et al showed associated circulating oxidized LDL with silent phase of atherosclerosis progression in clinically healthy men (16). In addition, according to Hulthe and Fagerberg (19), who proved the positive correlation between oxidized LDL concentration and atherosclerosis, we can assume that sour milk enriched with CLA and probiotics could lead to the formation of atherosclerotic plaques in vascular intima. Furthermore, commercial CLA supplements used for weight loss by obese people could have a negative effect on health because of increased cardiovascular morbidity and mortality risk.

Sour milk products enriched with CLA and probiotics have a positive effect on the metabolism of carbohydrates because they significantly decrease the concentration of glucose (Fig.2). Although many studies support the idea that CLA increases plasma glucose concentration because of induced insulin resistance (oxidative stress trigger impairs cellular insulin signalling) (9-11, 20, 21), the results of our study demonstrate that sour milk enriched with CLA and probiotics significantly decreases glucose concentration in the blood plasma of young people. We also found that glucose concentration was significantly lower in the blood plasma of the control group after subjects consumed the sour milk product. In the control group the decrease in plasma glucose was possibly due to sour milk itself and probiotics. We cannot, however, perform a detailed comparative analysis on both groups because of the uneven starting concentrations of glucose at

the beginning of the study. The possible cause of the decrease in glucose concentration is not likely because of diminished insulin resistance.

BMI changes were insignificant, possibly a result of the short study time, the small concentrations and mixed isomers of CLA used (2.5 g/0.5 l/day), or the participation of non-obese subject. Some scientific literature indicates that a significant decrease in BMI was achieved when much larger concentrations of CLA were used (3-7.2 g/day) (22). But it is worth nothing that usually the results of human clinical studies on the effects of CLA on body weight and composition are inconsistent (23). Plourde et al propose that the discrepancy between animal and human body composition studies is related to methodological differences and, to a lesser extent, to the CLA dose and isomers used (24). It is however much more likely that CLA actually does not affect body composition and fat deposition in humans, or does so only slightly.

We think that sour milk enriched with CLA and probiotics could be a product that significantly benefits health and the studies should be continued in the future. This product could start a new exciting CLA research period in which an endeavour will be made to minimize the negative effects and at the same time augment the positive effects of CLA. It is advisable to perform other studies of sour milk enriched with CLA and probiotics, but to select other CLA isomers and higher concentrations of CLA in sour milk. It would also be beneficial to enrich sour milk with antioxidant vitamins, and to use other probiotics with higher antioxidant action (possible candidate: Lactobacillus fermentum ME-3 (14)).

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