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# Abnormal lipoprotein oxylipins in metabolic syndrome and partial correction by omega-3 fatty acids

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ABSTRACT

Metabolic syndrome (MetSyn) is characterized by chronic inflammation which mediates the associated high risk for cardiovascular and other diseases. Oxylipins are a superclass of lipid mediators with potent bioactivities in inflammation, vascular biology, and more. While their role as locally produced agents is appreciated, most oxylipins in plasma are found in lipoproteins suggesting defective regulation of inflammation could be mediated by the elevated VLDL and low HDL levels characteristic of MetSyn. Our objective was to compare the oxylipin composition of VLDL, LDL, and HDL in 14 optimally healthy individuals and 31 MetSyn patients, and then to determine the effects of treating MetSyn subjects with 4 g/day of prescription omega-3 fatty acids (P-OM3) on lipoprotein oxylipin profiles. We compared oxylipin compositions of healthy (14) and MetSyn (31) subjects followed by randomization and assignment to 4 g/d P-OM3 for 16 weeks using LC/MS/MS. Compared to healthy subjects, MetSyn is characterized by abnormalities of (1) pro-inflammatory, arachidonate-derived oxylipins from the lipoxygenase pathway in HDL; and (2) oxylipins mostly not derived from arachidonate in VLDL. P-OM3 treatment corrected many components of these abnormalities, reducing the burden of inflammatory mediators within peripherally circulating lipoproteins that could interfere with, or enhance, local effectors of inflammatory stress. We conclude that MetSyn is associated with a disruption of lipoprotein oxylipin patterns consistent with greater inflammatory stress, and the partial correction of these dysoxylipinemias by treatment with omega-3 fatty acids could explain some of their beneficial effects.

## 1. Introduction

MetSyn is a combination of abdominal obesity, elevated triglycerides, mildly elevated blood pressure and fasting glucose, and low HDL-cholesterol [1] which taken together represents an increased risk for cardiovascular disease and co-morbidities [2]. While it remains controversial whether these risk factors act synergistically or independently, the dyslipidemia is clinically important since high TG and low HDL define a CVD risk independent of the classic lipid risk marker, LDL-cholesterol. After lifestyle changes, the primary means to reduce lipid-based risk is statin therapy however given the nature of MetSyn, this approach seems less appropriate since it leaves TG and HDL relatively un-corrected but reduces LDL-C, a dyslipidemia that is not always present in MetSyn.

Omega-3 fatty acids reduce serum triglycerides [3] by 20–30% in pharmaceutical doses [4,5], ameliorating one component of MetSyn – dyslipidemia. Alone, this effect seems insufficient to account for the reduction in mortality reported in the JELIS trial, where subjects with MetSyn taking 1.8 g/day EPA had a 50% reduction in relative risk for major coronary events [6]. Given the estimated contribution of hypertriglyceridemia to overall risk [7,8] this is a greater risk-reduction than would be expected (especially considering that the net change in TG levels was only 5%), and it suggests omega-3 fatty acid therapy reduces risk by other mechanisms.

One systemic effect of P-OM3 is to increase the abundance of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in tissues. They do the same for most [9,10], but not all [11] lipid mediators produced from these fatty acids. Production of lipid mediators by

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oxygenating enzymes such as cyclooxygenase, lipoxygenase or cytochrome p450 are a major means whereby polyunsaturated fatty acids (PUFA) exert their effects; collectively, the superclass of oxygenated lipid mediators is termed *oxylipins*. Oxylipins are transported throughout the plasma in lipoproteins where they are available for delivery to target tissues by the lipolytic actions of lipases [12]. We hypothesized that the MetSyn could also be characterized by an abnormal profile of lipoprotein oxylipins. In a previous study, P-OM3 favorably altered the vasoactive oxylipin pattern in VLDL [13]. Here we sought confirmation of this in the controlled trial setting and further asked whether intervention with P-OM3 improved abnormalities in lipoprotein oxylipins. We employed a targeted lipidomic approach, measuring the metabolites of multiple oxylipin-producing pathways, multiple parent fatty acids (FA) and each major lipoprotein subclass (HDL, LDL, and VLDL) in a placebo controlled trial.

## 2. Methods

### 2.1. Metabolic syndrome participants and study location

The study was conducted by Sanford Research/USD in cooperation with Sanford Clinic – Clinical Research Services in Sioux Falls, South Dakota, as originally reported [14]. The protocol was approved by the IRB at the University of South Dakota and registered at clinicaltrials.gov (NCT00286234). Informed consent was required.

### 2.2. Study design

This is an ancillary study [14] with the addition of a group of optimally healthy controls for comparison since 'healthy' oxylipin levels are unknown. Fig. 1 represents the flow diagram of the substudy reported here. The original study employed P-OM3 treatment along with extended release niacin (ERN) in a randomized, double-blind, placebo-controlled clinical trial, with a 2 × 2 factorial treatment design; for this study the ERN arm was not included.

### 2.3. Selection of metabolic syndrome subjects

As previously reported [14], after initial screening informed consent and a full screening panel were obtained. Inclusion criteria were: age 40–69 years; BMI 25–40 kg/m$^2$; fasting TG, 150–750 mg/dL; HDL-C > 10 mg/dL; and the ratio of TG/HDL-C > 3.5. Qualifying subjects began a 6-week, diet-stabilization, dual-placebo, run-in phase (single-blind) in which non-compliant subjects (< 80% compliant) were identified and excluded. Those completing the run-in phase were randomly assigned to treatment using permuted blocks of four with stratification for gender (Fig. 1). All subjects were > 80% compliant by pill count.

### 2.4. Healthy controls

In addition to the MetSyn subjects, we recruited a set of healthy controls for comparison with MetSyn. Our intention was for the subjects to be 'optimally' healthy rather than 'apparently' healthy, and so our recruitment criteria were stringent to reflect this. Recruitment took place during the same time as the MetSyn subjects were entering their final visit. Subjects were age-matched (aged 40–69) with inclusion criteria as: TG < 130, a TG/HDL ratio < 3.0 and BMI between 21 and 25. They could not be taking any lipid lowering medications (*e.g.* statins, fibrates, niacin, n-3 FAs, *etc.*) or vasoactive medications (*e.g.* ACE inhibitors, alpha or beta blockers, $Ca^{2+}$ channel blockers, or long acting nitrates, *etc.*). Subjects were screened by telephone and those qualifying were invited to a screening visit where informed consent was obtained, a medical history taken, height and weight measured and a fingerstick lipid and glucose panel obtained. Subjects still qualifying were invited to participate in the study.

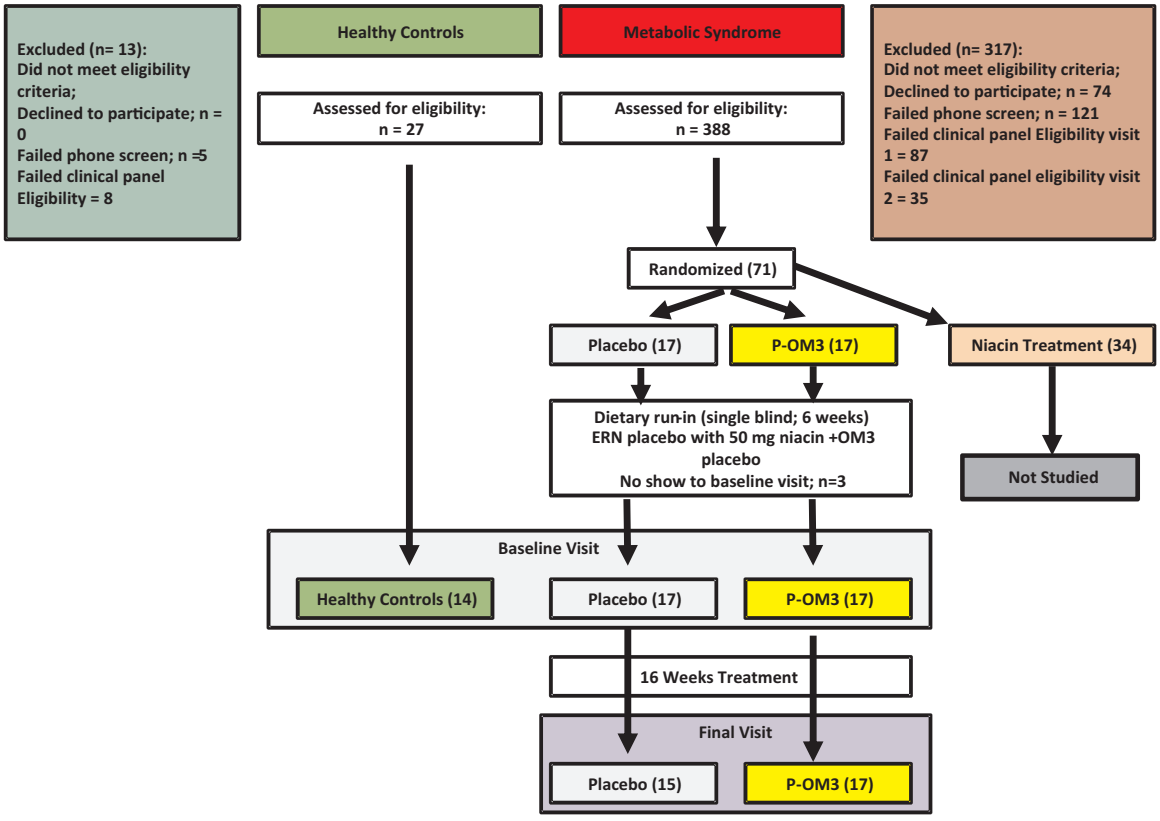


**Fig. 1. Subject recruitment**. Flow diagram showing the relationship between this ancillary trial and the parent trial. Based on the Consolidated Standards of Reporting Trial.