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Research Article

Multi-Targeted Prevention of Cardiovascular Disease Risk Factors with Reg´Activ Cholesterol Food Supplement

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Abstract

Background: Cardiovascular diseases are number one cause of death globally. Primary care settings is the optimal place to evaluate, notice and intervene. Beneficial modifying of the cardiometabolic profile has major impact for long-term success. Our previous open-label pilot study showed a complex positive influence of a food supplement Reg'Activ Cholesterol (RAC) on cardiometabolic parameters (CMP).

Objectives: To test: a) the effect of RAC to CMP in clinically asymptomatic volunteers with borderline-high values of glycosylated hemoglobin (HbA1c%), b) sustainability of the RAC effect on CMP (HbA1c%, homocysteine (Hcy), plasma cholesterol (Chol) and fractions, oxidized LDL (OxLDL), C-Peptide, ferritin, insulin, interleukin-1alfa, interleukin-6, leptin, plasminogen activator inhibitor-1, resistin and tumor necrosis factor alfa) implementing two time-points - 4 weeks and 8 weeks.

Methods: The model was a randomized double-blind placebo-controlled clinical trial (ISRCTN55339917).

Results and Conclusion: The level of Chol, LDL-cholesterol and oxLDL and HbA1c% decreased significantly and HDL-cholesterol increased significantly only in the study group after 4 and 8 weeks of consumption RAC containing LFME-3. The level of Hcy also decreased significantly in 8 weeks.

RAC has shown a complex positive effect on cardiovascular risk profile. Still investigations are needed to evaluate its long-term effects on clinical outcomes.

Trial Registration: ISRCTN55339917 http://www.isrctn.com/ISRCTN55339917.

Keywords: *Borderline Risk Profile; Food Supplements; Glycosylated Hemoglobin; LDL-Cholesterol; Oxidized LDL*

Abbreviations

Chol: Plasma Total Cholesterol; CMP: Cardiometabolic Parameters; CPEP: C-Peptide; CVD: Cardiovascular Diseases; FERR: Ferritin; HbA1c%: Glucosylated Hemoglobin; Hcy: Homocysteine; HDL-chol: HDL Cholesterol; IL-1α: Interleukin-1alfa; IL-6: Interleukin-6; INS: Insulin; LDL-chol: LDL cholesterol; LGI: Low-Grade Inflammation; LEPT: Leptin; LFME-3: *Lactobacillus fermentum* ME-3 (DSM 14241); OxS: Oxidative Stress; PAI-1: Plasminogen Activator Inhibitor-1; RAC: Reg´Active Cholesterol; RETN: Resistin; TNFα: Tumor Necrosis Factor Alfa

Introduction

Multimorbidity is getting increasing attention in primary care and with no sustainable management options we lose patient years and money.

The 3D (Dimensions of health, Depression and Drugs) approach to improve care for multimorbidity got to the first results presented in [1]. It was not associated with benefits in quality of life, illness burden or treatment burden. The authors stated that evaluation is needed based on whole-system change over a longer period of time [1].

The key to effective management and prevention of multimorbidity has to be the balancing of preventive and early diagnostic intervention with the patient's safety and limited in primary health care [2].

We approached the problem of multimorbidity from its roots: the common biochemical-pathophysiological pathway has to be targeted and changed in adequately selected subject groups to postpone the development of disease. In a group of clinically healthy subjects with borderline biochemical markers of cardiovascular disease and diabetes (DM2) risk we tested a multitargeted food supplement. The results of preliminary open label test setting have been published earlier [3]. Here we report the results of double-blind placebo-controlled setting study to evaluate the effect of a food supplement Reg'Activ Cholesterol (RAC) produced by VF Bioscience Company to improve cardiometabolic risk profile (CMP).

Dyslipidemia and especially an elevated LDL-chol are linked to the pathogenesis of CVD. The acknowledgement of the role of LDL oxidation, tightly associated with inflammation, is not new either [4]. The pathophysiological phenomena oxidative stress (OxS), chronic inflammation and glycation cause cardiometabolic changes that are common in the development of metabolic syndrome, diabetes and CVD [5]. The ATTICA study showed that a permanent low-grade/high-normal inflammation (LGI) and an advanced glycation both may enhance the development of CVD thus linking LGI and diabetes [6]. It is accepted that the ratio of blood triglycerides to HDL cholesterol (HDL-chol) (TG/HDL-chol) describes the insulin receptor resistance [7]. A possible role of a poor or unbalanced intestinal microbiota in atherosclerosis pathogenesis has also been described [8].

RAC is a complex designed of components that theoretically could positively affect OxS and LDL oxidation and/or glycation and/or other CMP and inflammatory markers. The components of RAC are: red yeast rice, ubiquinol, vitamin E, vitamins B6, B9, B12 and *Lactobacillus fermentum* ME-3 [3].

Lactobacillus fermentum ME-3 (DSM 14241) (LFME-3) is a strain isolated from a healthy child [9] that has both antimicrobial and antioxidative functional properties [10,11]. Several clinical studies [12-14] have shown health benefits of fermented products containing LFME-3. Our previous open-label pilot study [3] showed a complex positive influence of RAC on CMP.

Aim of the Study

The aim of the study was to test in placebo-controlled blind settings if RAC could have a clinically significant positive effect on cardiovascular disease risk factor profiles in clinically asymptomatic volunteers with borderline-high values of CMPs (alike [3]) (primary targets were LDL-chol, oxLDL and HbA1c%); b) sustainability of RAC influence on CMPs in two assay time points - 4 weeks and 8 weeks (the guidelines of European Food Safety Authority (EFSA)).

Materials and Methods

Subjects and study design

Within the setting of a larger clinical trial (ISRCTN55339917) [15] fifty clinically healthy volunteers with borderline values for risk factors for CVD were recruited (102 persons were assessed for meeting the inclusion/exclusion criteria) into a randomized, double blind placebo controlled (parallel) study (Figure 1).

We calculated the sample size based on the results of the open label setting experiment´s results [3]: if a parameter changes clinically significantly in at least 50% of participants, it should show also a statistically significant difference between the intervention group and control group. In our study the main primary outcome measures were the amount of LDL-chol, oxLDL and HbA1c% as they are generally accepted prognosis-related CMPs.

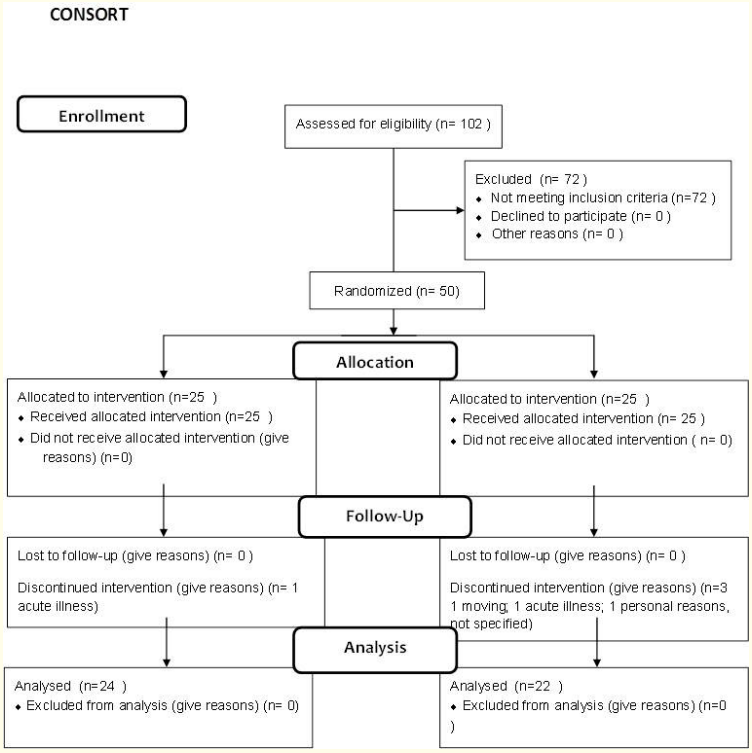

Figure 1: *The general scheme of study design (the CONSORT figure).*

Just like in [3], the inclusion criteria in the recruitment phase (open public call to participate) were age 50 - 75 years, BMI 24 - 30, being clinically healthy (asymptomatic). From those who responded the public call, people with HbA1c% level 5.7 - 6.4% were recruited.

The exclusion criteria were (also like in [3]) the following: a history of gastrointestinal disease, diabetes, food allergy, use of any antimicrobial agent or probiotics or an acute infection within the preceding 2 months; use of any regular concomitant medication including any non-steroidal anti-inflammatory drugs and antioxidant products or probiotics within at least the preceding 2 weeks; pregnancy or breastfeeding; any serious organ or systemic disease, eating disorder, extensive exercise, genetic hyperlipidemia, drug or alcohol abuse, smoking, active weight loss > 5 kg in prior 3 months; participation in other studies within the last 30 days/during the study and no wish to participate.

The blood samples were collected at Tartu University Clinics laboratory.

We concentrated on the possible influence on blood sugar metabolism (HbA1c%) and thus it was needed to keep this parameter equal in groups at the beginning of the study. According to literature, the screening to detect prediabetes and diabetes enables early prevention and intervention in asymptomatic, undiagnosed who could be detected with prediabetes using the ADA guidelines. HbA1c% is accepted as a good predictor for developing diabetes and according to the guidelines diabetes was defined as HbA1c% ≥ 6.5 and prediabetes was defined as HbA1c% ≥ 5.7 [16].

Then HbA1c% value was tested in 102 persons (until we reached the desired amount of 50 persons with HbA1c 5.7 - 6.4%). Then a list was generated with the ascending values of HbA1c. To get a HbA1c% - balanced at baseline test group and control group 4-member blocks of this list were randomly and in a double-blind manner assigned to A- and B-group, the B-group was later revealed as the test

group (after statistical analysis had been completed). All participants signed the written informed consent form. They had the option of withdrawing from the study at any time. The study protocol was approved by the Ethics Review Committee (ERC) on Human Research of the University of Tartu. The study was carried out in accordance with the principals of the Declaration of Helsinki. We measured the anthropometrical indices of the participants. The fasting blood samples for the analysis set were collected three times: at the beginning, after 4 weeks and at the endpoint of the trial period of administration of LFME3 containing capsules RAC for 8 weeks 2 capsules/day. The identical-looking placebo-capsules contained starch. The plasma samples were collected after an overnight fast and abstinence from any medications, tobacco, alcohol and tea or coffee. The samples were kept at -80°C until analyzed.

The tested supplement

RAC has been composed of components that influence different cardiovascular risk factors and is produced by VF Bioscience (Lille, France). Its components should theoretically positively affect OxS, LDL-chol amount, LDL oxidation, non-specific glycation of proteins, it combines *LF*ME-3 with others functional ingredients that have been used in food supplements for their antioxidative properties (red yeast rice, ubiquinol, vitamin E, vitamins B6, B9, B12) in adequate doses.

The strain *LF*ME-3 is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures GmbH) under registration number DSM 14241 [3].

Biochemical parameters determination

The parameters to describe the cardiovascular risk factors were selected according to the suggestions by the NDA of EFSA [17].

OxLDL was quantitated with the ELISA kit (Cat. No. 10-1143-01, Mercodia AB, Uppsala, Sweden) [18]. The Evidence Investigator™ Metabolic Syndrome Array1 (METS1) was used for simultaneous measurements of non-traditional parameters that still may affect CMP. The core technology is the Randox Biochip containing an array of discrete test regions of immobilized antibodies specific to different metabolic biomarkers. The chip is based on the sandwich chemiluminescent immunoassay (Randox Laboratories Ltd, United Kingdom, Crumlin catalogue number EV 3755) and simultaneous quantitative assay of C-Peptide (CPEP), ferritin, insulin, interleukin-1alfa (IL-1α), interleukin-6 (IL-6), leptin (LEPT), plasminogen activator inhibitor-1 (PAI-1), resistin (RETN) and tumor necrosis factor alfa (TNFα) from a single sample can be performed. The light signal generated from each of the test regions on the biochip is detected using digital imaging technology and compared to the corresponding signal from a stored calibration curve. To avoid false-high ferritin values in the statistical analysis hsCRP was tested simultaneously and if it had been over 3 mg/l the ferritin value would not have been included into statistical analysis. Analyses of plasma glucose and Chol, LDL-chol, HDL-chol, IL-6, TG and HbA1c%, also hsCRP were performed with standard laboratory methods using certified assays in the United Laboratories of the Tartu University Hospital, Estonia. Normal values for routine laboratory tests of the Nordic Reference Interval Project [19] were used as references.

Statistical analysis

We used Statistics for Windows, Stat Soft Inc. and Graph Pad PRISM Version 2.0, STATA-14.2 for calculations and graphs. All values were calculated and presented as mean and standard deviation (mean ± SD). Student's t-test was selected to be appropriate to evaluate statistically significant differences between the values before and after consumption of LFME-3 containing capsules. In all analyses, p values < 0.05 were considered to be statistically significant. Change of CPEP (4 and 8 weeks from 0 week) is presented with mean change and 95% confidence interval.

Results

At the beginning the control group and study group differed in the following parameters: Chol, LDL-chol, TG, OxLDL, TG/HDL ratio. It is unavoidable as the subjects were randomized based on the values of one parameter - no-one can predict the other parameters´ plasma values. Thus, we concentrated more on the differences in changes of parameters in (4 and) 8 weeks, not just to differences between groups.

The most impressive changes appeared in the test group in the decrease of Chol values on the account of LDL-chol (HDL-chol did increase), HbA1c% and oxLDL. Altogether the lipid fractions, HbA1c%, and Hcy - all known members of the CVD risk profile - changed for the more favorable pattern.

Repeating the results of the open-label setting [3], the level of total Chol as well as LDL-chol, oxLDL and HbA1c% decreased significantly in all participants and HDL-chol increased significantly after 4 and 8 weeks of consumption of RAC (Table 1). The level of homocysteine (Hcy), another risk factor of cardiometabolic diseases also decreased significantly (Table 2).

	Study group	Asymptomatic subjects n = 24		Placebo group	Asymptomatic subjects n = 22	
	0 week	4 weeks	8 weeks	0 week	4 weeks	8 weeks
HbA1c%	6.0 ± 0,38	5.8 ± 0.37 p = 1.173E-07	5.7 ± 0.38 p* = 8.042E-09; p** = 0.040	5.9 ± 0.2	5.8 ± 0.29	5.9 ± 0.23 p# = 0.02
Chol mmol/l	6.5 ± 1,4	5.6 ± 1.1 p = 1.079E-05	5.2 ± 1.1 p* = 8.911E-06	5.9 ± 1.1	6.0 ± 1	6.1 ± 1.2 p# = 0.04
LDL-chol mmol/l	4.5 ± 1.2	3.8 ± 0.9 p = 0,00017	3.4 ± 0.9 p* = 1.341E-05	3.9 ± 0.9	4.0 ± 0.9	4.0 ± 1
HDL-chol mmol/l	1.55 ± 0.33	1.64 ± 0.39 p = 0.01	1.65 ± 0.35 p* = 0.02	1.55 ± 0.49	1.61 ± 0.49	1.64 ± 0.54
TG mmol/l	1.57 ± 0.58	1.42 ± 0.60	1.29 ± 0.31 p* = 0.0023	1.72 ± 0,94	1.51 ± 0.60 p = 0.048	1.69 ± 0.89
Hcy µmol/l	12.4 ± 3.4	11.5 ± 2.9	10.7 ± 2.8 p* = 0.0004	12.4 ± 3.7	13.3 ± 4.9	12.4 ± 4.6
oxLDL U/l	87 ± 27	75 ± 21 p = 5.4234E-05	75 ± 23 p* = 0.00012	81 ± 22	80 ± 22	80 ± 23
Ratio TG/HDL-chol	1.08 ± 0.57	0.94 ± 0.54 p = 0.039	0.89 ± 0.38 p* = 0.0029	1.24 ± 0.74	1.09 ± 0.61 p = 0.079	1.21 ± 0.79 p# = 0.04

Table 1: *Cardio-metabolic markers at 0 point and after consumption of probiotic LFME-3 containing RAC capsules (the study group) and capsules without LFME-3 (the placebo group) (2 capsules/per day) 4 weeks and 8 weeks.*

p = 0 week/4 weeks; p = 0 week/8 weeks; p** = 4 weeks/ 8 weeks. All measured clinical parameters at 0 week for study group vs placebo group had no statistically different p values. p# is between study group and placebo group.*

The use of RAC also declined the insulin resistance marker TG/HDL-chol ratio and the latter one correlates inversely with the plasma level of small dense LDL particles. This ratio shows promise as an index of the atherogenic properties of the plasma lipid profile [7] (Table 2).

Consumption of LFME-3 containing RAC capsules caused a statistically significant decline of some other cardiometabolic markers like proinflammatory cytokine TNF-α (Table 2), but no changes considering leptin, PAI-1, resistin and IL-1α. As it is accepted that levels of CPEP above 1.6 ng/ml may predict insulin resistance [20] we implemented an additional approach. In both groups (study and placebo)

we divided the subjects into two groups, in the first group the persons had normal endemic reference value of CPEP (< 2) and the second group the subjects had CPEP > 2 (Figure 2). Using of RAC containing LFME-3 causes a shift of CPEP levels into normal range of values when levels of CPEP were initially higher than 2 ng/ml.

	Study group	Asymptomatic subjects n = 24		Placebo group	Asymptomatic subjects n = 22	
	0 week	4 week	8 week	0 week	4 week	8 week
CPEP ng/ml	1.57 ± 0.69	1.28 ± 0.47 p = 0.017	1.19 ± 0.46 p* = 0.009	2.5 ± 1.77	2.22 ± 1.54 p = 0.02 p^1 = 0.01	2.5 ± 2.0 p° = 0.004
INS µlU/ml	7.59 ± 2.85	6.58 ± 2.09	6.10 ± 1.83 p* = 0.04	9.18 ± 3.55	9.66 ± 5.82	9.64 ± 4.69
FERR ng/ml	100.3 ± 74.3	93.7 ± 75 p = 0.048	87.2 ± 66.8 p* = 0.0029	125.1 ± 95.8	118.5 ± 88.5 p^1 < 0.001	122.5 ± 89.3 p° < 0.001
TNF-α pg/ml	6.79 ± 2.51	6.06 ± 2.59 p = 0.0031	6.06 ± 2.59 p* = 0.045	6.75 ± 1.95	6,17 ± 1.92	6.29 ± 1.89
IL-1A pg/ml	0.35 ± 0.11	0.35 ± 0.08	0.35 ± 0.09	0.43 ± 0.16	0.42 ± 0.22	0.41 ± 0.12
LEPT ng/ml	5.3 ± 5.5	5.3 ± 5.3	5.5 ± 5.4	5.7 ± 7.0	5.3 ± 5.7	5.4 ± 6.0
PAI1 ng/ml	28.4 ± 5.0	26.3 ± 5.1	28.3 ± 5.2	28.3 ± 9.5	26.3 ± 8.2	27.6 ± 9.5
RETN ng/ml	3.4 ± 0.7	3.2 ± 0.5	3.4 ± 0.8	3.8 ± 0.9	3.4 ± 0.7	3.4 ± 0.7

Table 2: *The additional set of cardiometabolic parameters.*

p = 0 week/4 weeks; p = 0 week/8 weeks; Extent of changes between groups 0/4 weeks p^1 and p°.*

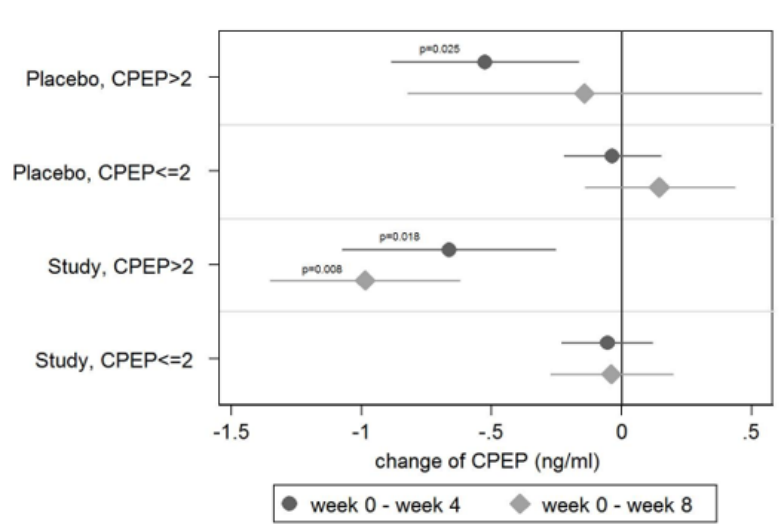

Figure 2: *Difference of CPEP in normal (< 2 ng/ml) and higher (> 2 ng/ml) values in placebo and study groups after consumption of probiotic LFM-3 containing RAC capsules, 2 capsules/per day, 4 weeks and 8 weeks.*

Discussion

When we go back from multiple diagnoses to common pathophysiological pathways it is possible to modify the course of several diagnostic units at once. If we detect the endangered subjects early we can postpone the progression of developing diseases for years.

Cardiometabolic changes are common in the development of diabetes and CVD. Therefore, to find a new approach for suppressing unhealthy shifts in CMPs is needed. Although our clinical trial was focused on quite broad set of CMPs we considered the changes in LDL-chol, oxLDL and HbA1c% to be primary outcomes as these parameters are generally acknowledged to be most predictive in CVD progression. The special accent of randomization was focused on HbA1c%. We also evaluated the sustainability of the RAC influence on every one of CMPs via implementation of two time points - 4 weeks and 8 weeks (the suggested time points by the guidelines of EFSA).

All blood lipid fractions, oxLDL, HbA1c% and Hcy changed in the study group for the more favorable pattern with acceptable sustainability. The use of RAC with LFME-3 also declined CPEP and the IR marker TG/HDL-chol. The latter has been shown to correlate inversely with the plasma level of small dense LDL particles thus also characterizing the atherogenicity of the plasma lipid profile [7].

Human health is closely linked to and dependent on the quality and variety of gut microbiota. The complex ecosystem inside the gut consists of complex interrelations between the host, nutrients, microbiological and environmental factors [8]. It has been established that the effect of probiotics on lipid metabolism parameters of the host can be quite strain and host specific [21,22]. The anti-oxidative and anti-atherogenic effects of LFME-3 have been tested for in several previous experiments in vitro, in animal models and human trials [10,12-14].

Probiotics may bind bile acids and thus decline Chol level [23].

Elevated OxS increases the production of oxLDL particles that are less likely to be recognizable to LDL receptors. The higher the LDL content the greater the possibility of production of oxLDL. Uptake of oxLDL particles by arterial wall macrophages leads to the formation of foam cells under the endothelium and atherosclerosis. The consumption of RAC capsules for 8 weeks decreased both the amounts of LDL and oxLDL, while the amount of HDL-Chol increased. The effect of monacolin K and LFME-3 may be the reasons for that [24].

The possible mechanisms of action of Hcy in contribution to vascular risk include endothelial dysfunction, inflammatory response, oxidation of LDL-chol, and platelet activation [25]. The level of Hcy declined in RAC users.

The ability of LFME-3 to decrease the levels of LGI (TNF-α) and HbA1c% was demonstrated in our study. The up-regulation of pro-inflammatory cytokines associated with OxS and inflammation may reduce glucose transporter type 4 (GLUT4) expression and translocation to the plasma membrane in human adipocytes and muscle cells, resulting in decreased insulin-stimulated glucose uptake [26]. The same may happen to GLUT2, the isoform of glucose transporter in glucose-sensing in pancreatic β-cells, liver [27].

The RAC formula contains monacolin K that inhibits HMG-CoA-reductase, the key enzyme of cholesterol synthesis.

Targeting hyperglycemia and OxS simultaneously may work in cooperation in correcting the lipid profile abnormalities (e.g. elevated LDL levels) and improve OxS which increases the susceptibility of LDL particles to oxidation and glycation [28]. This may prevent endothelial dysfunction and atherosclerosis. Intake of cysteine is the rate-limiting factor in human glutathione (GSH) biosynthesis. GSH is a regulator of many body functions and the principal cellular antioxidant. RAC contains L-cysteine and LFME-3 can transport and synthesize glutathione and has the ability of redox cycling of glutathione [11,14]. Vitamins B1, B6, B9 and B12 in the RAC composition have several cardiometabolic effects including the control of Hcy level.

Probably the simultaneous targeting of hyperglycemia and OxS could be at least as effective as intensive treatment of hyperglycemia in prevention of T2DM complications [28]. For decades the control of LDL-chol has been a cornerstone in CVD primary and secondary prevention. A reduction in OxS in addition to traditional cardiovascular risk factor control seems to be an ideal treatment strategy for T2DM

patients. As CVD and T2DM are chronic conditions needing a life-long attention and management there is a place for food supplements and functional foods in the complex management of them [29,30].

Conclusion

From our work we can conclude that the level of total Chol as well as LDL-chol and oxLDL decreased significantly in all participants and HDL-chol increased significantly after 4 and 8 weeks of consumption of LFME-3 containing food supplement RAC. Altogether the lipid fractions, HbA1c%, cellular level inflammation and Hcy - all known members of the CVD risk profile - changed for the more favorable pattern. Thus, it may be concluded that consumption of RAC suppresses the development of diabetes and changes the CVD risk factor profile.

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Conflict of Interest

The authors declare that they have no competing interests.

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