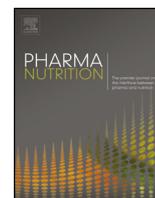




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Impact of systemic enzyme supplementation on low-grade inflammation in humans

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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 \times 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.

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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 ClinicalTrials.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).

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/cm² 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

Table 1
Composition of study products.

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

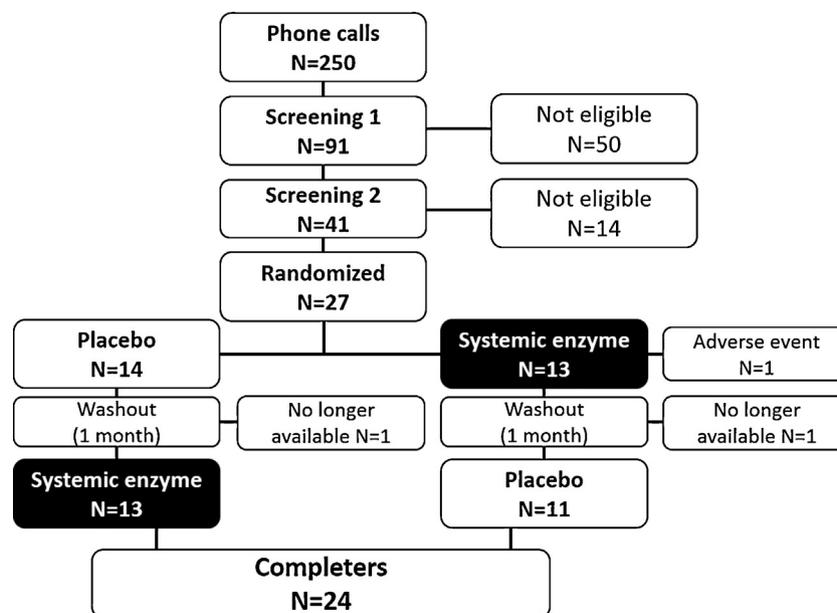


Fig. 1. Study flow chart.

databases. Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) [14], which takes into account physical activity as a whole (at work, at home, transportation and leisure). More detail on the procedure is provided in Supplementary material.

2.3.4. Pro- and anti-inflammatory gene expression measurement

PAXgene blood RNA kits (Qiagen, Mississauga, ON, Canada) were used to isolate mRNA from whole blood cells taken on the last day of each treatment phase. The concentration of the purified RNA was analysed using NanoDrop (Thermo Scientific, Wilmington, DE, USA). Complementary DNA (cDNA) synthesis was completed using 1 µg of total RNA and the High Capacity cDNA Kit (Life Technologies, Foster City, CA, USA). Gene expression was assessed by real-time PCR (RT-PCR) using Applied Biosystems Gene Expression Assays. Primers and TaqMan[®] probes were obtained from Applied Biosystems (interleukin-1beta (IL-1β): Hs01555410_m1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NF-κB1): Hs00765730_m1, peroxisome proliferator-activated receptor alpha (PPARA): Hs00947536_m1, tumor necrosis factor (TNF): Hs00174128_m1, TNF-receptor associated factor 3 (TRAF3): Hs00936781_m1), Applied Biosystems, Foster City, CA, USA). Each sample was analyzed in triplicate and calibrated to the ATCB housekeeping gene (Hs99999903_m1). Relative quantification was performed on an Applied Biosystems 7500 Real-Time PCR System and the $\Delta\Delta$ CT calculation method was used to assess the mean fold expression difference (MFED) between the two treatments [15].

2.3.5. Pro- and anti-inflammatory markers and serum lipids

Serum CRP levels were measured using the Behring Latex-Enhanced (highly sensitive) CRP assay on the Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer as described previously [16]. Serum adiponectin was measured by ELISA (B-Bridge International Inc., #K1001-1). Serum IL-6 was assessed by immunoassay (R&D system, #HS600B). Monocyte chemoattractant protein-1 (MCP-1) was assessed by immunoassay (R&D system, #DCP00). Inter-assay

coefficients of variations (CV) for each of these assays were <1% (CPR), 5.2% (adiponectin), 7.8% (IL-6) and 5.7% (MCP-1). Serum cholesterol (C), triglycerides and HDL-C were assessed on a Roche/Hitachi Modular according to the manufacturer's specifications and using proprietary reagents. Plasma LDL-C concentrations were calculated using the Friedewald Equation. CVs for cholesterol, LDL-C, TG and HDL-C were all below 3%.

2.4. Safety and intolerance symptoms

Complete blood count, blood clotting and liver and kidney functions were assessed after each of the 4-week supplementation periods. Subjective tolerance ratings of the frequency and intensity of side effects were obtained by questionnaire administered on site at the beginning and end of each treatment period. On each occasion, participants indicated whether each side effect was absent (0), of mild intensity (1), of moderate intensity (2) or of severe intensity (3).

2.5. Statistical analyses

Statistical analyses were undertaken in a blinded fashion without knowledge of treatment allocation. The primary analyses consisted in comparing the expression levels of anti- and pro-inflammatory genes in whole blood cells between systemic enzyme supplementation and placebo. The secondary analyses consisted in assessing the change in the serum inflammatory markers and lipid levels with treatment. Data were analyzed using the PROC MIXED procedure for repeated measures in SAS (version 9.3; SAS, Inc., Cary, NC). Treatment and sex were considered as fixed effects and subject was considered as random effect. Interaction of treatment by sex was investigated systematically for each study outcome by introducing the appropriate term into the mixed models. There was no significant treatment by sequence interaction for any of the study outcomes. Variables were log-transformed if needed prior to statistical analysis. In such cases, geometric means are presented. Differences have been considered significant at $P \leq 0.05$ (two-tailed). Analyses were undertaken on a

per protocol basis, i.e. only in subjects with complete data ($N = 24$), excluding dropouts.

3. Results

3.1. Subject characteristics at baseline

Table 2 shows the baseline characteristics of the 15 women and 9 men who completed the study. Supplemental Table 1 (Supplementary material) shows their physical activity levels as well as usual dietary habits. Mean age (S.D.) of participants was 45.5 (17.1) years. Six of the women were postmenopausal. With respect to ethnicity, 23 subjects were Caucasian and 1 was Asian. All participants were non-smokers. Mean systolic and diastolic blood pressures, blood lipids and glucose status were in the normal range. Average serum CRP concentration at screening was 2.99 (1.90) mg/L and was slightly higher in women than in men.

3.2. Systemic enzyme supplementation and inflammation

Table 3 summarizes the impact of 4-week systemic enzyme supplementation vs. placebo on whole blood expression levels of inflammatory genes. There was no difference between treatments in the expression of any of the selected genes. As shown in Table 4, 4-week supplementation with systemic enzymes vs. placebo significantly reduced serum IL-6 concentrations ($p = 0.04$) but had no effect on mean serum CRP ($p = 0.47$), MCP-1 ($p = 0.39$) and adiponectin levels ($p = 0.73$). A significant treatment by sex interaction was observed for CRP ($p = 0.007$) as well as for IL-6 ($p = 0.02$). As shown in Fig. 2, 4-week supplementation with systemic enzymes compared with placebo significantly reduced serum CRP ($p = 0.03$) and IL-6 ($P = 0.008$) in men but not in women ($p \geq 0.08$).

3.3. Systemic enzyme supplementation and blood lipids

As shown in Table 5, there was no effect of systemic enzyme supplementation on lipid levels, with the exception of serum cholesterol, which was increased slightly but significantly by 3.1% ($P = 0.05$). Participants' weight, waist circumference and blood pressure remained unchanged with treatments (not shown).

Table 2
Characteristics of study participants at screening.

Variables	All ($N = 24$)	Women ($N = 15$)	Men ($N = 9$)
Age (years)	45.5 (17.1)	44.1 (17.2)	47.8 (17.8)
Body mass index (kg/m ²)	26.7 (5.2)	26.2 (6.0)	27.6 (3.8)
Waist circumference (cm)	90.8 (14.4)	86.7 (14.4)	97.8 (12.3)
Percent fat (%)	33.6 (9.3)	36.6 (9.9)	28.6 (5.8)
Total abdominal fat (kg)	2.37 (1.17)	2.23 (1.27)	2.59 (1.00)
Systolic blood pressure (mmHg)	112.3 (12.9)	107.6 (12.5)	120.1 (9.6)
Diastolic blood pressure (mmHg)	65.5 (9.3)	63.7 (8.0)	68.4 (10.9)
C-reactive protein ^a (mg/L)	2.99 (1.90)	3.37 (1.98)	2.36 (1.66)
Fasting glycemia (mmol/L)	5.03 (0.51)	4.91 (0.48)	5.22 (0.54)
Total cholesterol (mmol/L)	5.48 (1.00)	5.62 (0.92)	5.26 (1.15)
LDL-cholesterol (mmol/L)	3.07 (0.82)	3.07 (0.85)	3.07 (0.81)
HDL-cholesterol (mmol/L)	1.76 (0.45)	1.95 (0.44)	1.45 (0.29)
Triglycerides (mmol/L)	1.41 (0.68)	1.30 (0.60)	1.59 (0.81)
Cholesterol/HDL-cholesterol ratio	3.28 (0.95)	2.99 (0.72)	3.76 (1.12)
Metabolic syndrome (%)	12.5	0	33.3

Values are presented as means (S.D.) except for metabolic syndrome prevalence.

^a Average of 2 measurements taken on 2 different occasions.

Table 3

Relative expression of genes related to inflammation after supplementation with systemic enzymes for 4 weeks vs. placebo supplementation.

Gene	Systemic enzymes	Placebo	Fold change ^a	P_{tx}
IL-1β	7.07 (0.14)	7.04 (0.22)	0.98	0.82
NFγB1	6.89 (0.08)	6.83 (0.13)	0.96	0.50
PPAR-α	9.47 (0.09)	9.43 (0.12)	0.97	0.70
TNF	8.60 (0.09)	8.61 (0.12)	1.01	0.92
TRAF3	8.16 (0.08)	8.17 (0.15)	1.01	0.87

Values are presented as means (standard error of the means). Each gene expression was calibrated to the ATCB housekeeping gene. Higher values indicate lower gene expression.

Analyses presented there are based on the PROC MIXED procedure in SAS on $N = 24$ subjects. P value from the main effect of treatment in the mixed model. Abbreviations: IL-1 β , interleukin-1 β ; NF γ B1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PPAR- α , peroxisome proliferator-activated receptor alpha; TNF, tumor necrosis factor; TRAF3, TNF-receptor associated factor 3.

^a Fold change in gene expression in the systemic enzymes vs. placebo = $2^{-(\text{mean "delta Ct mean" from the systemic enzymes} - \text{mean "delta Ct mean" from the placebo})}$.

3.4. Compliance and side effects

Compliance as assessed by number of tablets returned to the research team was very high (95%) and was comparable between the two treatments (not shown). There was no difference between the two treatments on safety parameters assessed (liver and kidney functions, Supplemental Table 2). There was no difference between treatments in number of red cells, white cells and platelets. There was a significant reduction in the absolute count of lymphocytes after systemic enzyme supplementation ($P = 0.03$) while the relative count of lymphocytes remained unchanged ($P = 0.07$, Supplemental Table 3). Occurrence of side effects was low and similar between the two treatments, with the exception of fatigue intensity rating and frequency, which was slightly higher during systemic enzyme supplementation than during placebo ($p = 0.04$ both for intensity and frequency, not shown).

4. Discussion

Several studies on inflammation related to arthritis and post-surgery have reported beneficial anti-inflammatory effects of supplementation with systemic enzymes. Less is known about this treatment modality on subclinical inflammation associated with obesity and metabolic syndrome. Our hypothesis was that supplementation with systemic enzymes attenuates inflammation in men and women with subclinical inflammation and that this effect can be perceived at the gene expression level as well as at the

Table 4

Serum levels of inflammatory markers after supplementation with systemic enzymes for 4 weeks vs. placebo.

Inflammation variables	Systemic enzymes	Placebo	% Difference	P_{tx} ^a
CRP (mg/L) ^b	2.17 (2.45)	2.16 (2.13)	0.5%	0.47
IL-6 (pg/mL)	1.25 (0.89)	1.41 (0.98)	-11.3%	0.04
MCP-1/CCL2 (pg/mL)	367.6 (113.1)	356.3 (100.7)	3.1%	0.39
Adiponectin (μ g/mL) ^b	8.43 (1.53)	8.53 (1.50)	-1.2%	0.73

Values are presented as means (S.D.) and percentage of change from placebo.

Analyses presented there are based on the PROC MIXED procedure in SAS on $N = 24$ subjects.

Abbreviations: CRP, C-reactive protein; IL-6, interleukine-6; MCP-1/CCL2, monocyte chemoattractant protein.

^a P value from the main effect of treatment in the mixed model. Models for CRP and IL-6 included treatment, sex and treatment by sex interaction; Model for MCP-1/CCL2 included treatment only; model for adiponectin included treatment and sex.

^b Analyses performed on log-transformed values. Geometric means are presented in such cases.

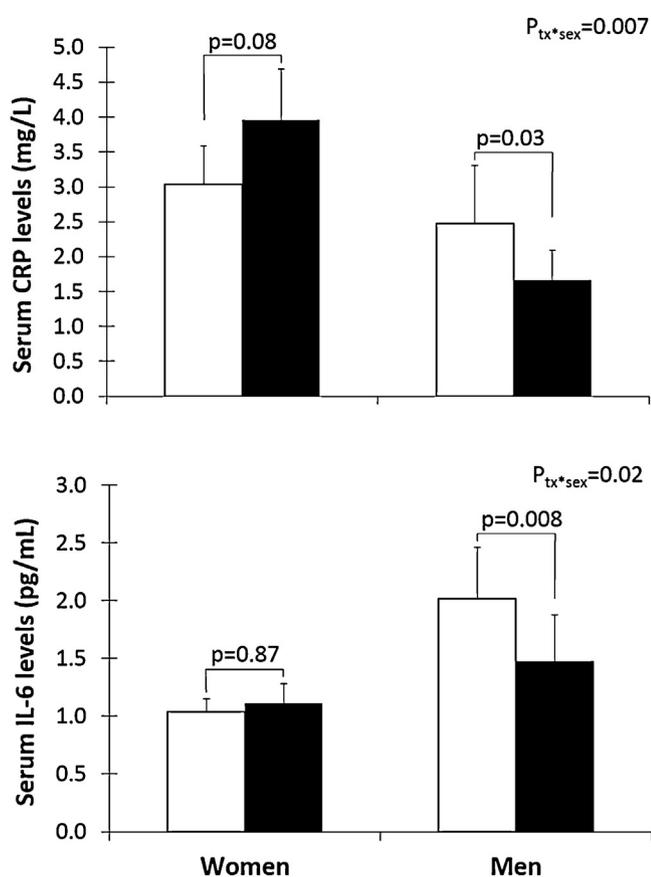


Fig. 2. Serum CRP and IL-6 levels in women and men after a 4-week supplementation with systemic enzymes (black bars ■) or placebo (open bars □). Values are presented as means (standard error of the means).

level of blood biomarkers of inflammation. Data suggest that supplementation with a mix of systemic enzymes (Wobenzym®) for 4 weeks has no effect on expression levels of genes related to inflammation in whole blood cells. However, systemic enzyme supplementation may have subclinical anti-inflammatory effects, as evidenced by a reduction in serum IL-6 concentrations. Although our study was not originally designed specifically to investigate differences between men and women, data revealed a significant treatment by sex interaction for IL-6 and CRP concentrations. Specifically, reduction in both of these inflammatory markers was significant in men but not in women.

While previous studies having investigated systemic enzymes in acute inflammatory conditions have shown similar anti-

inflammatory effects in both men and women [8,17], other evidence have highlighted sex differences in inflammation processes and levels [18–20]. Sex differences in CRP concentrations may be due at least partly to the impact of estrogens as premenopausal women have higher levels of CRP compared to men [18] and hormone replacement therapy has also been associated with increased CRP concentrations [21,22]. Data from our sample are compatible with this concept that women tend to have higher serum CRP levels than men (+42% higher CRP in women compared with men). Recent evidence has also suggested that both gene expression and inflammatory biomarkers may respond differently to dietary change [23,24]. Further studies are therefore needed in a context of subclinical inflammation to validate the observation that men may be more responsive than women to certain anti-inflammatory therapies, such as systemic enzyme supplementation.

The apparent disconnect between the absence of change in inflammatory gene expression and the change in inflammatory marker levels in men also needs to be discussed. Changes in gene expression induced by a dietary intervention generally occur rapidly, before changes in protein level can be observed. However, protein levels reflect the balance between both RNA and protein production and turnover. In that context, the correlation between the concentrations of proteins with their corresponding mRNAs is not always strong [25]. In the present study, systemic enzymes were supplemented for a period of 4 weeks, which should have been long enough to induce changes in gene expression levels, especially considering that small but significant anti-inflammatory effects were observed. It is possible that the effect of systemic enzymes on gene expression occurred acutely, i.e. after each supplementation of the tablets, and that it was not perceived when gene expression levels were measured at the end of the treatment period. It is also likely that the potential anti-inflammatory effect of systemic enzyme supplementation was not mediated by the particular genes and transcription factors investigated in this study, or that whole blood cells do not reflect the anti-inflammatory changes occurring in other tissues after supplementation with systemic enzymes.

Mechanisms through which systemic enzyme products such as Wobenzym® may have anti-inflammatory effects have been proposed [11,26,27]. Talaieva and Bratus speculated that systemic enzymes may act indirectly through reduction of inflammation-associated reactive oxygen species generation, and/or through the effect of other products in the formulation such as rutin, a flavonoid with antioxidant properties [28]. Others have suggested that these proteases may form complexes with cytokines through the interaction of specific anti-proteases widely available in human blood and promote their clearance from inflamed tissues via endocytotic and phagocytic routes [29,30]. Other mechanisms, such as interactions with cell surface receptors and signal transduction events have been suggested [29]. One of the systemic enzyme ingredients, bromelain, has been shown to have anti-inflammatory effects as evidenced by an attenuated IL-1β, IL-6 and TNF-α from THP-1 cells after LPS stimulation [31].

At the beginning and at the end of each treatment period, participants were invited to indicate the frequency and intensity of side effects over the last 4 weeks. Participants reported slightly but significantly more fatigue while being supplemented with systemic enzyme than placebo both for intensity and frequency. However, there was no difference in the frequency of other side effects between systemic enzyme supplementation and placebo. There was also no difference between the two treatments on safety parameters of liver and kidney function. The reduction in the absolute count of lymphocytes observed with the systemic enzyme treatment was significant but remained in the normal range. Moreover, the absolute count was not different and the white cells,

Table 5

Lipid profile after supplementation with systemic enzymes for 4 weeks vs. placebo supplementation.

Lipid variables	Systemic enzymes	Placebo	% difference	P_{tx}^a
Total cholesterol (mmol/L)	5.61 (0.98)	5.44 (1.03)	3.1%	0.05
LDL-C (mmol/L)	3.22 (0.74)	3.11 (0.77)	3.6%	0.17
HDL-C (mmol/L)	1.74 (0.45)	1.71 (0.48)	1.2%	0.40
Triglycerides (mmol/L) ^b	1.28 (1.55)	1.21 (1.59)	5.5%	0.20
Total cholesterol:HDL-C ratio	3.40 (0.93)	3.37 (1.00)	0.9%	0.72

Values are presented as means (S.D.) unless stated otherwise. Analyses presented there are based on the PROC MIXED procedure in SAS on $N=24$ subjects.

Abbreviation: C, cholesterol.

^a P value from the main effect of treatment in the mixed model. Models included treatment only.

^b Analyses performed on log-transformed values. Geometric means are presented.

platelets and hemoglobin counts remained similar. This is consistent with previous data and a long history of use having established that systemic enzymes such as Wobenzym® are considered safe.

This study has strengths and limitations. The crossover randomized double-blind design of this study is a major strength. Statistics were also performed in a blinded fashion, reducing the risk of biases. Markers of inflammation were measured twice at screening and after each treatment phase, which contributed to reduce intra-individual variability and hence increased statistical power. Compliance was very high (>95%), with only one participant with a compliance below 80% (79.8%). The number of subjects who completed the study was high (24/27). This is to the best of our knowledge the first study to have documented the side effects and the safety of a chronic use (4 weeks) of systemic enzymes in healthy volunteers with subclinical inflammation. On the other hand, the sample size was limited and not specifically calculated to investigate potential treatment by sex interaction in the response to treatment. The study design did not allow us to investigate the impact of systemic enzyme supplementation on inflammation in an acute setting, i.e. immediately after ingestion of the supplement.

In conclusion, data from this study suggest for the first time that short-term supplementation with systemic enzymes may attenuate inflammatory processes in healthy individuals with subclinical inflammation, and that this effect may be more important in men than in women. This apparent difference in inflammatory responses to systemic enzyme supplementation between men and women needs to be further assessed in future studies.

5. Disclosure

This study was supported through funding from Atrium Innovations. Study products were provided by Mucos Pharma GmbH & Co. KG (Oberhaching, Bavaria, Germany). Sponsors had no role to play in running the study, in data analysis and in decision to publish this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.phanu.2015.04.004>.

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