Original Article

MOLECULAR METABOLISM

Statin dose reduction with complementary diet therapy: A pilot study of personalized medicine

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ABSTRACT

Objective: Statin intolerance, whether real or perceived, is a growing issue in clinical practice. Our aim was to evaluate the effects of reduced-dose statin therapy complemented with nutraceuticals.

Methods: *First phase*: Initially, 53 type 2 diabetic statin-treated patients received a supplementation with fish oil (1.7 g EPA + DHA/day), chocolate containing plant sterols (2.2 g/day), and green tea (two sachets/day) for 6 weeks. *Second phase:* "Good responders" to supplementation were identified after multivariate analysis (n = 10), and recruited for a pilot protocol of statin dose reduction. "Good responders" were then provided with supplementation for 12 weeks: standard statin therapy was kept during the first 6 weeks and reduced by 50% from weeks 6–12.

Results: *First phase*: After 6 weeks of supplementation, plasma LDL-C (−13.7% ± 3.7, $P = .002$) and C-reactive protein (−35.5% ± 5.9, $P = .03$) were reduced. Analysis of lathosterol and campesterol in plasma suggested that intensity of LDL-C reduction was influenced by cholesterol absorption rate rather than its synthesis. *Second phase*: no difference was observed for plasma lipids, inflammation, cholesterol efflux capacity, or HDL particles after statin dose reduction when compared to standard therapy.

Conclusions: Although limited by the small sample size, our study demonstrates the potential for a new therapeutic approach combining lower statin dose and specific dietary compounds. Further studies should elucidate "good responders" profile as a tool for personalized medicine. This may be particularly helpful in the many patients with or at risk for CVD who cannot tolerate high dose statin therapy.

Trial registration: ClinicalTrials.gov, NCT02732223.

Keywords Atherosclerosis; Omega-3 fatty acids; Plant sterols; Polyphenols; Responders

1. INTRODUCTION

Cardiovascular disease (CVD), particularly atherosclerosis, is the major cause of morbidity and mortality throughout the world. The main target for CVD prevention is LDL-cholesterol (LDL-C) lowering, usually attained by statins [1]. Although statins are considered a first-line lipid-lowering therapy and have contributed to clinical event reduction, residual cardiovascular risk remains high among statin-treated patients [2]. The most recent study with PCSK9 antibodies (FOURIER trial) supported the LDL-C hypothesis, which is "the lower the better", showing that this new class of drugs can further decrease the rate of clinical events [3]. However, results have also shown that despite extreme LDL-C reduction, absolute outcomes (MI) were still high after PCSK9 inhibition atop statin therapy [3]. Furthermore, atherosclerotic plaque regression with PCSK9 antibody was previously shown to be only 1% [4], highlighting the need to address risk factors beyond lipids, such as inflammation and oxidative stress. This approach is especially important under diabetic conditions, in which plaque regression is impaired in pre-clinical models even when LDL-C is normalized [5].

Novel add-on therapies to maximally tolerated statin have also focused on the atheroprotective properties of HDL, especially its function on reverse cholesterol transport. However, the effectiveness of HDL-targeted agents is still controversial [6]. Although combination therapies have proven necessary [7], and despite increasing efforts in drug development, a critical issue that undermines CVD prevention is the high prevalence of statin undertreatment due to real or perceived

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Abbreviations: ARA, arachidonic acid; CAT, catalase; CEC, cholesterol efflux capacity; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EGCG, epigalocatechin gallate; EPA, eicosapentaenoic acid; GPx, glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; n-3 FA, omega-3 fatty acids; PPARα, peroxisome proliferator-activated receptor alpha; SOD, superoxide dismutase; SREBP-1c, sterol regulatory element-binding protein-1c; TG, triglycerides

Received January 15, 2018 • Revision received February 5, 2018 • Accepted February 13, 2018 • Available online xxx

https://doi.org/10.1016/j.molmet.2018.02.005

adverse effects [8]. Statin intolerance leads to discontinuation or suboptimal statin use; afflicted patients have 50% higher risk of coronary events than those with good statin adherence [9]. Convincing these patients, who claim muscle pain or weakness, to continue the statin therapy is a major challenge in clinical practice.

The combination of nutraceuticals with statin treatment could help overcome these limitations [10,11]. Bioactive compounds such as omega-3 fatty acids (n-3 FA), plant sterols, and polyphenols are naturally occurring molecules with great potential to reduce atherosclerosis progression by reducing inflammation, LDL-C, and oxidative stress, respectively [12–15]. Although the biological effects of these single compounds have been extensively evaluated over the last decade [14,16,17], studies regarding bioactive compound combinations are still scarce, and the combined action of such agents in atherosclerosis is poorly understood [18]. More interestingly, the potential of reducing statin dose in conjunction with complementary diet therapy has never been investigated and could contribute to reduce adverse effects associated to high statin dosages and increase treatment adherence.

In the present study, we first evaluated the effects of daily consumption of a combined supplementation of n-3 FA (fish oil supplement), plant sterols (enriched chocolate) and polyphenols (green tea) on biomarkers of inflammation, lipidemia and oxidative stress in type 2 diabetic patients treated with statins. We then conducted a pilot study to evaluate — in terms of plasma lipid profile, inflammatory, and oxidative markers — the effects of statin dose reduction by the combined supplementation compared to standard statin therapy (full dose), in a sub-group of patients who better responded to the initial intervention.

Table 1 — Subjects characteristics.

	n	Mean ± SEM
Gender (M/F), n	19/34	—
Ethnicity (White; Black; Asian)	42; 10; 1	—
Age (y)	—	63.8 ± 0.9
Systolic blood pressure (mm Hg)	—	130.7 ± 2.9
Diastolic blood pressure (mm Hg)	—	80.2 ± 1.7
Heart rate (bpm)	—	69.9 ± 1.7
Medical follow up (y)	—	10.4 ± 0.9
Drugs		
Atorvastatin (20; 40; 80 mg)	5; 12; 6	—
Sinvastatin (10; 20; 40 mg)	1; 16; 13	—
Metformin 500–2550 mg (1–3 daily)	46[a]	—
Gliclazide 30–120 (1–3 daily)	12[a]	—
Aspirin (100 mg)	39	—
Diuretic	42	—
Angiotensin converting enzyme (ACE) inhibitor	14	—
Angiotensin II receptor antagonist	26	—
Beta-blocker	18	—
Calcium channel blocker	15	—
Alpha2 adrenergic agonist	2	—
Vasodilator	2	—
Antiocoagulant	2	—
Antiarrhythmic agent	2	—
Thyroid hormone thyroxine	7	—
Hyporuricemic agent	6	—
Proton pump inhibitor	13	—

[a] 5 subjects were on treatment with both metformin and gliclazide.

2. METHODS

2.1. Subjects

Participants with established dyslipidemia and diabetes were recruited from Dante Pazzanese Institute of Cardiology (São Paulo, Brazil). The primary selection criteria were current statin (simvastatin or atorvastatin) and hypoglycemic treatment (metformin and/or gliclazide). Exclusion criteria were patients who were taking n-3 FA, plant sterol, or green tea supplements; patients with poorly controlled diabetes (HbA1c $>$ 7.5%) or dyslipidemia (LDL-C $>$ 100 mg/dL); patients with atherosclerotic cardiovascular disease (ASCVD); pregnant females; patients who presented any congenital cardiac disorders or uncontrolled endocrine, renal; or hepatic disease; patients with excessive alcohol consumption. Based on these criteria, 53 subjects (male $n = 19$ and female $n = 34$) were enrolled and completed the first phase of the trial. Patient characteristics are shown in Table 1. The study was approved by the Institutional Review Board of the Dante Pazzanese Institute of Cardiology (CAAE 27349114.5.3001.0067) and all patients provided informed written consent prior to inclusion. ClinicalTrials.gov ID was NCT02732223.

2.2. Study design

First phase: a crossover intervention was carried out. The protocol was single-blinded once the quantification of ingestion markers in plasma samples was indicative of subject's treatment. Initially, subjects were randomly assigned to receive nutraceuticals or a control treatment for 6 weeks. Daily treatment with nutraceuticals (NTR) consisted of seven fish oil softgels (1.7 g of EPA + DHA), two dark chocolate truffles containing plant sterol esters (2.2 g/day), and two green tea sachets (~170.8 mg epigalocatechin gallate (EGCG)/day). Control treatment (CON) consisted of seven soy bean oil softgels, two regular dark chocolate truffles, and two anise tea sachets. As green tea and anise tea taste different, participants were informed in the beginning of the protocol that the tea taste would change along the study. The chocolate containing plant sterols was developed with collaboration of Chocolife Indústria e Comércio de Alimentos Funcionais Ltda, São Paulo [19]. The plant sterols PinVita™ES (70% β-sitosterol) from DuPont™ Danisco® Food Ingredients were purchased from MasterSense Ing. Alim. Ltda. (São Paulo, Brazil). The fish oil and placebo supplements (1 g softgels) were donated by Bionatus Laboratório Botânico Ltda. (São José do Rio Preto, São Paulo). The green tea and anise tea sachets (1.6 and 2.0 g each) were donated by Leão Alimentos e Bebidas (São Paulo, Brazil). Detailed description of chemical composition and oxidative status of food components are available in Appendix S1 (Supporting Information). Participants were instructed to consume 1 softgel after breakfast, 3 softgels, and 1 chocolate truffle twice a day (after main meals) and to drink two cups of tea per day. After a 6-week washout period, the groups were changed following the cross-over design for 6 weeks more. During each step, the subjects maintained their habitual routine and diet. Prescribed drugs were kept without any change throughout the study.

Second phase: Multivariate statistical tools were applied to identify subjects who overall better responded to the combined supplementation provided in the first phase of the trial. Cluster analysis was performed including C-reactive protein, malondialdehyde and LDL-C concentration as active variables. Via this analysis, two sub-groups were identified with the aim of classifying patients that showed greater and lesser degrees of responses to the nutraceuticals intervention. Subjects that made up the so-called responder ("R"; n = 10) and non-responder ("NR"; n = 10) sub-groups were invited to participate in the second phase of the study. A pilot protocol of statin dose reduction by complementary diet therapy was carried out in the "R" sub-group only. Initially, "R" and "NR" subjects were recruited for a first visit, when baseline blood samples were collected and nutraceuticals (NTR) were provided only to responders. The "R" sub-group completed 6 weeks of NTR during which they maintained their prescribed dose of statin. Responders then reduced their statin dose 50%, and completed 6 more weeks of NTR.

