A Pilot Trial: Fish Oil and Metformin Effects on ApoB-Remnants and Triglycerides in Women with Polycystic Ovary Syndrome

Donna Vine¹, Ethan Proctor¹, Olivia Weaver¹, Mahua Ghosh², Katerina Maximova³, Spencer Proctor¹.

¹Metabolic and Cardiovascular Diseases Laboratory, ²Department of Endocrinology and Metabolism, Faculty of Medicine and Dentistry, ³School of Public Health, University of Alberta, Edmonton, Alberta, Canada.

Address correspondence and request for reprints to:

Dr. Donna Vine
Metabolic and Cardiovascular Diseases Laboratory
Division of Human Nutrition,
Faculty of Agriculture, Life and Environmental Sciences
University of Alberta, Edmonton, AB
Canada, Tel: 780-492-4393
Email: donna.vine@ualberta.ca

Acknowledgements: The study was funded in part by Canadian Health Research Institute, Women and Children’s Health Research Institute and Alberta Diabetes Institute.

Disclosure Summary: The authors have nothing to disclose

Registered at clinicaltrials.gov (NCT04116203)
Abstract

Context: Women with PCOS have increased incidence of atherogenic dyslipidemia and increased incidence of cardiovascular disease (CVD). Interventions to target atherogenic dyslipidemia are limited in women with PCOS to reduce CVD risk.

Objective: To determine the effect of high dose fish oil (FO), metformin and FO as an adjunct therapy to metformin (FO-metformin) for 12 weeks on fasting and non-fasting plasma lipids and ApoB-remnants in young women with the metabolic syndrome (MetS) and PCOS.

Design, setting and participants: Participants were randomized into three interventions; i) FO, ii) metformin and iii) FO-metformin in an open-label parallel pilot trial in young women aged 18-30yrs with MetS and PCOS.

Main Outcome Measures: Plasma lipids and ApoB (48 and 100)-lipoproteins and triglycerides (TG) were measured in the fasted and postprandial state following a high-fat meal at baseline and post-intervention.

Results: FO-metformin significantly lowered fasting plasma TG by >40% compared to FO and metformin treatments. Fasting plasma apoB48 was lowered 40% in FO-metformin and 15% in the FO treated groups from baseline to post-intervention. ApoB48 AUC, ApoB48 IAUC, ApoB100 AUC and ApoB100 IAUC decreased in all groups from baseline to post-intervention, however these findings did not reach statistical significance.

Conclusions: The findings of this pilot trial show high dose FO and FO-metformin combination therapy tend to lower fasting and post-prandial plasma TG and ApoB-lipoprotein remnants compared to metformin, however the study is limited by small sample size. These results may be clinically significant in individuals with PCOS for management of atherogenic dyslipidemia.

Keywords: PCOS, Obese, Metabolic Syndrome, fish oil, metformin, triglycerides, ApoB-lipoproteins, postprandial lipemia, non-fasting lipids, lipids
Introduction

Cardiometabolic risk factors of obesity, dyslipidemia and insulin resistance are highly prevalent in adolescents and young women with polycystic ovary syndrome (PCOS) (1,2). These risk factors persist throughout the lifespan of women with PCOS, increasing the risk of cardiovascular disease (CVD), Type 2 Diabetes and other co-morbidities (3-7). Large population studies have shown a 2-fold higher incidence of dyslipidemia and CVD in women with PCOS (5,6,8,9). In young women (18-45 yrs) with PCOS and the metabolic syndrome (MetS) there is an increased incidence of atherogenic dyslipidemia and premature atherosclerotic cardiovascular disease (ACVD), including endothelial and cardiac dysfunction, and increased carotid intimal medial thickness (cIMT) (10-19). A causal risk factor in the development of atherosclerosis and CVD is atherogenic dyslipidemia, and 65-80% of women with PCOS are reported to have an adverse lipid profile (20,21).

Atherogenic dyslipidemia includes elevated fasting and non-fasting triglycerides (TG) and total apolipoprotein (Apo)-B, and decreased fasting HDL-C concentrations (22,23). Fasting and non-fasting plasma ApoB-remnant cholesterol lipoproteins have been causally associated with increased incidence of ischemic cardiovascular events (24,25). Remnant cholesterol refers to remnants of triglyceride rich Apo-B-lipoproteins; chylomicron-ApoB48 from the intestine and very low density lipoprotein-ApoB100 derived from the liver (24). ACVD is caused by an accumulation of ApoB-lipoprotein remnant cholesterol in the subendothelium-intima and thickening of the smooth muscle layer in the arterial wall (26-28). ApoB-cholesterol remnants are associated with early ACVD, ‘residual risk’ of CVD and increased ischemic CVD events (24). Progression of ACVD over decades leads to end stage ischemic CVD, including coronary and cerebral artery disease (24,26). We have been the first to show that obese adolescents with PCOS and MetS (13-17yrs) have elevated fasting and non-fasting TG and plasma ApoB-remnant cholesterol lipoproteins (1,29). These results indicate that adolescents and young women with PCOS and the MetS may be at high-risk for premature development of ACVD (1,29). Currently we lack evidence-based research for effective and safe treatments that target ApoB-remnant cholesterol lipoproteins in young women with PCOS (2,5,30).

The metabolic syndrome (MetS) has higher prevalence in adolescents and young females (18-50 yrs) with PCOS compared to age and BMI matched controls, and has been shown to be between 24-45% in different populations (3,30-32). Individuals with PCOS and MetS are at a 4-fold higher risk of developing T2D (5,6). Furthermore, those with PCOS and T2D have a 3-fold higher risk of developing ACVD compared to those without diabetes (5). The first-line of intervention in overweight-obese PCOS patients is to reduce body weight by targeting dietary-lifestyle habits (2). However, the PCOS
and MetS phenotype is often metabolically resistant to dietary-lifestyle interventions (33-35). Metformin is commonly used as standard of care for treatment of insulin resistance and the prevention of transition to Type 2 Diabetes in PCOS (2,36). Metformin may improve insulin sensitivity, reduce glucose production, and reduce androgens via steroidogenic enzyme pathways, and contribute to improvement in the insulin mediated upregulation of lipogenesis leading to dyslipidemia (36,37). However meta-analyses have shown metformin does not improve fasting plasma lipids in PCOS, particularly in the PCOS and MetS phenotype (30,36,38,39). Fish oil (FO) contains omega-3 long chain fatty acids, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), and FO has been shown to effectively target fasting TG, non-fasting plasma TG and total apoB-lipoproteins in the MetS (40-43). Meta-analyses have shown FO lowers fasting plasma TG and apoB-lipoproteins by 15-45%, and FO specifically targets the non-fasting or post-prandial excursion in plasma TG following a high fat meal (42,44). Evidence to date suggests FO supplementation may reduce fasting plasma TG in PCOS women (45-50). However, the efficacy of FO or combination FO-metformin treatment to target both fasting and non-fasting excursions in TG and ApoB-lipoprotein remnants in the PCOS and MetS phenotype has not been investigated.

The aim of this pilot trial was to investigate the efficacy of a polytherapeutic first-line intervention approach of healthy diet-lifestyle counselling with either high dose fish oil, metformin, or combination fish oil + metformin treatment on fasting and non-fasting plasma TG, and ApoB48 and ApoB100-lipoprotein remnant concentrations in young women with PCOS and the MetS. We hypothesized that a combination of fish oil + metformin, compared to fish oil or metformin alone, would lower fasting and non-fasting plasma TG, and ApoB48- and ApoB100-lipoprotein remnant concentrations in women with PCOS and the MetS.

Methods

Study Design

The trial was an open-label parallel randomized design. Upon enrolment eligible participants completed a signed informed consent and were assigned using the block method (Sealed envelope™) to one of the following interventions for 12 weeks; 1) metformin (1.5mg/day, n=8)), FO (high dose containing 2.52g EPA, 1.68g DHA/day, n=13)) or 3) FO-metformin (n=8). Participant selection was based on inclusion criteria of consecutive patients that consented to be in the study over a specific time interval of 12 mths. Original sample size calculations to achieve a clinically significant reduction of 15% in 12 weeks in the primary outcome of fasting plasma TG were n=28 with p<0.05 and 80% power to detect a significant difference. We aimed to recruit n=38 participants.
allowing for a 30% dropout or failure to comply to intervention. Final participant numbers in each intervention group using random permuted block method were as follows metformin n=8, FO n=13, FO-metformin n=8. Prior to baseline measurements participants completed 24hr food records for 3 days to reflect their usual dietary intake (2 weekdays and 1 weekend day)(51). Diet records were used by a Registered Dietitian to counsel participants on nutrition and lifestyle according to Eat Well Live Well with Health Canada’s Food Guide, as part of recommendations for first-line standard of care (2). Compliance to interventions was assessed by our institutional clinical trial management team in which compliance was determined by documenting number of capsules consumed by participant (daily log) and counting of remaining capsules. The study has 98% compliance. The study was approved by the University of Alberta Human Ethics Board (Pro00059201), Health Canada Natural Health Product Directorate and Health Canada Drug Directorate (HC6-24-C191998), and registered at clinicaltrials.gov (NCT04116203).

Study Participants and Inclusion Criteria

Participants were young women with PCOS aged 18-30yrs recruited from endocrine clinics and the greater community in Edmonton, Alberta, Canada. Inclusion criteria included; i) a PCOS diagnosis using the Rotterdam PCOS Diagnostic Criteria in adults with two of the following criteria met; oligo- or anovulation, clinical and/or biochemical hyperandrogenism, or polycystic ovaries on ultrasound, and exclusion of related endocrine disorders (2); ii) features of the MetS; BMI >25 kg/m², fasting plasma TG >150mg/dL and/or total apoB>0.08g/L, the presence of glucose intolerance or impaired insulin sensitivity (fasting glucose 100-125 mg/dL (6.1-6.9 mM), OGT 2 hr >7.8-11 mM and/or insulin >15μM/mL. Participants were excluded if they were pregnant, lactating or attempting to get pregnant or taking fertility drugs. In those taking oral-contraceptives, metformin, non-required medications, nutraceuticals or special diets were required to withdraw from these if medically appropriate and a washout period of 8 weeks was initiated before starting the intervention. The health and diet aspects for each participant were reviewed as part of routine standard of care before approval for participation in the study.

Anthropometric, body composition and blood pressure measurements

Anthropometric assessments were done on the same clinical visit day at baseline and post-intervention by trained research staff using standardized measurement protocols, as previously described (1,29). In brief, height was measured without shoes to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Limited, UK). Waist circumference (WC) was measured to the nearest 1 mm at the level of the umbilicus using a non-stretch measuring tape. All anthropometric measurements were completed three times and average values were calculated. Body composition including weight, fat mass, fat-free mass, % fat mass and % fat-free mass was determined using the BOD POD system (COSMED/Life Measurement, Inc. USA) (29,52).
Baseline and post-intervention assessment of fasting plasma lipids, biochemical and endocrine parameters

Fasting plasma lipids, biochemical and endocrine parameters were performed at University of Alberta Hospital Outpatient Laboratory using standard protocols (29). Total cholesterol (TC), LDL-C, HDL-C and TG were measured using enzymatic colorimetric assays at (29,52). Plasma endocrine hormone profile (using fasting blood collected between 08:00 and 11:00 hr) with assay limit of quantification (LQ) and intra-assay coefficient of variation (ICV) using the same lot number included; total T (LQ:0.2 nmol/l, ICV:2.4-3.3%, Roche Diagnostics, Cobas e801 ECLIJA), androstenedione (LQ:1.0 nmol/l, ICV:8.8-12.1%, DIASource RIA), dehydroepiandrosterone (DHEAS) (LQ:0.5nmol/l, ICV:2.5-5%, Roche Diagnostics, Cobas e801 ECLIJA) and sex hormone binding globulin (SHBG) (LQ:0.8nmol/l, ICV 6.8-7.2%, Roche Diagnostics, Cobas e801 ECLIJA). Free T was calculated using Vermeulen equation(53). The free androgen index (FAI) was calculated, FAI=100 (Total T/SHBG) (29). Fasting and non-fasting plasma insulin and glucose were assessed using an oral glucose tolerance test (2hr, 75g glucose). The homeostasis model assessment of insulin-resistance (HOMA-IR) was calculated by fasting insulin (mU/mL) x fasting glucose (mmol/L) / 22.5, and insulin resistance was defined by a HOMA-IR reading >4 (29,54).

Baseline and post-intervention postprandial lipid and ApoB-lipoprotein metabolism following a high-fat meal

At baseline and following the 12 wk intervention a high-fat meal test was undertaken by each participant. Following a 12-hour overnight fast a blood sample was taken and then subjects consumed a high-fat milk shake meal providing 0.61g lipid/kg body weight to assess non-fasting postprandial lipid metabolism (29). The meal contained cream (35% milk fat) and Ensure® Calorie Plus Meal Replacement Drink (Abbott Laboratories, Canada) to provide approximately 62.5% energy as fat, 30% energy as carbohydrate and 7.5% energy as protein. Blood samples following consumption of the meal were collected into EDTA-coated vacutainers at 2, 4, 6 and 8 hr, and plasma prepared by centrifugation (3500 rpm, 4 °C for 10 min) and stored at -80 °C for lipid and ApoB-lipoprotein analysis. Area Under the curve (AUC) and incremental AUC (iAUC) (non-fasting response) for lipids and apoB-lipoproteins were determined using GraphPad 8.0 (San Diego, US)(29,52).
Quantification of plasma ApoB-lipoproteins

ApoB48- and ApoB100-lipoproteins were quantified using an adapted Western blot method as previously described (1,29,52). Briefly, total plasma proteins were separated on a 3–8% NUPAGE Tris-acetate polyacrylamide gel (Invitrogen, USA) and then transferred onto a polyvinylidene difluoride membrane (0.45 μm, ImmobilonPTM, Millipore, USA). Membranes were incubated with a primary polyclonal antibody specific for ApoB (dilution 1:200, RRID:AB_92217)(55) and a secondary antibody tagged with horseradish peroxidase (dilution 1: 500, RRID:AB_628490) (56). ApoB48 and B100 bands were visualized by enhanced chemiluminescence (ECL Advance, Amersham Biosciences, UK) and quantified using linear densitometric comparison to a known mass of purified human ApoB48 and ApoB100 standards (1,29).

Statistical Analyses

Normality of continuous variables was assessed by the D’Agostino & Pearson omnibus normality test. Differences between intervention groups were examined using ANOVA and repeated-measures ANOVA for comparing baseline to post-intervention results, followed by Bonferroni post-tests for multiple comparisons when data assumed a normal Gaussian distribution. To explore simple relationships between primary outcomes of fasting and non-fasting TG and apoB-lipoproteins, Pearson (for normally distributed data that assume linearity and homoscedasticity) and Spearman (for data not normally distributed) correlation coefficients were used. Select multiple linear regression models were used to further examine the relationship between primary lipid outcomes and predictor variables of interest. Assumptions of normality, linearity, limited multicollinearity, and homoscedasticity were applied, and variables did assume a normal distribution of residuals. Akaike information criterion statistics were used to assess overall model fit. The level of statistical significance was set at p<0.05. All statistical and graphical analyses were performed using GraphPad™ Prism 9.0 (GraphPad Software, Inc. USA).

RESULTS

Anthropometry, Endocrine and Insulin-Glucose Parameters

The body weight, BMI, waist circumference, waist-to-hip ratio, % total fat mass and fat free mass were not significantly different between groups at baseline and post-intervention (Table 1). Within the FO-metformin group there were reductions (8-14%) in total body weight, BMI and % fat mass...
from baseline to post-intervention but this did not reach statistical significance. There were no significant differences in endocrine hormones between treatment groups at baseline or post-intervention. Within groups, SHBG increased by >30% in the metformin treated group, was reduced by >20% in the fish oil group and there was no change in the FO-metformin group, however these did not reach statistical significance. Free T tended to increase in within all groups from baseline following the intervention, and FAI increased markedly in the fish oil group by 70%. There was no significant differences in insulin, glucose, OGT (2hr) or HOMA-IR at baseline or post-intervention between groups. Fasting insulin and HOMA-IR tended to be lower by 17-30% within all groups at baseline compared to post-intervention and these findings may be of clinical significance in individuals, however there was no statistical difference within groups.

Fasting Plasma Lipids and ApoB-Lipoproteins

There was no difference in fasting plasma concentrations in LDL-C, HDL-C or non-HDL-C and apoB48 or apoB100-remnant lipoproteins between groups at baseline or post-intervention (Table 2). Metformin compared to the other treatments was shown to significantly reduce fasting plasma total cholesterol by 10% from baseline to post-intervention. Within the FO-metformin group fasting plasma TG was reduced by >40% from baseline to post-intervention, and in the FO intervention there was a trend for a reduction in TG by 20% from baseline to post-intervention, however these did not reach statistical significance. There was also trend for fasting plasma concentrations of ApoB48 to be lowered by 15% in the FO and by 40% in the FO-metformin group from baseline to post-intervention, which may be clinically significant in individuals, however these findings did not reach statistical significance (Table 2 and Figure 1).

Postprandial Response in Plasma lipids and ApoB-Lipoproteins following a High-Fat Meal

The postprandial response in plasma ApoB-lipoproteins and TG following the high-fat meal is shown in Table 2 and Figure 1. Between groups there was not significant difference in postprandial response at baseline or post-intervention. However, within groups FO and FO-metformin treatments decreased total plasma TG_{AUC} by 15% and 40%, respectively, however these findings did not reach statistical significance. Plasma TG_{AUC}, representing the incremental response in plasma TG from fasting concentration following the high-fat meal tended to decrease in all groups from baseline to post-intervention, by 12% in metformin, 20% in FO and 54% in the FO-metformin intervention groups. Similarly plasma ApoB48_{AUC} and ApoB48_{IAUC}, representing intestinal chylomicron secretion and metabolism in response to the high-fat meal, decreased 7-17% within all groups from baseline to post-intervention. The decrease in ApoB48_{AUC} and ApoB48_{IAUC} was greatest within the FO-
metformin group, however these findings were not significantly different. ApoB100\textsubscript{AUC} and ApoB100\textsubscript{iAUC}, representing hepatic very low density lipoprotein metabolism following the high-fat meal, were shown to modestly decrease by 5-12% in the FO group and 12-15% in the FO-metformin group from baseline to post-intervention compared to metformin treatment alone, however these reductions did not reach statistical significance.

**Correlation and Regression Analysis of Fasting and Non-Fasting Plasma Triglycerides, ApoB-lipoproteins, Body Weight and Insulin Indices**

Total body weight, BMI and %fat mass tended to be reduced to a greater extent in the FO-metformin group compared to metformin and FO groups, therefore we tested if % change in these parameters was correlated with % change in fasting and non-fasting plasma lipids and ApoB-lipoproteins from baseline to post-intervention (Table 3). A decrease in %fat mass was shown to be positively correlated with a decrease in fasting plasma TG. Furthermore, based on our current data of trends for improvements in fasting insulin and HOMA-IR in all intervention groups, we also explored the correlation of these indices with % change in fasting and non-fasting plasma lipids and ApoB-lipoproteins from baseline to post-intervention. We found no significant correlations of % change in insulin or HOMA-IR with % change in fasting and non-fasting plasma lipids and ApoB-lipoproteins from baseline to post-intervention (Table 3). We further performed a multiple linear regression model analyses with the dependant primary variable of % change in fasting TG from baseline to post-intervention with predictive variables of % change in %fat mass and insulin. These parameters were also chosen on the basis of our previous findings and the pathophysiological relationship of these variables (1,29). In the model we found no interaction effect of % change in insulin on the ability of %fat mass to predict % change in plasma TG (data not shown).

**Discussion**

Premature onset of ACVD, including cIMT and endothelial dysfunction, is increased in young women with PCOS (11-16,57). Population studies have shown women with PCOS have a 2-fold higher incidence of CVD, including hypertension and ischemic vascular disease (5,8,9). Atherogenic dyslipidemia, including elevated fasting and non-fasting TG and apoB-lipoprotein cholesterol remnants, are causative risk factors in the development of ACVD and end-stage ischemic CVD events (24,25,58). The aim of this pilot trial was to investigate the potential effect of metformin compared
to high dose FO or combination FO-metformin treatment on fasting and non-fasting plasma TG, and apoB-lipoprotein remnant concentrations in young women with PCOS and the MetS. Consistent with our findings in youth and adolescents with PCOS and MetS, we have shown that young women (18-30yrs) with PCOS and MetS have elevated fasting and postprandial plasma TG, ApoB48 and ApoB100-lipoprotein remnants (1,29,52). The results of this small pilot study demonstrate a strong trend for FO and FO-metformin combination treatment to reduce fasting TG and apoB48-lipoprotein remnants compared to mono-therapy with metformin in young women with PCOS and MetS. Reductions in fasting and postprandial plasma TG and apoB48-lipoprotein remnants were 15-40% following the high dose FO and FO-metformin treatments. Despite a lack of statistical difference between groups, reductions in these risk factors can represent significant clinical improvement in individuals. For example, a decrease in plasma TG and ApoB-remnants by 1mmol/L or >20% is associated with significant reductions in clinical CVD risk (24,59). Furthermore, elevated non-fasting plasma apoB48-remnants have been observed in normolipidemic and hyperlipidemic females with coronary heart disease and it provides an early subclinical biomarker of ACVD and ‘residual risk’ of CVD events (25,60,61). Long term studies are required to track PCOS women with fasting and non-fasting hypertriglyceridemia and apoB-remnant dyslipidemia to determine if they present with increased morbidity and mortality from CVD and ischemic events later in life compared to women without PCOS.

We implemented first-line recommendations of dietary-lifestyle counselling and found no significant improvement between groups in body weight, BMI, % fat mass or waist circumference. However, the FO-metformin group showed a tendency for reductions in body weight, %fat mass and BMI. In correlation analyses we showed only % fat mass was positively associated with improvements in fasting plasma TG, consistent with previous studies, but was not associated with improvements in other lipids or apoB-lipoproteins (1,29). These results suggest improvements at least in fasting plasma TG may be dependent on improvements in %fat mass following interventions. The FO-metformin interventions was shown to have a greater improvement in fasting plasma insulin and HOMA-IR compared to metformin and FO groups, however correlation analyses showed no association of these variables with fasting and non-fasting lipids and apoB-lipoproteins. In regression analyses, we further explored the effect of insulin and % fat mass on fasting and non-fasting lipids and apoB-lipoproteins. We found adjustment of these variables did not impact the primary lipid and ApoB-lipoprotein outcomes. These results suggest that improvements in fasting and non-fasting and apoB-lipoproteins, and non-fasting plasma TG were independent of improvements in % fat mass and insulin indices.
The mechanisms and treatments to target increased atherogenic dyslipidemia and ACVD risk remain understudied in PCOS (5,62). We have shown that androgens via the androgen receptor can exacerbate lipogenesis and dyslipidemia in conditions of insulin resistance and obesity (63,64). The clinical challenge in PCOS is that individuals often have IR and obesity, and together with hyperandrogenemia this appears to exacerbate ApoB-remnant dyslipidemia and premature development of ACVD (3,5,13,19,62,65). To date there have been limited interventional studies that specifically target ApoB-remnant dyslipidemia and subclinical ACVD in PCOS (2,66). Our findings in this study are consistent with meta-analysis with metformin mono-therapy that show no significant improvements in fasting plasma lipids in PCOS, particularly those with obesity (34,36). However we did show trends for improvements in all fasting plasma lipids with metformin treatment. Our data is also consistent with meta analyses that show FO lowers fasting plasma TG and ApoB-lipoproteins by 15-45%, and FO specifically targets the non-fasting excursion in plasma TG following a lipid-meal (42,67). We have shown that FO can reduce intestinal secretion of apoB48 and TG through inhibition of the nuclear receptor SREBP-1c in an animal model of MetS (68). The ANCHOR study showed high dose EPA-ester can lower plasma TG, total apoB and non-HDL cholesterol (an indirect marker of remnant cholesterol) in older high-risk women with T2D on statin therapy (69). Previous trials with low dose omega-3 fatty acids (<1g EPA/DHA) have shown mixed effects, including no effect on Major Adverse Cardiac Events and significant reductions in secondary outcomes, such as heart attack and coronary heart disease risk (70,71). These studies examined older participants (>50-90yrs) taking statins and those with pre-existing health outcomes such as diabetes, coronary heart disease and stroke (70,71). Long term interventions with high dose EPA (>2-4g, FO or EPA-ester or EPA/DHA) have shown reduced incidence of end-stage CVD events in older patients on statin therapy with hypertriglyceridemia and diabetes (44,72-75). A longer term trial is needed in young and older women with PCOS to test the efficacy of high dose FO to reduce plasma ApoB-lipoprotein remnants and TG, and the development and progression of subclinical ACVD.

The limitations of this pilot trial are the small sample size and large interindividual variation in fasting and postprandial response to the high-fat meal leading to a lack of statistical significance in post-intervention results between groups. Standard available hospital anti-body based methods for quantification of steroid hormones were used and this is a limitation of the study. We also did not determine the presence of ACVD, such as cIMT or endothelial vascular function, at baseline or post-intervention. In young women aged 18-30 years increased incidence of premature ACVD has been reported (9,13-16), however this age group is young to observe advanced atherosclerotic lesions and ischemic CVD events, as these are observed in later life (5,76). Three months is a short period of time to target prevention or progression of ACVD, as this disease develops over decades and is
usually diagnosed in mid to late adulthood (>50 years) following an ischemic or vascular event (3,5,76). In pre-clinical studies we have explored the mechanisms of FO and metformin on lipogenic mechanistic pathways, however in this study we did not explore mechanisms (64,68). FO is known to have anti-inflammatory effects (44), however we did not measure inflammatory or cardiac function indices in this pilot trial.

The findings of this pilot trial show high dose FO and FO-metformin combination therapy tend to lower fasting and post-prandial plasma TG and ApoB-lipoprotein remnants to a greater extent compared to metformin alone in young women with PCOS and MetS. These results may be clinically significant for management of fasting and non-fasting atherogenic dyslipidemia and prevention of early ACVD in these individuals. PCOS women with MetS are at higher risk for the development of ACVD, and nutrition-lifestyle counseling combined with FO-Metformin and/or other treatments used in PCOS may form a polytherapeutic approach to prevent early ACVD (76).

Acknowledgements

The study was supported by bridge and pilot funding from the Women and Children’s Health Research Institute (DV), Alberta Diabetes Institute (DV) and Canadian Institute for Health Research (DV). EP and OW were funded by NSERC Summer Studentships.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
References

1. Vine DF, Beilin L, Burrows S, Huang R-C, Hickey M, Hart R, Proctor SD, Mori TA. ApoB48-Lipoproteins Are Associated with Cardiometabolic Risk in Adolescents with and without Polycystic Ovary Syndrome. J Endoc Soc 2020; 4:1-12

2. Teede HJ, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertility Sterility 2018; 110:364-379

3. Pinola P PK, Piltonen TT, Puurunen J., Vanky E S-PI, Stener-Victorin, E LHA, Ravn P, Skovsager Andersen, M GD, Mellembakken JR, Ruokonen, A TJ, Morin-Papunen LC. Normo- and hyperandrogenic women with polycystic ovary syndrome exhibit an adverse metabolic profile through life. Fertil Steril 2017; 107:e2788–2795

4. Bellver J, Robles A, Muñoz E, Martínez F, Landeras J, García-Velasco J, Fontes J, Álvarez M, Álvarez C, Acevedo; Group of interest in Reproductive Endocrinology (GIER) of the Spanish Fertility Society (SEF). Polycystic ovary syndrome throughout a woman’s life. J Assist Reprod Genet 2018; 35:25-39

5. Glintborg D, Nybo M, Abrahamsen B, Andersen M. Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. Cardiovasc Diabetol 2018; 17:37

6. Rubin KH, Glintborg D, Nybo M, Abrahamsen B, Andersen M. Development and Risk Factors of Type 2 Diabetes in a Nationwide Population of Women With Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2017; 102:3848-3857

7. Azziz R, Marin C, Hoq L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. J Clin Endocrinol Metab 2005; 90:4650-4658

8. Okoroh EM BS, George MG, Craig Hooper W. Assessing the intersection of cardiovascular disease, venous thromboembolism, and polycystic ovary syndrome. Thromb Res 2015; 136:1165-1168

9. Ding DC, Wang JH, Lin SZ, Sung FC. Coronary artery disease risk in young women with polycystic ovary syndrome. Oncotarget 2018; 9:8756-8764

10. Sprung VS AG, Cuthbertson DJ, Pugh CIA, Aziz N, Green DJ, Cable NT & Jones H Endothelial function measured using flow-mediated dilation in polycystic ovary syndrome: a meta-analysis of the observational studies. Clin Endocrinol 2013; 78-80

11. Talbott EO, Guzik DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Art. Thromb. Vasc. Biol. 2000; 20:2414-2421

12. Meyer ML, Malek AM, Wild RA, Korytkowski MT, Talbott EO. Carotid artery intima-media thickness in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012; 18:112-126

13. Kosmala W, O’Moore-Sullivan TM, Plaksej R, Kuliczkowska-Plaksej J, Przewlocka-Kosmala M, Marwick TH. Subclinical impairment of left ventricular function in young obese women: contributions of polycystic ovary disease and insulin resistance. J Clin Endocrinol Metab 2008; 93:3748-3754
14. Vural B, Caliskan E, Turkoz E, Kilic T, Demirci A. Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. Hum Reprod 2005; 20:2409-2413

15. Luque-Ramirez M, Mendieta-Azcona C, Alvarez-Blasco F, Escobar-Morreale HF. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. Hum Reprod 2007; 22:3197-3203

16. Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, Batakis N, Phenekos C, Dionyssiou-Asteriou A. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90:2740-2746

17. Yildirim E, Yuksel UC, Celik M, Bugan B, Gokoglan Y, Ulubay M, Gungor M, Koklu M. Echocardiographic evaluation of diastolic functions in patients with polycystic ovary syndrome: A comparative study of diastolic functions in sub-phenotypes of polycystic ovary syndrome. Cardiol J 2017; 24:364-373

18. Orrio F, Palomba S, Cascella T, De SB, Di BS, Russo T, Labella D, Zuolo F, Lombardi G, Colao A. Early impairment of endothelial structure and function in young normal-weight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004; 89:4588-4593

19. Usselman CW, Yarovinsky TO, Steele FE, Leone CA, Taylor HS, Bender JR, Stachenfeld NS. Androgens drive microvascular endothelial dysfunction in women with polycystic ovary syndrome: role of the endothelin B receptor. J Physiol 2019; 597:2853-2865

20. Wild RA. Dyslipidemia in PCOS. Steroids 2012; 77:295-299

21. Wild RA, Rizzo M, Clifton S, Carmina E. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. Fertil Steril 2011; 95:1073-1079 e1071-1011

22. Contois JH, McConnell JP, Sethi AA, Csako GD, S. Hoefner, D. M. Warnick, G. R. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Clin Chem 2009; 55:407-419

23. Anderson T et al. Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult: Application of GRADE and Evidence Review. Canadian Cardiovascular Society 2016;

24. Varbo A, Benn M, Tybaerg-Hansen A, Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol 2013; 61:427-436

25. Fruchart JC, Sacks FM, Hermans MP. Implications of the ACCORD lipid study: perspective from the Residual Risk Reduction Initiative (R(3)i). Curr Med Res Opin 2010; 26:1793-1797

26. Nelson AJ PE, Pagidipati NJ. Atherosclerotic cardiovascular disease and heart failure: Determinants of risk and outcomes in patients with diabetes. Prog Cardiovasc Dis 2019; S0033-0620(19)30098-2

27. Proctor SD, Vine DF, Mamo JC. Arterial retention of apolipoprotein B(48)- and B(100)-containing lipoproteins in atherogenesis. Curr Opin Lipidol 2002; 13:461-470

28. Borén J WK. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. Curr Opin Lipidol 2016; 27:473-483
29. Vine DF, Wang Y, Jetha MM, Ball GD, Proctor SD. Impaired ApoB-Lipoprotein and Triglyceride Metabolism in Obese Adolescents With Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2017; 102:970-982

30. Newman C, Boord J, Cariou B, Chait A, Fein H, Ginsberg H, Goldberg I, Murad HS. Lipid Management in Patients with Endocrine Disorders: An Endocrine Society Clinical Practice Guideline. Journal Clinical Endocrinology and Metabolism 2020; 105:1-70

31. Barber TM, Andreou A, Franks S. Polycystic ovary syndrome: insight into pathogenesis and a common association with insulin resistance. Clin Med 2016; 16:262-266

32. Hart R, Mori T, Huang RC, Norman RJ, Franks S, Sloboda D, Beilin L, Hickey M. Extent of metabolic risk in adolescent girls with features of polycystic ovary syndrome. Fertil Steril 2011; 95:2347-2353

33. Lim SS, Norman RJ, Davies MJ, Moran LJ. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obes Rev 2013; 14:95-109

34. Oyesanya AO, van Wely M, Clarke MJ. Life-style modification, non-pharmacological and pharmacological strategies for obese subfertile women. Cochrane Database of Systematic Reviews 2010; 11

35. Pasquali R, Cavazza C, Ibarra Gasparini D, Ciampaglia W, Cognigni GE, Pagotto U. Heterogeneity in the responsiveness to long-term lifestyle intervention and predictability in obese women with polycystic ovary syndrome. Eur J Endocrinol 2011; 164:53-60

36. Naderpoor N, de Courten B, Misso ML, Moran LJ, Teede HJ. Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. Hum Reprod Update 2015; 21:560-574

37. Nestler JE. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med 1996; 335:617-623

38. Mehrabian F, Mohamadkhani M, Moeinoddini M, Karimzadeh P. Comparison of the effects of metformin, flutamide plus oral contraceptives, and simvastatin on the metabolic consequences of polycystic ovary syndrome. J Res Med Sci 2016; 23:7

39. Sun J, Cai R, Sun H, Zhou Y, Wang P, Huang R, Xia W, Wang S. An investigation into the therapeutic effects of statins with metformin on polycystic ovary syndrome: a meta-analysis of randomised controlled trials. Bmj 2015; 5:e007280

40. Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003; 44:455-463

41. Kelley DS, Siegel D, Vemuri M, Chung GH, Mackey BE. Docosahexaenoic acid supplementation decreases remnant-like particle-cholesterol and increases the (n-3) index in hypertriglyceridemic men. J Nutr 2008; 138:30-35

42. Oscarsson J. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. Lipids in Health & Disease 2017; 16:149-162

43. Jimenez-Gomez Y, Marin C, Peerez-Martinez P, Hartwich J, Malczewska-Malec M, Golabek I, Kiec-Wilk B, Cruz-Teno C, Rodriguez F, Gomez P, Gomez-Luna MJ, Defoort
C, Gibney MJ, Perez-Jimenez F, Roche HM, Lopez-Miranda J. A low-fat, high-complex carbohydrate diet supplemented with long-chain (n-3) fatty acids alters the postprandial lipoprotein profile in patients with metabolic syndrome. J Nutr 2010; 140:1595-1601

44. Kris-Etherton PM, Bowen KJ, Skulas-Ray AC, Jackson KH, Petersen KS, Harris WS. Recent Clinical Trials Shed New Light on the Cardiovascular Benefits of Omega-3 Fatty Acids. Methodist Debakey Cardiovasc J 2019; 15:171-178

45. Phelan N, O'Connor A, Kyaw Tun T, Correia N, Boran G, Roche HM, Gibney J. Hormonal and metabolic effects of polyunsaturated fatty acids in young women with polycystic ovary syndrome: results from a cross-sectional analysis and a randomized, placebo-controlled, crossover trial. Am J Clin Nutr 2011; 93:652-662

46. Nasri K, Aghadavod E, Taghizadeh M, Asemi Z. The Effects of Omega-3 Fatty Acids Supplementation on Gene Expression Involved in the Insulin and Lipid Signaling Pathway in Patients with Polycystic Ovary Syndrome. Horm Metab Res 2017; 49:446-451

47. Cussons AJ, Watts GF, Mori TA, Stuckey BGA. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: A randomized controlled trial employing proton magnetic resonance spectroscopy. Journal of Clinical Endocrinology and Metabolism 2009; 94:3842-3848

48. Vargas ML, Almario RU, Buchan W, Kim K, Karakas SE. Metabolic and endocrine effects of long-chain versus essential omega-3 polyunsaturated fatty acids in polycystic ovary syndrome. Metabolism: Clinical & Experimental 2011; 60:1711-1718

49. Mohammadi E, Rafraf M, Farzadi L, Asghari-Jafarabadi M, Sabour S. Effects of omega-3 fatty acids supplementation on serum adiponectin levels and some metabolic risk factors in women with polycystic ovary syndrome. Asia Pac J Clin Nutr 2012; 21:511-518

50. Rahmani E SM, Ebrahimi FA et al. The effects of omega-3 fatty acids and vitamin E co-supplementation on gene expression of lipoprotein(a) and oxidized low-density lipoprotein, lipid profiles and biomarkers of oxidative stress in patients with polycystic ovary syndrome. Mol Cell Endocrinol 2017; 439:247-255

51. Kirkpatrick S, Hobin E, Solbak N, Wallace A, Haines J, Mayhew A, Orr S, Raina P, Robson P, Sacco J, Whelan H. Lessons from Studies to Evaluate an Online 24-Hour Recall for Use with Children and Adults in Canada. Nutrients 2017; 9 pii: E100. doi: 110.3390/nu9020100.

52. Wang Y, Nzekwu MM, Maximova K, Vine DF, Jetha M, Ball GB, Proctor SD. Elevated remnant lipoproteins may increase subclinical CVD risk in pre-pubertal children with obesity: A case control study. Pediatric Obesity 2013; 8:376-384

53. Vermeulen A, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endo Metab 1999; 84:3666-3672.

54. Matthews DR, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-419

55. RRID:AB_92217 hsorRA.

56. RRID:AB_628490 hsorRA.

57. Orio F, Palomba S, Spinelli L, Cascella T, Tauchmanova L, Zullo F, Lombardi G, Colao A. The cardiovascular risk of young women with polycystic ovary syndrome: an
observational, analytical, prospective case-control study. J Clin Endocrinol Metab 2004; 89:3696-3701

58. Varbo A, Nordestgaard BG, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Benn M. Nonfasting triglycerides, cholesterol, and ischemic stroke in the general population. Ann Neurol 2011; 69:628-634

59. Nordestgaard BG BM, Schnohr P and Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama 2007; 298:299-308

60. Meyer E, Westerveld HT, de Ruyter-Meijstek FC, van Greevenbroek MM, Rienks R, van Rijn HJ, Erkelens DW, de Bruin TW. Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with greater than 70% stenotic coronary artery disease: a case-control study. Atherosclerosis 1996; 124:221-235

61. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Elevated Remnant Cholesterol Causes Both Low-Grade Inflammation and Ischemic Heart Disease, While Elevated Low-Density Lipoprotein Cholesterol Causes Ischemic Heart Disease without Inflammation. Circulation 2013; 128:1298-1309

62. Torres Fernandez ED AK, Syed M, Maranon RO, Romero D, Yanes Cardozo LL. Long-Lasting Androgen-Induced Cardiometabolic Effects in Polycystic Ovary Syndrome. J Endocr Soc 2018; 2:949-964

63. Vine DF, Shi D, Proctor SD. Insulin and testosterone are associated with elevated intestinal secretion of lipids and lipoproteins in a rodent model of the metabolic syndrome and polycystic ovary syndrome. Journal Diabetes and Metabolism 2014; 5:391-399

64. Kupreeva M, Lehner R, Watts R, Ghosh M, Proctor SD, Vine DF. Metformin and Flutamide Differentially Modulate Insulin- Glucose, Lipid and Apo-B Lipoprotein Metabolism, and Reproductive-Endocrine Function in a PCOS-prone rodent model. Am J Phys Endo Metab 2019; 316:E16-33

65. Cardozo L, Reckelhoff J. Cardiometabolic Features of PCOS: Role of Androgens. Physiology 2017; 32:357-366

66. Studen K PM. Cardiometabolic risk in polycystic ovary syndrome. Endocr Connect 2018; 7:R238-R251

67. Lopez-Huertas E. The effect of EPA and DHA on metabolic syndrome patients: a systematic review of randomised controlled trials. Br J Nutr 2012; 107 Suppl 2:S185-194

68. Lu J, Borthwick F, Hassanali Z, Wang Y, Mangat R, Ruth M, Shi D, Jaeschke A, Russell JC, Field CJ, Proctor SD, Vine DF. Chronic dietary n-3 PUFA intervention improves dyslipidaemia and subsequent cardiovascular complications in the JCR:LA- cp rat model of the metabolic syndrome. Br J Nutr 2011; 105:1572-1582

69. Brinton EA, Guyton JR, Philip S, Doyle RT, Juliano RA, Mosca L. Lipid Effects of Icosapent Ethyl in Women with Diabetes Mellitus and Persistent High Triglycerides on Statin Treatment: ANCHOR Trial Subanalysis. J Womens Health 2018; 27:1170-1176

70. Bowman L, Wallendszus K et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus.ASCEND Study Collaborative Group. N Engl J Med 379:1540-1550

71. Masuda D, Yamashita S. Postprandial Hyperlipidemia and Remnant Lipoproteins. J Atheroscler Thromb 2017; 24:95-109
72. Alexander D et al. Eicosapentaenoic and Docosahexaenoic Long-Chain Omega-3 Fatty Acids and Coronary Heart Disease Risk. Mayo Clin Proc 2017; 92:15-29
73. Siscovick DS, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, Kris-Etherton PM, Jacobson TA, Engler MB, Alger HM, Appel LJ, Mozaffarian D; American Heart Association Nutrition Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Epidemiology and Prevention; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; and Council on Clinical Cardiology. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory From the American Heart Association. Circulation 2017; 135:e867-e884
74. Yokoyama M, Matsuzaki M. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007; 369:1090-1098
75. Bhatt D, Miller M, Brinton E, Jacobson T, Ketchum S, Doyle R, Juliano R, Jiao L, Granowitz C, Tardif J-C, and Ballantyne C, M.D. REDUCE-IT Investigators. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med 2019; 380:11-22
76. Rossello FV, Oliva B, Sanz J, Friera L, López-Melgar B, Mendiguren J, Pezzi E, Bueno H, Fernández-Ortiz F, Ibanez B, Ordovás J. Association Between Body Size Phenotypes and Subclinical Atherosclerosis Journal of Clinical Endocrinology & Metabolism 2020; 105
Figure 1. Fasting (A) and Non-fasting (B) response to a high-fat meal test in plasma triglycerides, ApoB100- and ApoB48-lipoproteins at baseline and post-intervention treatment for 12 weeks with Metformin (n=8), Fish Oil (n=13) and Fish Oil-Metformin (n=8) in women with PCOS. Mean ± SEM. *p<0.05
Table 1. Fasting anthropometric, endocrine, insulin and glucose concentrations at baseline and post-intervention treatment for 12 weeks with Metformin, Fish Oil or Fish Oil-Metformin in women with PCOS

|                  | Metformin (n=8) | Fish Oil (n=13) | Fish Oil-Metformin (n=8) |
|------------------|-----------------|-----------------|--------------------------|
|                  | Baseline (n=29) | Baseline        | Post (%)                 |
|                  | Age (yr)        | 25.0±1.8        | 25.5±1.2                 | 28.7±1.7                  |
|                  | Body Weight (kg) | 109.8±4.8       | 117.9±7.6                | 116.1±7.4 (-1)            | 104.5±5.7                 | 105.7±5.6 (1) | 111.6±10.3 | 102.8±7.8 (-8) |
|                  | BMI (kg/m²)     | 38.6±1.4        | 41.9±1.3                 | 41.2±0.9 (-1)            | 35.9±1.8                 | 36.4±1.8 (1) | 40.5±3.6  | 37.1±2.3 (-8) |
|                  | WC (cm)         | 45.7±1.1        | 47.8±2.3                 | 48.1±2.3 (<1)            | 43.8±1.2                 | 44.1±1.3 (<1) | 47.3±2.5  | 46.6±2.0 (-1) |
|                  | WHR             | 0.9±0.01        | 0.9±0.02                 | 1.0±0.03 (+10)           | 0.9±0.02                 | 0.9±0.02 (0)   | 0.9±0.02  | 1.0±0.02 (+10) |
|                  | Fat Mass (kg)   | 50.4±2.8        | 56.3±4.7                 | 55.8±4.5 (<1)            | 46.6±3.9                 | 47.9±4.5 (3)  | 51.8±6.4  | 44.8±3.9 (-14) |
|                  | Fat Free Mass (kg) | 59.4±1.6      | 61.3±3.2                 | 60.1±3.4 (-2)            | 58.2±1.9                 | 56.6±2.3 (3)  | 59.6±4.3  | 58.2±4.2 (-2) |
|                  | Androstetandione (nmol/L) | 8.8±0.7 | 7.2±0.8                 | 7.8±1.8 (8.0)            | 9.3±0.9                  | 11.1±1.1 (16)  | 9.6±1.7  | 10.2±0.6 (6) |
|                  | Testosterone (nmol/L) | 1.4±0.1     | 1.3±0.2                 | 1.3±0.4 (0)             | 1.5±0.2                  | 1.6±0.1 (6)    | 1.4±0.2  | 1.5±0.2 (7) |
|                  | Free T (pmol/L)  | 27.4±2.8        | 23.8±4.1                 | 25.2±8.1 (6)            | 30.8±6.0                 | 34.7±4.6 (13) | 26.8±3.9  | 29.2±2.3 (9) |
|                  | SHBG (nmol/L)   | 34.4±3.6        | 35.6±6.0                 | 55.6±6.9 (36)           | 39.0±5.5                 | 30.1±4.9 (-22) | 29.3±7.2  | 30.0±6.3 (2) |
|                  | FAI             | 4.7±0.6         | 4.4±0.9                 | 5.1±1.7 (16)            | 3.9±0.9                  | 6.6±0.9 (70)  | 5.7±1.2  | 5.9±0.9 (3) |
|                  | DHEAS (umol/L)  | 7.6±0.6         | 7.6±1.2                 | 6.4±1.2 (-16)           | 8.2±1.0                  | 8.4±1.1 (2)   | 6.7±1.1  | 7.2±1.2 (8) |
|                  | Insulin (pmol/L)| 215.0±28.0      | 217.1±46.4               | 180.1±52.2 (-17)        | 223.4±60.3               | 181.1±20.8 (-19) | 203.1±43.7 | 144.3±19.0 (-29) |
|                  | Glucose (mmol/L) | 4.9±0.1        | 4.8±0.2                 | 4.7±0.1 (-2)           | 4.9±0.1                  | 4.8±0.2 (-2)   | 4.9±0.1  | 4.6±0.2 (-6) |
|                  | OGT (2hr mmol/L)| 5.9±0.2        | 5.9±0.4                 | 6.4±0.8 (8)            | 6.0±0.3                  | 6.1±0.5 (2)    | 5.9±1.2  | 5.7±0.6 (3) |
|                  | HOMA-IR         | 7.8±1.1         | 7.9±1.8                 | 6.3±1.9 (-20)           | 8.1±2.2                  | 6.5±0.8 (-20)  | 7.4±1.7  | 5.0±0.8 (-32) |

Values are presented as mean±SEM and variables were normally distributed. SHBG, sex hormone binding globulin; FAI, free androgen index; DHEAS, dehydroepiandrostendione; HOMA-IR, OGT (2hr), oral glucose tolerance at 2hr, homeostatic model assessment of insulin resistance; WC, waist circumference; WHR, waist hip ratio; T, testosterone. %, mean percentage change from baseline within group.
Table 2. Fasting and Non-fasting lipid and apoB-concentrations at baseline and post-intervention treatment with Metformin, Fish Oil or Fish Oil-Metformin for 12 weeks in women with PCOS.

| Metformin (n=8) | Fish Oil (n=13) | Fish Oil-Metformin (n=8) |
|----------------|----------------|-------------------------|
| TG (mmol/L)    | Baseline       | All groups (n=29)       |
|                |                 |                         |
|                | 2.5±0.2        | 2.2±0.2                 |
| TG (AUC)       | 2459±260       | 2388±583                |
| TG (iAUC)      | 825±128        | 955±217                 |
| TG (mmol/L)    | 5.3±0.2        | 5.4±0.2                 |
| HDL-C (mmol/L) | 1.0±0.0        | 1.1±0.1                 |
| LDL-C (mmol/L) | 3.4±0.2        | 3.4±0.2                 |
| Non-HDL-C (mmol/L) | 4.6±0.2 | 4.2±0.2 |
| ApoB48 (ug/ml) | 24.6±2.4       | 22.3±4.8                |
| ApoB48 (AUC)   | 188.3±17.4     | 187.1±34.4              |
| ApoB48 (iAUC)  | 43.8±6.2       | 40.1±9.5                |
| ApoB100 (g/ml) | 2.8±0.2        | 2.8±0.2                 |
| ApoB100 (AUC)  | 2518±2140      | 27240±4824              |
| ApoB100 (iAUC) | 5682±857       | 6810±1900               |

Values are presented as mean±SEM and and variables were normally distributed. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high density lipoprotein cholesterol, AUC, area under the curve; iAUC, incremental area under the curve. AUC and iAUC represent non-fasting response following a high-fat meal test. %, mean percentage change from baseline within group.
Table 3. Correlation of fasting and non-fasting plasma triglycerides and body weight from baseline to post-intervention following 12 weeks of treatment with Metformin, Fish Oil or Fish Oil-Metformin in women with PCOS.

| Variable  | % BMI | % BW | %, FM(%) | % Ins | %HOMA-IR |
|-----------|-------|------|----------|-------|----------|
| **Fasting** |       |      |          |       |          |
| % TG       | 0.03  | 0.20 | 0.49*    | -0.05 | 0.07     |
| % TC       | 0.05  | 0.24 | 0.07     | 0.37  | 0.03     |
| % LDL-C    | 0.05  | 0.23 | 0.23     | 0.33  | 0.28     |
| % HDL-C    | 0.01  | -0.02| -0.28    | 0.37  | 0.28     |
| % Non-HDL-C| 0.07  | 0.28 | 0.18     | 0.29  | 0.26     |
| % ApoB48   | 0.01  | 0.02 | -0.02    | -0.08 | -0.14    |
| % ApoB100  | 0.03  | -0.15| 0.04     | -0.09 | 0.17     |
| **Non-fasting** |       |      |          |       |          |
| % TG_{AUC} | 0.08  | 0.09 | 0.27     | -0.01 | -0.13    |
| % ApoB48_{AUC} | 0.25  | 0.26 | -0.04    | 0.08  | -0.02    |
| % ApoB100_{AUC} | 0.02  | 0.01 | 0.04     | 0.19  | 0.17     |

Variables are % change in variable from baseline to post-intervention.

BMI, body mass index. BW, body weight. HOMA-IR, homeostatic model assessment of insulin resistance. *p<0.01
