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Impact of systemic enzyme supplementation on low-grade inflammation in humans

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ABSTRACT

Systemic enzyme therapy has been shown to be efficient in treating pain and inflammation associated with injury or musculoskeletal disorders. However, whether systemic enzyme supplementation also attenuates subclinical inflammation remains to be investigated.

In this randomized controlled trial, we investigated the impact of systemic enzyme supplementation on inflammatory gene expression as well as on markers of inflammation in 24 adult men and women with subclinical inflammation (serum C-reactive protein [CRP] levels >1 mg/L and <10 mg/L). Participants were supplemented with systemic enzymes (Wobenzym®450 FIP from bromelain and 1440 FIP from trypsin, 6 tablets/d) or placebo for periods of 4 weeks separated by a 4-week washout period.

Systemic enzyme supplementation had no impact on expression levels of whole blood cell inflammatory genes compared with placebo but significantly reduced serum IL-6 levels ($p = 0.04$). However, there was a significant sex × treatment interaction for IL-6 ($p = 0.02$) and CRP ($p = 0.007$). Specifically, both serum IL-6 and CRP concentrations were significantly reduced in men ($p \leq 0.03$) but not in women ($p \geq 0.08$).

This study suggests that short-term supplementation with systemic enzymes may attenuate subclinical inflammation, with perhaps greater effects among men than among women.

1. Introduction

Inflammation is being increasingly recognized as a key etiological factor in the development of atherosclerosis and subsequent cardiovascular disease (CVD) [1] and is frequently found co-segregating with obesity and metabolic syndrome [1–3]. C-reactive protein (CRP) has been used extensively as a non-specific marker of the acute phase response for decades [4]. Data have further shown that CRP is a powerful predictor of CVD outcomes in epidemiological studies [5]. Indeed, studies that have investigated the predictive value of subclinical CRP levels have been relatively consistent in showing that individuals with high serum CRP levels (>3.0 mg/L) are at greater risk of CVD compared to individuals with lower (<1.0 mg/L) CRP levels, independent of gender and plasma cholesterol concentrations [6]. Other blood markers of active subclinical inflammation include monocyte chemotactic protein (MCP-1), adiponectin and interleukins (IL) such as IL-6 [7].

Systemic enzyme therapy, which involves the oral delivery of primarily proteolytic enzymes in combination with rutin and administered in the absence of food, has been recommended for many years for the treatment of pain and inflammation associated with musculoskeletal disorders, arthritis and post-surgery [8–10]. However, the impact of systemic enzyme supplementation on subclinical inflammation associated with metabolic syndrome and obesity is less known. In rabbits fed a lipid-rich, metabolic syndrome-inducing diet for 8 weeks, supplementation with systemic enzymes significantly reduced serum CRP concentrations [11]. To the best of our knowledge, no study has yet documented the impact of systemic enzyme supplementation on subclinical inflammation in humans.

The objective of this study was to examine the impact of systemic enzyme supplementation on inflammatory gene expression in whole blood cells and on blood markers of inflammation in men and women with subclinical inflammation. We hypothesized that systemic enzyme supplementation for 4 weeks down-regulates the expression of genes associated with inflammation

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in whole blood cells and reduces the concentrations of inflammatory biomarkers.

2. Material and methods

2.1. Study design

The study was conducted as a double blind, crossover, randomized, placebo controlled trial at the Institute of Nutrition and Functional Foods (INAF) in Québec City, Canada. Participants were supplemented with systemic enzymes in the form of Wobenzym® or placebo for periods of 4 weeks each in random order, with a 4-week washout between the two treatment phases. Treatment sequence was assigned to participants via the use of random sequence of numbers. Allocation to treatment sequence was concealed by a secure computer-assisted method enabling preservation of assignments until enrollment was confirmed. The study sponsor held the trial codes, which were disclosed after completion of the statistical analyses. Study products (Wobenzym® and placebo) were supplied by Mucos Pharma GmbH & Co. KG (Oberhaching, Bavaria, Germany). The systemic enzyme product was delivered in tablets each providing 90 mg (450 FIP units) bromelain from pineapple, 48 mg (1440 FIP units) trypsin from bovine and porcine pancreas, and 100 mg rutin from *Sophora japonica*. The placebo contained no active ingredients. Both the enzyme product and placebo contained the same inactive ingredients, were enteric coated, and were white film coated to ensure blinding (titanium dioxide) (Table 1). Participants were instructed to consume 6 tablets/day, 45 min before a meal for 4 weeks. The study protocol was approved by Université Laval's Research Ethics Board and is registered at ClinicalTrial.gov # NCT01848808.

2.2. Subjects

Men and women were recruited from the general population in the Québec City metropolitan area through paper advertisements and electronic newsletters. To be eligible, participants needed to be aged between 18 and 75 years and have serum CRP levels >1 mg/L and <10 mg/L on 2 separate days at screening. Exclusion criteria were: hypersensitivity to components of the systemic enzyme supplement, severe congenital or acquired coagulation disorders (e.g. hemophilia, in dialysis patients) or liver damage, pregnancy or breastfeeding, planned surgical operations during the study, any clinical signs or laboratory evidence for severe inflammatory, endocrine, renal/pulmonary, neurological, cardiovascular, metabolic, gastrointestinal, hematological, or psychiatric condition and active malignancy of any type other than basal cell carcinoma. Other exclusion criteria were current use of anticoagulants or platelet aggregation inhibitors, chemotherapeutic agents, antibiotics, medication for lipids, diabetes, hypertension, inflammation, autoimmune diseases, mood disorders or NSAID within 1 month of entering the study, excessive alcohol consumption (more than two drinks per day for men, one for women) or alcoholism, smoking, drug use and history of drug abuse, as well as current use of supplements or natural health products.

A total of 250 subjects were screened by phone and 91 of them were invited to a first screening visit. Forty-one potentially eligible subjects, based on a first serum CRP level of >1 mg/L, were invited to the 2nd visit to complete the screening process, including a second assessment of serum CRP. A total of 27 subjects met all eligibility criteria (10 men and 17 women). One female interrupted her participation because of adverse event (gastritis). There were two other dropouts due to lack of availability during the study. Thus, 24 subjects completed the study (see study flow chart at Fig. 1).

Table 1
Composition of study products.

	Wobenzym®	Placebo
Active ingredients		
Bromelain, mg/tablet	90	0
Trypsin, mg/tablet	48	0
Rutin, mg/tablet	100	0
Other ingredients (binders)	Yes	Yes
Enteric coating (pH resistant)	Yes	Yes
White coating (titanium dioxide)	Yes	Yes

2.3. Measurements

Subjects were instructed to avoid intense physical exercise 36 h before blood samples were taken and came to the clinical investigation unit after a 12 h overnight fast. Inflammatory gene expression in whole blood cells was assessed on samples collected at the end of each treatment phase. Serum concentrations of inflammatory markers and lipid levels were measured twice on two consecutive days after each treatment. The mean of the two post-treatment measurements was used in the analyses. General health assessment (complete blood count, liver and kidneys function), blood pressure, anthropometric measurements (height, weight, waist and hip girths, and body composition) as well as medical history were assessed prior to randomization. Participants also completed a questionnaire assessing diet and physical activity, as well as occurrence of any side effects during the study, as detailed below.

2.3.1. Anthropometry and blood pressure

Anthropometric measurements (body weight, height, waist and hip girths) were collected according to standardized procedures [12] at the first screening visit as well as before and after each phase. Systolic and diastolic blood pressures were averaged from 3 measurements taken after a 10 min rest in the sitting position using an automated blood pressure monitor (Omron, HEM-907XL).

2.3.2. Body composition assessed with dual-energy X-ray absorptiometry (DXA)

Baseline body composition was measured prior to initiating the first treatment phase with dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, GE Lunar Corporation, Madison, WI, USA). The scanner was calibrated before each measurement session against the standard calibration block supplied by the manufacturer for possible baseline drift. A quality-control test to monitor the reproducibility and stability of data was also performed before each session using a spine phantom provided by the manufacturer. The value from the quality-control test was plotted on graphs, and the score of each measurement was required to be within ± 0.05 g/cm^2 of the baseline result. More detail on the procedure is provided in Supplementary material.

2.3.3. Diet and physical activity

Eligible subjects received instructions from a registered dietitian regarding the forbidden use of specific supplements and medication during the study and to keep their nutritional and physical activity habits constant. Dietary intake during the study was assessed on three occasions using a validated web-based food-frequency questionnaire (FFQ) [13]: 1- at study entry; 2- after the first treatment and 3- after the second treatment. This validated FFQ inquires on food intake over the last 4 weeks, which is consistent with treatment duration in this study. Data from these questionnaires were analyzed using the Nutrient Data System software based on a mix of Canadian and FDA-produced nutrient

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