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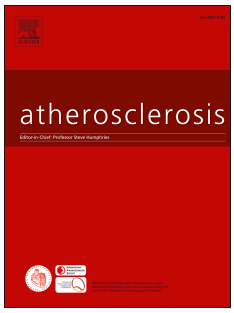

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Effects of Niacin and Omega-3 Fatty Acids on the Apolipoproteins in Overweight Patients with Elevated Triglycerides and Reduced HDL Cholesterol

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Highlights

- Niacin plus omega-3 therapy was tested in overweight dyslipidemic patients
- This combination is more potent in normalizing apolipoprotein parameters
- These changes are consistent with effects of this combination on plasma lipids
- Combo therapy normalized apoC3 glycosylation

ABSTRACT

Objective - Prescription omega-3 acid ethyl esters (P-OM3) and extended release niacin (ERN) both have beneficial effects on plasma lipids and lipoproteins. The purpose of this study was to describe the effects of mono- and combination (Combo) therapy of these agents in patients with the metabolic syndrome.

Methods - Very low density (VLDL), intermediate/low density (IDL/LDL, hereafter LDL), and high density lipoproteins (HDL) were isolated from 56 overweight patients with elevated triglyceride/HDL-C ratios at baseline and after 16 weeks of treatment with placebo, ERN (2g/day), P-OM3 (4g/day), or Combo and then analyzed by quantitative electrophoresis for apolipoproteins (apo) A1, A2, B, C2, C3 and E. Total plasma concentrations and the ratios of each apo with apoB (in VLDL and LDL) and with apoA1 (in HDL) were calculated. An apoC3 glycosylation index (a ratio between di- and mono-sialylated isoforms) was also determined in plasma and in each lipoprotein fraction.

Results - ERN reduced plasma apoB (-11%, $p<0.05$). Combo increased LDL apoE/apoB ratio (64%, $p<0.01$) and LDL apoA1/apoB (91%, $p<0.05$). ERN increased the apoC3 glycosylation index only in HDL (37%, $p<0.05$), whereas P-OM3 and Combo increased the index in whole plasma (48% and 49%, respectively, $p<0.05$ for both) and in every lipoprotein class (VLDL: 26%, $p<0.01$ and 26%, $p<0.05$; LDL: 55%, $p<0.01$ and 61%, $p<0.01$; HDL: 43%, $p<0.001$ and 44%, $p<0.001$, respectively). All findings were significant after adjustment for age, sex, body mass index (BMI), smoking, medications, and baseline apo value.

Conclusions - ERN produced a beneficial reduction in plasma apoB. The enrichment of LDL with apoE and apoA1 was unique to the Combo group and might be beneficial owing to the atheroprotective properties of apoE and HDL2 (a likely source of apoA1 in LDL fraction). The effect of therapies on the apoC3 glycosylation index is a novel finding, the implications of which will require further study.

Keywords: apolipoproteins; extended release niacin; prescription omega-3 acid ethyl ester

INTRODUCTION

The Metabolic Syndrome (MetSyn) afflicts approximately 23% of American adults [1]. MetSyn is diagnosed based on the co-occurrence of three out of five conditions: abdominal obesity (present in 56% of US adult population [1]), elevated fasting triglyceride levels (characteristic of 24% American adults [1]), low levels of high density lipoprotein cholesterol (HDL-C), hypertension, and elevated fasting plasma glucose [2]. Subjects with MetSyn are at increased risk for developing coronary heart disease (CHD) and type 2 diabetes [3,4]. While lifestyle changes (diet and exercise) can improve many of the manifestations of MetSyn or prevent its development, pharmacotherapy is often required to normalize hypertriglyceridemia [5], hypertension [6], and hyperglycemia [7] in MetSyn patients.

Niacin and omega-3 fatty acids each have beneficial effects on plasma lipids [8-13]. Niacin (nicotinic acid) is one of only two approved drugs (along with fibrates) prescribed to elevate HDL-C [14]. Owing to the additional effect of niacin in the reduction of low density lipoprotein cholesterol (LDL-C) it can be administered as an additional therapy when the target LDL-C value cannot been reached with statins alone, or as a monotherapy to patients intolerant to statins [15]. Niacin can also reduce markers of systemic inflammation (C-reactive protein and lipoprotein-associated phospholipase-A) and affects the remodeling of LDL particles, increasing large buoyant and decreasing small dense LDLs. These are all potentially anti-atherosclerotic properties [16]. Omega-3 acid ethyl esters, at their approved pharmaceutical dose of 4 g/d, lower plasma triglycerides (TG) by slowing the hepatic production and secretion of very low density lipoproteins (VLDL) and by increasing VLDL-TG clearance from circulation [17]. Prescription omega-3 fatty acids (P-OM3) reduce TG in mildly hypertriglyceridemic patients receiving statin therapy without altering levels of LDL-C or HDL-C [5]. P-OM3 was reported to alter the size, concentration, and/or composition of lipoproteins that may have anti-atherothrombotic implications [18].

The efficacy of both niacin and P-OM3 in reducing the cardiovascular (CV) events, however, has recently been brought into question. The AIM-HIGH trial which used ERN was stopped early for

the lack of the effect on hard CV endpoints [19], and in the OMEGA trial, P-OM3 failed to reduce the rate of sudden cardiac death (or other CV events) after acute myocardial infarction [20]. In a recent meta-analysis of omega-3 treatment studies there was no overall effects on total CV events [21]. Therefore, despite the compelling evidence of the effects of niacin and omega-3 fatty acids in the reduction of the CV events in several earlier studies [10,22-24], it remains to be established in which patient population and with which treatment regimen niacin and/or omega-3 therapy will reduce CV events and death. In this regard, we believe that the long-term cardioprotective effects of these agents have to be evaluated in patients who are not on guideline-directed therapeutic regimens such as the majority in this study.

We have recently demonstrated that P-OM3 and extended release niacin (ERN) in combination has beneficial effects on plasma lipids and lipoproteins beyond the benefits of mono-therapy [25]. Because apolipoproteins (apos) play a major role in regulating plasma lipoprotein metabolism, in the present study we explored the impact of these two agents, alone and in combination, on plasma and lipoprotein-specific apo composition. Based on our previous report that overweight patients with hypertriglyceridemia and low HDL-C displayed an alteration in the post-translational glycosylation of several apolipoproteins, including apoC3 compared to healthy controls [26], we also focused on the effects of these agents on apoC3 glycosylation in this trial.

METHODS

Ethics Statement

Human study protocols were approved by the Institutional Review Board of the University of South Dakota. Written informed consent was obtained from all study participants.

Study Subjects

This study included 56 subjects with MetSyn who participated in a trial testing the effects of P-OM3 and ERN, alone and in combination, on lipoprotein and vascular endpoints in MetSyn, (NCT00286234) [25]. Subjects were enrolled during time period from December 2007 through April 2008. Inclusion criteria were body mass index (BMI) greater than 25 kg/m^2, TG greater than 150 mg/dl, and HDL-C less than 50 mg/dl. In the original study, sixty subjects were randomized to treatment with dual placebo, P-OM3 (4 g/day), ERN (2 g/day), or combination in a 16-week, double-blind trial. In the present report, four subjects were excluded because of an insufficient amount of plasma for lipoprotein preparation or a technical failure in the lipoprotein preparation.

Lipoprotein Preparations, 1D Electrophoresis, and Protein Identification

All subjects fasted for at least 8 hours prior to blood collection. Lipoprotein preparations, 1D electrophoresis, and protein identification methods were described previously [26]. By this method, six classic apolipoproteins: apoA1, A2, B, C2, C3, and E were consistently identified. Accordingly, in this study we focused our quantitative analysis on these targets. Briefly, lipoproteins were isolated from EDTA plasma by sequential ultracentrifugation in densities 1.006; 1.063; and 1.21 g/ml corresponding to VLDL, IDL/LDL, and HDL fractions as previously described [27], and stored frozen (-80^{0}C) until analysis. Lipoprotein fractions (4.5 μg of protein) were subjected to gradient SDS-PAGE (4-20% Peptide gels, BioRad, Hercules, CA) and stained with Sypro Orange (Invitrogen, Grand Island, N.Y.). Gels were scanned using Typhoon scanner at 532/555nm excitation/emission wave lengths and analyzed using Image Quant version 5.0. Intensities of all bands were measured as area under the curve with baseline adjusted manually. The absolute amount of protein in each band was calculated based on its fraction of total protein loaded on the gel (4.5 μg per lane). Protein identification was aided by LC-MS/MS, MALDI-TOF, and comparative 2D electrophoresis as described [26]. Multiple isoforms were detected for apoB, apoC3, and apoE, the total amounts of these proteins (sum of isoforms) were considered in this analysis. The ratio between two major glycosylated apoC3 isoforms: di-sialo (also known as apoC3-2) over mono-sialo (apoC3-1) was used as an index of glycosylation.

The total concentrations of each apo in plasma were determined by summing the concentrations in VLDL, LDL, and HDL obtained from sequential ultracentrifugation. ApoA1, A2, C2, C3, and E were also measured directly in a subset of plasmas using commercial Human Apolipoprotein Magnetic Bead Multiplex Immuno-Assay (EMD Millipore, Danvers, MA).

Lipid analysis

Whole plasma TG, VLDL-C, LDL-C, and HDL-C were measured by the VAP Lipid Panel utilizing Vertical Auto Profile technology at Atherotec Diagnostics Lab (Birmingham, Alabama).

Statistics

GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used to calculate mean ± SD, repeated measures ANOVA models, and to compare baseline and final values employing Bonferroni-corrected pair-wise post-tests. JMP8 software (SAS Institute Inc., Cary, NC, USA) was used to calculate multiple linear regression models, in which the effect of treatment group assignment on the apo parameter change from baseline (expressed as %) were computed in the presence of the following covariates: age, sex, BMI, smoking status, use of anti-hypertensive medications, use of statins, and the corresponding log-transformed apo parameter value at baseline. Significance was accepted at $p < 0.05$.

RESULTS

Table S1 shows characteristics of 56 subjects included in this sub-analysis**.** This table was recalculated using data previously reported in the parent study of 60 subjects [25] to ensure that the baseline parameter distributions were not skewed and the reported effects were still present after exclusion of four subjects for the technical reasons from this sub-study.

Total plasma apo concentrations

Total plasma concentrations of apoA1, A2, B, C2, C3, and E were calculated from the quantitative 1D electrophoresis measurements in VLDL, LDL, and HDL density ultracentrifugation fractions according to the method reported earlier [26]. A subset of plasma samples (n=26 subjects, randomly selected) was also analyzed by the direct multiplex immuno-assay for apoA1, A2, C2, C3, and E (using MILLIPLEX MAP Human Apolipoprotein Magnetic Bead Panel from EMD Millipore, Billerica, MA) to establish a correlation between two methods. Significant correlations were found in the levels of apoA1, A2, C2, and C3 measured in plasma directly (immuno-assay) or calculated from ultracentrifugation/1D electrophoresis **(Figure S1).**

Plasma concentrations of apos calculated from ultracentrifugation/1D-electrophoresis method were used for further analysis. The two-way ANOVA analyses of the effects of treatments followed by the pair-wise comparisons (baseline vs. final) within each treatment group indicated that Placebo and P-OM3 alone had no effects on total plasma apo concentations; ERN alone decreased apoB; Combo decreased apoB, apoC2 and apoC3 and increased apoE **(Table 1)**. When analyses were done using percent changes from baseline adjusting for covariates (age, sex, BMI, smoking, medications, and log-transformed baseline apo level), the apoB effect of ERN was confirmed (-11%, $p<0.05$), but all other apo changes became non-significant **(Table 1)**.

Analysis of the relative apo composition of individual lipoprotein fractions

With respect to the per particle apo composition of each lipoprotein fraction (relative to apoB for VLDL and LDL and to apoA1 for HDL), mono-therapies (ERN or P-OM3) had no significant effects in unadjusted models; 13% increase in HDL apoC3/apoA1 by P-OM3 ($p<0.05$) was detected after adjustment for covariates **(Table 2)**. Combo affected three parameters according to unadjusted ANOVA: it enriched LDL fraction with apoE and with apoA1, and it reduced apoC3 in HDL. A 61% increase in apoE/apoB ratio of LDL ($p<0.01$) and 91% increase in apoA1/apoB ratio ($p<0.05$) remained significant after adjustment for other covariates in this study, but the effect on apoC3/apoA1 ratio in HDL was lost (**Table 2**).

Glycosylation of apoC3

The glycosylation index of apoC3 in plasma was calculated as a total amount of di-sialo apoC3 in VLDL, LDL, and HDL divided by the total amount of mono-sialo isoform in these fractions. P-OM3 and Combo had significant effects on plasma apoC3 glycosylation index before and after adjustment for covariates and contributed to about 50% increase (48% in P-OM3, $p<0.05$; and 49% in Combo, $p<0.05$%). P-OM3 and Combo also increased apoC3 glycosylation index in every lipoprotein fraction according to both unadjusted and fully adjusted statistical models. The changes from baseline were as follows: P-OM3 increased the apoC3 glycosylation index in VLDL by 26% ($p<0.01$), in LDL by 55% ($p<0.01$), and in HDL by 43% ($p<0.001$); Combo increased the index in VLDL by 26% ($p<0.05$); in LDL by 61% ($p<0.01$), and in HDL by 44% ($p<0.001$, **Table 3)**. The effect of ERN on apoC3 glycosylation appeared to be less significant in unadjusted models **(Table 3)** and was only present in HDL after adjustments for age, sex, BMI and other covariates (37% increase, $p<0.05$, **Table 3**).

DISCUSSION

The purpose of this study was to determine the effects of ERN, P-OM3, and their combination on plasma concentrations of apolipoproteins and on the apolipoprotein composition of VLDL, IDL/LDL, and HDL in patients with features of the MetSyn. Because of the small size of this study we analyzed the results using two approaches, and only those observations found by both approaches were considered reliable. Primary findings were that ERN reduced plasma apoB, and Combo enriched LDL with apoE and apoA1. We also made the unexpected observation that these agents altered apoC3 glycosylation patterns. ERN increased the apoC3 glycosylation index in HDL, whereas P-OM3 and Combo increased the index in all lipoprotein fractions. The possible mechanisms for these effects and their potential implications for CV disease risk will be considered below.

The decrease in total plasma apoB by ERN is likely to be beneficial in patients with the elevated TG. ApoB levels appear to be a better predictor of future CVD than LDL-C [28] and is a target for lipid

lowering therapies aimed at CVD risk reduction [29]. The AIM-HIGH study reported a modest reduction of apoB [30], which is consistent with this study.

We did not find any effects of P-OM3 on the levels of apolipoproteins in plasma or in lipoprotein fractions. Similarly, in a larger study (N=503) of Japanese subjects with hypertriglyceridemia, one year treatment with P-OM3 plus statin did not show significant changes in plasma apoA1 and apoB in comparison with statin monotherapy [31]. Two recent studies in statin-treated subjects with hypertriglyceridemia found a reduction in plasma apoC3 after addition of P-OM3 treatment compared to statins alone [18,32]. In patients with acute myocardial infarction P-OM3 treatment resulted in the reduction in total apoC3 and apoC3 in non-apoB particles [33]. The major effect of P-OM3 in this study was found in apoC3 glycosylation but not the total plasma apoC3 or its distribution between particles. Both the small size of this study relative to those noted above, and our use of multivariable modeling (absent from the other studies) may explain these different findings.

With Combo treatment, we observed the enrichment of LDL fraction with apoE, which is likely to be beneficial because apoE can aid in LDL clearance (since apoE is the primary ligand for the apoB/E receptor), stimulate cholesterol efflux from macrophages, and modulate immune function [34]. These actions should slow the development of hypercholesterolemia and atherosclerosis.

We also found that Combo increased apoA1 in the LDL density fraction. This might reflect the comigration of larger HDL particles (i.e., HDL2) into the LDL density region. An effect of niacin on HDL size has been previously observed in patients treated in combination with statin [35], and in the primary report from the present study, the HDL2 sub-fraction was increased in the Combo group [25]. Because the HDL2 particles appear to be cardioprotective in healthy adults [36], we speculate that the increased presence of apoA1 in the LDL fraction might be beneficial.

Novel effects of ERN and P-OM3 on apoC3 glycosylation

We reported earlier significantly lower ratios of di-sialo/mono-sialo glycol-isoforms of apoC3 across all lipoprotein classes in MetSyn subjects in comparison to age-sex matched optimally healthy controls [26]. In this study we found that the apoC3 glycosylation index was increased by ERN treatment and essentially achieved normal levels with P-OM3 and Combo therapy. ApoC3 glycosylation has not yet been studied in the context of short-term, lipid-lowering therapies such as ERN and P-OM3. ApoC3 glycosylation is reversibly associated with BMI [37]. It was shown to be increased after bariatric surgery or in patients with type 2 diabetes on metformin therapy [37]. Severe calorie restriction in women with obesity was another stimulus that increased di-sialylated apoC3 isoform while reducing mono-sialylated apoC3 in VLDL [38]. Our study indicates that short-term pharmacological interventions can increase apoC3 glycosylation index in plasma and across all lipoprotein classes, which is likely to be independent of apo levels because we have not observed any dramatic changes in total plasma apolipoproteins. It is plausible though that the efficiency on apoC3 glycosylation simply reflects the quality of protein synthesis and co-translational modifications such as physiological glycosylation, which affects secreted proteins and is catalyzed by the enzymes residing in the endoplasmic reticulum and the Golgi apparatus. ApoC3 is present in three glycol isoforms: a minor non-glycosylated isoform (commonly denoted apoC3-0), and two glycosylated isoforms, which differ in the number of sialic acid residues attached (mono-sialylated and di-sialylated isoforms, apoC3-1 and apoC3-2). The amount of non-glycosylated apoC3 is increased in patients with uremia by almost two fold [39]. Sialylation of lipoproteins appears to be protective against coronary heart disease (CHD) because lipoproteins isolated from healthy individuals contain more apo-associated sialic acid compared to subjects with atherosclerosis [40-43]. The significance of apoC3 glycosylation in CVD is not clear and requires further investigation. A simple MALDI-TOF mass-spectrometry method, requiring only a few microliters of plasma, is available to accurately measure apoC3 glycosylation [37] and could be applied for larger clinical trials. We hope that our identification of an apoC3 glycosylation defect in the cohort of

overweight dyslipidemic patients at baseline [26] and the response of this marker to lipid lowering therapies would stimulate epidemiological and basic research into this potential CVD marker.

Niacin and omega-3 have recently been tested in large clinical trials (AIM-HIGH, HPS2-THRIVE and OMEGA) with hard cardiovascular primary endpoints including nonfatal myocardial infarction and death from coronary causes [19,20,44]. These studies were designed based on the prior clinical knowledge indicating that niacin increases HDL-C and reduces triglycerides, and further that of omega-3 fatty acids reduce triglycerides. Unfortunately, no benefits on the hard cardiovascular endpoints were observed in these clinical studies. The possible reasons for these failures have been discussed at length [45-48]. The limited apo data generated in AIM-HIGH yielded confusing results: baseline apoB and the apoB/apoA1 ratio were significantly predictive of CV events only for the placebo group but not in the niacin group [30]. The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT), which used fibrates to increase HDL-C, was more successful in reducing CVD events [49]. In it, both apoA1 and apoB were better predictors of CV events and mortality compared to HDL-C or triglycerides [49]. No information is currently available regarding other apos in CV risk prediction nor are there any associations of apo posttranslational modifications such as glycosylation with CV outcomes in clinical trials.

The purpose if our small clinical trial was to assess the effects of combination therapy with fish oil and niacin on the lipid and lipoprotein endpoints in asymptomatic participants with elevated BMI, high TG, and reduced HDL-C (the components of MetSyn), the majority of whom were naïve to lipid lowering medications. In the original study we indeed observed more beneficial changes in lipid endpoints in subjects on the combination therapy compared to either mono-therapy [25]. Whether this benefit of Combo therapy can be translated into CVD risk reduction in MetSyn patients or in overweight and mildly dyslipidimic individuals is unknown.

In conclusion, we found a clear beneficial effect of ERN in the reduction of plasma apoB; and a unique and potentially atheroprotective positive effects of combinational (ERN plus P-OM3) therapy on apoE in LDL fraction. Intriguingly, combo treatment increased the detection of apoA1 in LDL density cut, which might be consistent with the increase in more buoyant atheroprotective HDL2 subfraction reported by us earlier [25]. We identified a novel effect of ERN and P-OM3 therapy on apoC3 glycosylation. The potential benefits of increased apoC3 glycosylation with lipid-lowering therapies require further investigation.

Supporting Information

Table S1. Subjects' characteristics (mean ± SD) and parameter distributions after exclusion of four subjects of the original study [25] for the technical reasons.

Figure S1. Correlations between apolipoprotein concentrations measured in plasma directly by Multiplex Immuno-Assay and calculated from 1D electrophoresis of lipoprotein fractions obtained by sequential ultracentrifugation. A, apoA1; B, apoA2; C, apoC2; D, apoC3; E, apoE; black – baseline; gray – final.

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Table 1. Total plasma apo levels calculated from 1D electrophoresis (mg/dL; mean ± SD), and their % change from baseline (mean[95%CI]).

apo	Time	Placebo (n=14)	ERN (n=14)	P-OM3 (n=15)	Combo (n=13)	p value
apoA1	baseline	93.2 ± 17.9	102.8 ± 21.3	100.9 ± 18.3	94.7 ± 18.5	
	final	98.1 ± 16.4	115.4 ± 19.6	100.1 ± 17.5	109.5 ± 23.6	0.07 [a]
	% change	0.3 (-14; 15)	14 (1.2; 27)	-4.6 (-18; 8.9)	7.5 (-8.1; 23)	0.05 [b]
apoA2	baseline	30.2 ± 6.8	33.7 ± 6.1	32.9 ± 8.6	31.9 ± 7.4	
	final	32.3 ± 6.7	36.5 ± 6.9	31.3 ± 7.4	34.4 ± 8.2	0.12 [a]
	% change	11 (-2.7; 25)	13 (0.4; 25)	-1.1 (-14; 12)	10 (-5.0; 25)	0.17 [b]
apoB	baseline	89.7 ± 14.3	102.4 ± 28.0	84.7 ± 25.7	113.6 ± 17.3	
	final	91.8 ± 17.7	87.1 ± 21.4 **	85.5 ± 19.7	102.7 ± 20.9 *	0.005 [a]
	% change	5.2 (-4.4; 15)	-11 (-20; -1.7) [#]	3.5 (-6.1; 13)	-1.5 (-13; 9.5)	0.01 [b]
apoC2	baseline	9.4 ± 1.8	9.1 ± 2.6	8.3 ± 2.5	10.7 ± 3.7	
	final	9.2 ± 1.8	9.0 ± 2.8	8.0 ± 2.3	8.5 ± 2.6 ***	0.03 [a]
	% change	-1.2 (-14; 11)	-3.4 (-15; 8.2)	-8.8 (-21; 3.4)	-19 (-33; -4.8)	0.06 [b]
apoC3	baseline	35.7 ± 14.6	30.0 ± 9.3	32.0 ± 10.9	38.1 ± 15.1	
	final	33.7 ± 13.5	29.7 ± 10.3	33.8 ± 12.4	31.5 ± 10.8 **	0.02 [a]
	% change	3.8 (-10; 18)	2.5 (-10; 16)	8.0 (-5.5; 22)	-9.0 (-25; 6.9)	0.15 [b]
apoE	baseline	7.2 ± 2.0	9.0 ± 2.7	9.1 ± 2.9	7.3 ± 2.1	
	final	7.5 ± 1.7	7.9 ± 1.9	9.0 ± 2.9	9.0 ± 2.4 **	0.0009 [a]
	% change	2.0 (-13; 17)	-8.9 (-23; 5.2)	-0.4 (-15; 15)	17 (-0.1; 34)	0.02 [b]

[a] unadjusted model - repeated measures two-way ANOVA followed by post-hoc analysis comparing baseline and final means, * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. baseline; [b] fully adjusted model - linear regression analysis of the % apo parameter change from baseline and the treatment group (as an independent variable with four levels), adjusted for age, sex, smoking, medications, BMI, and log-transformed baseline apo concentrations followed by post-hoc analysis comparing ERN, P-OM3, or Combo to Placebo, [#] $p<0.05$ vs. Placebo.

Table 2. Apo composition of VLDL, LDL, and HDL fractions (µg/mg [a], mean ± SD) and changes in apo composition as an effect of treatment.

Apo	Time	Placebo (n=14)	ERN (n=14)	P-OM3 (n=15)	Combo (n=13)	p value
VLDL						
apoA1/apoB	baseline	79.5 ± 78.0	76.6 ± 44.1	73.8 ± 36.3	57.5 ± 28.3	
	final	83.2 ± 80.7	71.0 ± 31.2	85.3 ± 77.5	72.0 ± 49.5	0.65 [b]
	% change	9.3 (-19; 38)	3.4 (-23; 30)	2.1 (-26; 30)	30 (-2.8; 62)	0.26 [c]
apoC2/apoB	baseline	466 ± 270	424 ± 127	450 ± 478	325 ± 117	
	final	439 ± 222	336 ± 83	396 ± 178	384 ± 196	0.36 [b]
	% change	4.8 (-15; 25)	-9.5 (-28; 9.0)	6.6 (-13; 26)	20 (-2.6; 42)	0.06 [c]
apoC3/apoB	baseline	1347 ± 861	1071 ± 303	1344 ± 1614	870 ± 256	
	final	1326 ± 1078	911 ± 199	1240 ± 553	1029 ± 474	0.66 [b]
	% change	-2.2 (-23; 19)	-7.3 (-27; 12)	16 (-4.4; 36)	19 (-4.5; 42)	0.05 [c]
apoE/apoB	baseline	243 ± 191	217 ± 67	274 ± 192	149 ± 64	
	final	241 ± 191	198 ± 66	271 ± 106	224 ± 67	0.05 [b]
	% change	3.4 (-25; 32)	-7.8 (-34; 18)	9.2 (-19; 38)	47 (15; 79) [#]	0.007 [c]
LDL						
apoA1/apoB	baseline	66.5 ± 42.7	91.3 ± 47.8	81.2 ± 62.5	51.0 ± 31.6	
	final	65.6 ± 47.3	96.8 ± 43.9	83.1 ± 42.8	97.0 ± 67.1 **	0.02 [b]
	% change	-19 (-83; 44)	28 (-30; 86)	3.2 (-57; 63)	91 (20; 163) [#]	0.01 [c]
apoC2/apoB	baseline	8.9 ± 2.6	9.1 ± 4.7	8.7 ± 5.0	10.3 ± 5.3	

	final	7.9 ± 3.1	7.6 ± 3.3	7.9 ± 3.1	8.3 ± 5.1	0.78 [b]
	% change	3.6 (-19; 27)	3.6 (-18; 25)	14 (-8.2; 37)	0.6 (-25; 26)	0.67 [c]
apoC3/apoB	baseline	88.6 ± 67.8	61.8 ± 32.1	87.2 ± 56.0	86.3 ± 38.3	
	final	75.4 ± 65.1	51.5 ± 26.4	80.9 ± 41.4	68.6 ± 35.4	0.76 [b]
	% change	-6.7 (-29; 15)	-8.2 (-29; 13)	13 (-8.5; 34)	-2.9 (-28; 22)	0.23 [c]
apoE/apoB	baseline	30.3 ± 14.3	40.2 ± 17.7	53.8 ± 51.8	27.9 ± 14.0	
	final	27.8 ± 11.3	38.2 ± 19.3	48.1 ± 37.8	43.0 ± 16.5 **	0.002 [b]
	% change	-1.7 (-33; 29)	-0.8 (-29; 28)	-1.6 (-32; 29)	64 (30; 98) [##]	0.0003 [c]
<u>HDL</u>						
apoA2/apoA1	baseline	345 ± 44	364 ± 42	347 ± 53	359 ± 51	
	final	351 ± 51	341 ± 41	335 ± 39	343 ± 42	0.34 [b]
	% change	6.9 (-0.6; 14)	1.2 (-5.7; 8.2)	3.6 (-3.8; 11)	5.5 (-2.9; 14)	0.50 [c]
apoB/apoA1	baseline	42.7 ± 45.6	36.6 ± 27.9	20.3 ± 17.9	34.2 ± 35.5	
	final	51.7 ± 43.7	26.8 ± 19.7	23.6 ± 19.4	29.6 ± 20.1	0.25 [b]
	% change	69 (8; 129)	1.3 (-54; 57)	42 (-15; 99)	38 (-28; 104)	0.18 [c]
apoC2/apoA1	baseline	65.3 ± 15.7	61.3 ± 16.9	57.5 ± 15.5	74.7 ± 28.3	
	final	59.4 ± 12.8	60.2 ± 18.1	57.5 ± 16.1	62.1 ± 23.6	0.05 [b]
	% change	-11 (-23; 0.7)	-6.2 (-17; 4.8)	-4.3 (-16; 7.2)	-19 (-33; -5.8)	0.10 [c]
apoC3/apoA1	baseline	225 ± 63	193 ± 49	206 ± 68	235 ± 98	
	final	208 ± 57	190 ± 56	225 ± 74	207 ± 88 **	0.001 [b]
	% change	-2.2 (-12; 7.7)	1.1 (-8.1; 10)	13 (3.0; 22) [#]	-6.4 (-18; 4.8)	0.004 [c]

apoE/apoA1	baseline	38.0 ± 9.7	42.2 ± 12.0	42.2 ± 11.0	36.0 ± 8.1	
	final	39.8 ± 8.4	36.0 ± 5.6	45.5 ± 18.6	38.3 ± 10.7	0.03 [b]
	% change	1.3 (-16; 19)	-11 (-27; 5.6)	6.6 (-11; 24)	0.6 (-19; 20)	0.24 [c]

[a] normalized to apoB (VLDL and LDL) or apoA1 (HDL); [b] repeated measures two-way ANOVA of paired (baseline and final) measures, p value is shown for the interaction between time and treatment; ** $p < 0.01$ vs. baseline; [c] linear regression analysis of the % apo parameter change from baseline and the treatment group (as an independent variable with four levels), adjusted for age, sex, smoking, medications, BMI, and log-transformed apo at baseline followed by post-hoc analysis comparing the effects of ERN, P-OM3, or Combo to Placebo; [#] $p<0.05$, [##] $p<0.01$ vs. Placebo

Table 3. ApoC3 glycosylation index in plasma and in VLDL, LDL, or HDL density fractions (mean ± SD) and % changes in apoC3 glycosylation index adjusted for covariates (mean; 95%CI).

Fraction	Time-point	Placebo (n=14)	ERN (n=14)	P-OM3 (n=15)	Combo (n=13)	p value
plasma	baseline	0.50 ± 0.16	0.40 ± 0.10	0.49 ± 0.11	0.48 ± 0.11	
	final	0.50 ± 0.14	0.50 ± 0.14 *	0.70 ± 0.33 ***	0.75 ± 0.31 ****	0.003 [a]
	% change	-9 (-28; 11)	39 (19; 58)	48 (33; 66) [#]	49 (30; 69) [#]	0.0002 [b]
VLDL	baseline	0.61 ± 0.17	0.53 ± 0.11	0.60 ± 0.10	0.60 ± 0.11	
	final	0.62 ± 0.14	0.66 ± 0.11 *	0.78 ± 0.28 **	0.82 ± 0.28 ***	0.03 [a]
	% change	-12 (-35; 10)	16 (-4; 37)	26 (3; 49) [##]	26 (0; 51) [#]	0.006 [b]
LDL	baseline	0.53 ± 0.22	0.41 ± 0.12	0.49 ± 0.11	0.44 ± 0.14	
	final	0.51 ± 0.18	0.56 ± 0.23	0.75 ± 0.51 **	0.77 ± 0.33 **	0.02 [a]
	% change	-25 (-70; 20)	32 (-8; 72)	55 (11; 99) [##]	61 (12; 109) [##]	0.002 [b]
HDL	baseline	0.46 ± 0.16	0.36 ± 0.10	0.45 ± 0.12	0.45 ± 0.11	
	final	0.45 ± 0.14	0.51 ± 0.15 *	0.66 ± 0.31 ***	0.77 ± 0.32 ****	0.002 [a]
	% change	-18 (-47; 11)	37 (11; 63) [#]	43 (15; 72) [###]	44 (12; 76) [###]	0.0002 [b]

[a] repeated measures two-way ANOVA of paired (baseline and final) measures, p value is shown for the interaction between time and treatment; * p<0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 vs. final; [b] linear regression analysis of the % apoC3 glycosylation index change (from baseline) and the treatment group (as an independent variable with four levels) adjusted for age, sex, smoking, medications, BMI, and log-transformed apoC3 glyco-isoform ratio at baseline followed by post-hoc comparisons of ERN, P-OM3, or Combo to Placebo; [#] p<0.05; [##] p<0.01; [###] p<0.001 vs. Placebo.

Highlights

- Niacin plus omega-3 therapy was tested in overweight dyslipidemic patients
- This combination is more potent in normalizing apolipoprotein parameters
- These changes are consistent with effects of this combination on plasma lipids
- Combo therapy normalized apoC3 glycosylation

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Supporting Information

Table S1. Subjects' characteristics (mean ± SD) and parameter distributions after exclusion of four subjects of the original study [1] for technical reasons.

Variable	Time	Placebo (n=14)	ERN (n=14)	P-OM3 (n=15)	Combo (n=13)
Age (years) [a]	baseline	49.6 ± 12.9	47.0 ± 11.3	46.3 ± 11.1	48.6 ± 6.6
Male Sex (N, %) [b]	baseline	8 (57%)	8 (57%)	8 (53%)	9 (69%)
Smokers (N, %) [b]	baseline	1 (7%)	2 (14%)	1 (7%)	0 (0%)
Anti-hypertens. Med (N, %) [b]	baseline	3 (21%)	4 (29%)	3 (20%)	3 (23%)
Statins Use (N, %) [b]	baseline	3 (21%)	2 (14%)	3 (20%)	0 (0%)
MetSyn-related parameters [c]					
BMI (kg/m^2)	baseline	29.8 ± 2.5	32.7 ± 4.6	34.0 ± 3.7	33.1 ± 4.4
	final	30.1 ± 2.3	32.7 ± 4.8	34.4 ± 4.0	33.2 ± 4.4
TG (mg/dL)	baseline	249 ± 156	193 ± 66	206 ± 74	213 ± 76
	final	261 ± 176	164 ± 61	180 ± 81	147 ± 46 ***
HDL-C (mg/dL)	baseline	40.6 ± 7.4	44.3 ± 9.8	43.7 ± 7.5	40.0 ± 8.1
	final	41.2 ± 6.2	49.4 ± 11.4 **	43.4 ± 10.7	47.9 ± 9.7 ****
LDL-C (mg/dL)	baseline	121 ± 24	151 ± 45	112 ± 36	141 ± 29
	final	118 ± 20	139 ± 51	112 ± 33	140 ± 33
VLDL-C (mg/dL)	baseline	30.4 ± 11.4	28.9 ± 8.4	29.7 ± 9.7	35.5 ± 13.6
	final	30.8 ± 10.5	23.6 ± 6.1 *	25.9 ± 7.5	23.8 ± 5.6 ****
Total Chol (mg/dL)	baseline	192 ± 28	224 ± 55	185 ± 42	209 ± 46
	final	197 ± 37	199 ± 43	179 ± 38	196 ± 36
Systolic BP (mmHg)	baseline	129 ± 11	133 ± 15	133 ± 11	133 ± 10
	final	127 ± 13	127 ± 11	133 ± 17	128 ± 8
Diastolic BP (mmHg)	baseline	81 ± 7	81 ± 9	84 ± 7	84 ± 7
	final	80 ± 10	79 ± 10	81 ± 6	80 ± 7
Fasting Glucose (mg/dL)	baseline	95 ± 13	100 ± 11	103 ± 10	104 ± 13
	final	99 ± 8	104 ± 10	102 ± 12	107 ± 14
HGB A1c (%)	baseline	5.4 ± 0.5	5.7 ± 0.4	5.5 ± 0.4	5.5 ± 0.4
	final	5.6 ± 0.4	5.8 ± 0.4	5.6 ± 0.4	5.8 ± 0.4

[a] one-way ANOVA; [b] Chi-square test; [c] two-way ANOVA on paired (baseline vs. final) parameter values; * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, Bonferroni-corrected post-test, baseline vs. final

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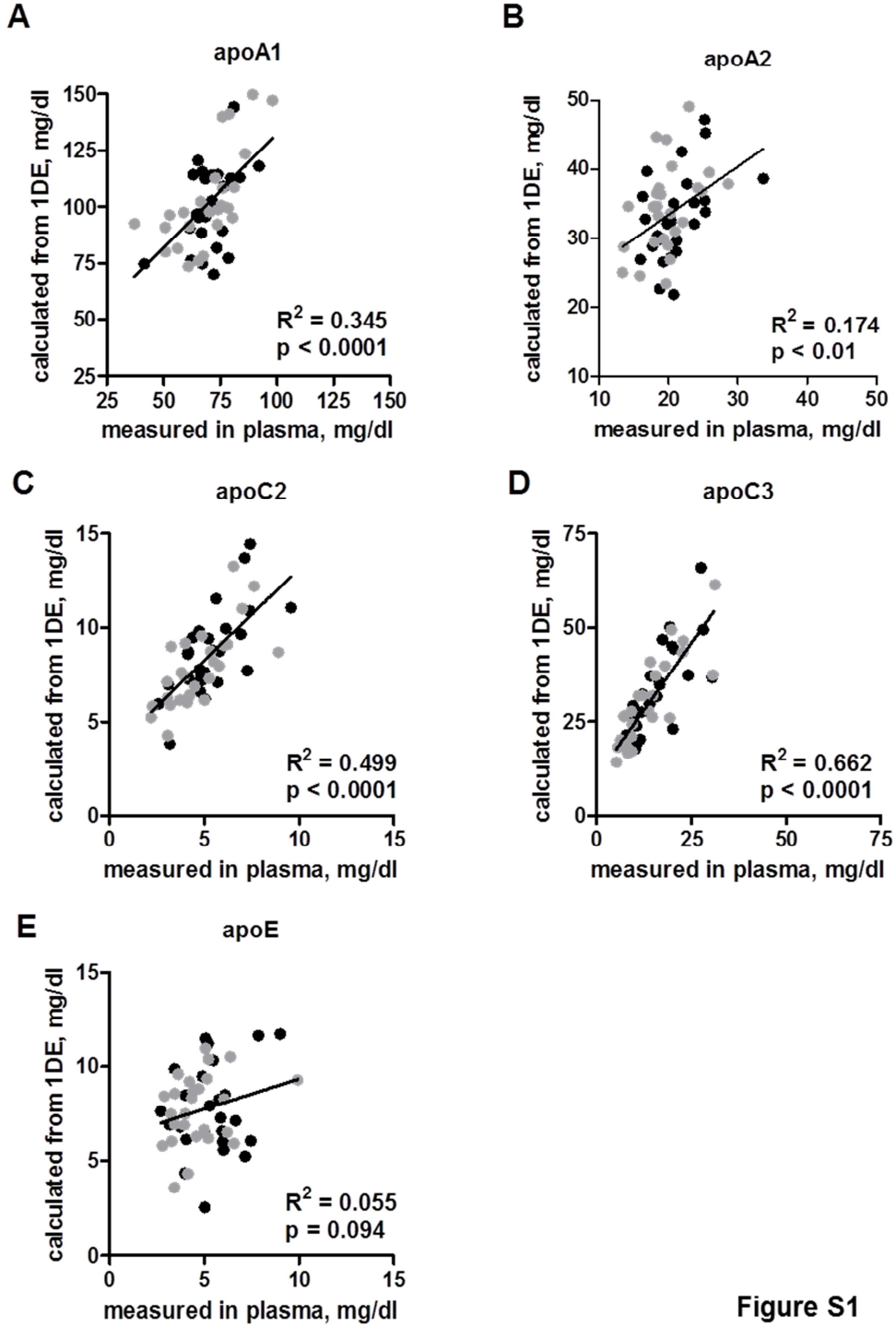

Figure S1. Correlations between apolipoprotein concentrations measured in plasma directly by Multiplex Immuno-Assay and calculated from 1D electrophoresis of lipoprotein fractions obtained by sequential ultracentrifugation. A, apoA1; B, apoA2; C, apoC2; D, apoC3; E, apoE; black – baseline; gray – final.

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