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Vine D, Proctor E, Weaver O, Ghosh M, Maximova K, Proctor S. A Pilot Trial: Fish Oil and Metformin Effects on ApoB-Remnants and Triglycerides in Women with Polycystic Ovary Syndrome. Journal of the Endocrine Society. 2021;bvab114.

What We Know, Think We Know, or Are Starting to Know

Within the complex heterogeneity of Polycystic Ovary Syndrome [PCOS], much of the focus is often on external phenotype presentations of the condition: hirsutism, acne, and central adiposity. The internal focus, i.e., the physiological focus, often emphasises the elevated androgen and insulin resistant characteristics of the pathophysiology. However, these characteristics also lead to a significantly altered risk for cardiovascular disease [CVD] [(1)].

The shift toward central abdominal distribution of adipose tissue and increased visceral fat levels in the hyperandrogen/hyperinsulin PCOS phenotype was previously thought to explain the differences in CVD risk between PCOS and age-matched women without PCOS [(2)]. This is because central adiposity correlates with the 'atherogenic lipoprotein phenotype' *, which is independent of PCOS *per se*, and is characterised by elevated triglycerides, low HDL levels, and high LDL levels [which is typically smaller, dense LDL in this phenotype], and which strongly advances atherosclerosis [(3)].

However, recent analyses have shows that even when body weight is matched to women without PCOS, women with PCOS display higher triglycerides, lower HDL, and higher LDL [(4)]. This pattern of atherogenic lipoproteins is exacerbated by obesity in women with PCOS [(4)]. Nevertheless, elevated androgens appear to drive the presence of atherogenic lipoproteins in PCOS, which may be independent of differences in body weight alone [(4)]. These factors may explain the higher risk of CVD events in premenopausal women with PCOS [(2)].

Interventions for targeting risk factors and underlying metabolic physiology in PCOS include both pharmacological and nutritional supplements, and there is interest in potential combination therapies. The omega-3 fatty acids EPA and DHA have an established effect on reducing triglyceride levels [(5)]. Metformin has a dual effect in PCOS. It reduces symptoms of hyperandrogens, and improves insulin sensitivity [(6)]. Metformin has been shown to improve the overall lipid profile - lower triglycerides, increase HDL, and lower LDL - in women with PCOS [(4,6)]. The present study investigated the effects of omega-3 supplementation and metformin on cardio-metabolic risk factors in women with PCOS.

*Geek Box: Atherogenic Dyslipidemia

The 'atherogenic lipoprotein phenotype' is the technical term describing a particular pattern of blood cholesterol levels that is considered a significant risk for cardiovascular disease. The pattern includes high levels of triglycerides, high levels of LDL-cholesterol, and low levels of HDL-cholesterol. This profile is considered highly 'atherogenic', meaning it is implicated in the process of lipoproteins carrying cholesterol [i.e., VLDL and LDL] penetrating the arteries, leading to atherosclerosis. 'Dyslipidemia' is the fancy term for this adverse lipid profile. What it describes is a profile that looks like this:

- *Low HDL: because HDL is needed to clear cholesterol from circulation, low HDL is problematic for heart disease risk. When excess TGs are offloaded onto HDL, HDL becomes broken down in the liver and removed from circulation.*
- *High LDL: all LDL particles, irrespective of size, are capable of penetrating into the arteries. However, in the ALP, LDL remodels into smaller, denser particles. They carry less cholesterol than larger LDL, but as more of them penetrate the arteries, they lead to as much cholesterol being trapped in the artery wall, generating atherosclerosis.*
- *High Triglycerides: high triglycerides is important to the development of the ALP, as it is when HDL and LDL become overburdened with carrying triglycerides that they are forced to remodel. Further, as triglycerides carried in chylomicrons [fat from the diet] and VLDL [fat synthesised in the liver] are broken down, this creates what are known as 'remnant particles', which are also atherogenic.*

Traditionally, cardiovascular disease [CVD] risk has been focused on high LDL alone, however, we now know the ALP phenotype is also a significant risk for CVD, and that indirect processes, i.e. the impact of refined carbohydrate/added sugars on liver fat accumulation, insulin resistance, and the resulting increase in circulating free fatty acids, and new triglyceride formation, are important factors, in addition to the impact of dietary fat.

Relevant to this discussion is also Apolipoprotein-B [ApoB for short], which may be subdivided into ApoB-48 and ApoB-100. ApoB is highly relevant, because it is lipoproteins containing ApoB, like LDL or Lp(a), that are atherogenic. Thus, ApoB provides a more direct measure of all atherogenic lipoproteins in circulation, not just those that are LDL. We can further differentiate ApoB-containing lipoproteins by their source, i.e., dietary intake of fat or endogenous synthesis of fat in the liver. ApoB-48 containing lipoproteins are secreted from the intestines, and thus reflects dietary fat intake. On the other hand, ApoB-100 is derived from very-low-density lipoprotein [VLDL] that is synthesised in the liver, and thus reflects endogenous synthesis of new triglycerides. The remnants of both ApoB-48 and ApoB-100 lipoproteins contribute to the overall circulation of ApoB-containing lipoproteins, which drives atherosclerosis.

The Study

This was a pilot, open-label study [more under ***Key Characteristic***, below]. Participants were randomised to one of three treatments, which ran parallel to each other:

- Metformin [Met-only]: 1.5mg per day
- Omega-3 [FO]: 2.52g EPA + 1.68g DHA per day
- Metformin+Fish Oil [FO+Met; combination of the above doses of metformin and fish oils, respectively]

The study lasted 12-weeks. At baseline, and again after the intervention, participants underwent body composition measures, blood samples for blood lipids and other hormones [i.e., insulin], and also underwent a high-fat test meal, after which blood samples were collected every 2hr for up to 8hr after the test meal, to investigate post-prandial fat metabolism.

The primary outcome was change in fasting triglycerides from baseline to post-intervention. Secondary outcomes included non-fasting [i.e., post-prandial] triglycerides, blood cholesterol levels, androgens and insulin.

Results: 8 women completed the Met-only intervention, 13 completed the FO intervention, and 8 completed the FO+Met intervention. At baseline, mean BMI was 38.6, body weight 109.8kg, and hyperandrogens and insulin were present. Participants were highly insulin resistant [HOMA-IR value of <1.0 is insulin sensitive, baseline average in this study was 7.8].

- ***Body Composition:*** There were no significant differences between groups observed over 12-weeks, or within group differences for the Met-only or FO groups. The FO+Met group showed an 8% reduction in body weight and BMI, respectively, and 14% [51.8kg to 44.8kg, ~7kg total] reduction in fat mass [not statistically significant].
- ***Endocrine and Glucose:*** There were no statistically significant differences between groups in insulin levels, glucose, or insulin resistance [HOMA-IR] over 12-weeks. The FO+Met group did show the greatest magnitude of change, however, with a 29% reduction in fasting insulin and 32% reduction in HOMA-IR. There were no significant differences in androgens between groups, but for within-group differences the Met-only group increases sex-hormone binding globulin by 36%.
- ***Fasting Lipids:*** There were no statistically significant differences between groups in fasting LDL, HDL, or ApoB, over 12-weeks. However, the FO+Met group showed the greatest magnitude of effect with a reduction of 40% in fasting triglycerides, and a 40% reduction in fasting ApoB-48.

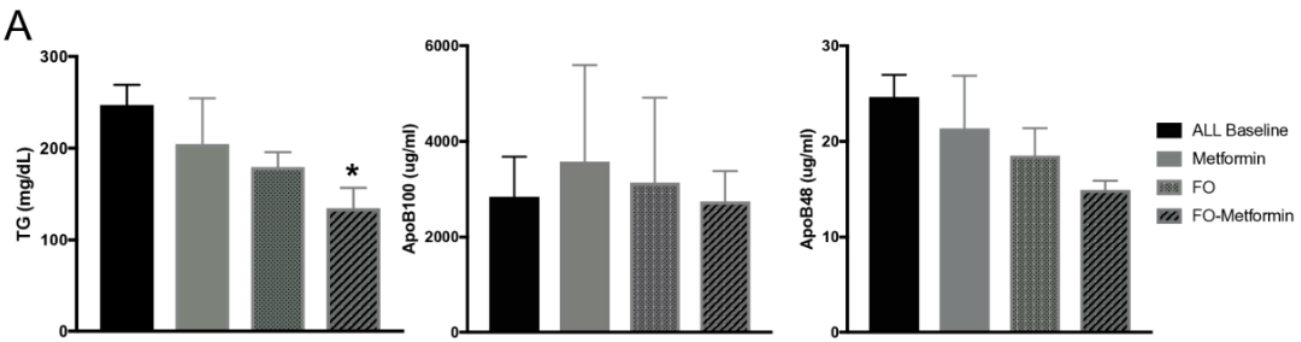

Figure *from paper illustrating the change in fasting triglycerides [far left], ApoB-100 [middle], and ApoB-48 [far right] from baseline to post-intervention. The asterisk above the FO-Met group on the far-left graph for fasting triglycerides would usually indicate statistical significance, yet the authors are painfully unclear on this in the written results section and do not have an accompanying written legend for this figure in the paper. Whether statistically significant or not, it is clear that the combination of FO+Met had a greater impact on reducing fasting triglycerides and ApoB-48 compared to the other interventions.*

- ***Post-prandial Lipids:*** There were no statistically significant differences between groups in post-prandial triglycerides or ApoB-containing lipoproteins, over 12-weeks. However, the greatest magnitude of effect was observed in the FO+Met group, including a 40% decrease in post-prandial triglycerides, and 32% reduction in post-prandial ApoB-48 [more under ***Interesting Finding***, below], and 15% reduction in post-prandial ApoB-100.

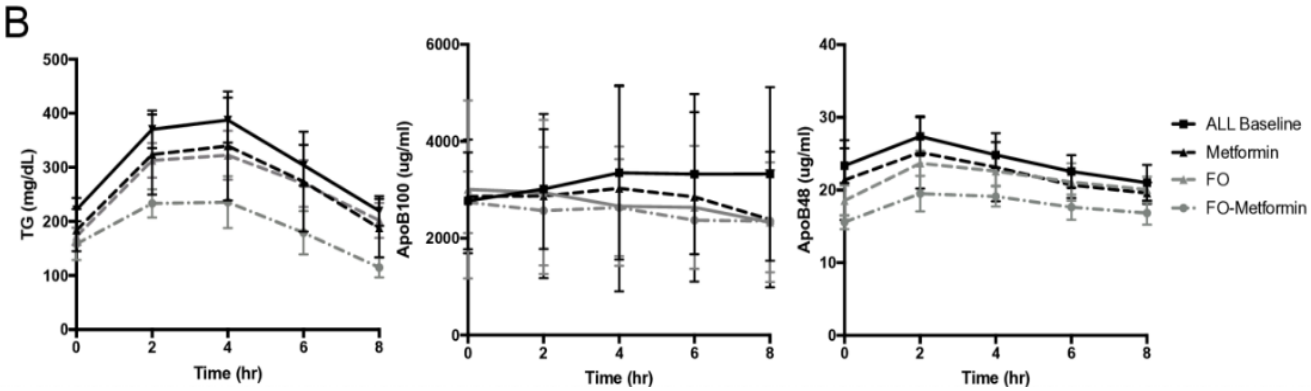

Figure *from paper illustrating the change in fasting triglycerides [far left], ApoB-100 [middle], and ApoB-48 [far right] from baseline to post-intervention. The asterisk above the FO-Met group on the far-left graph for fasting triglycerides would usually indicate statistical significance, yet the authors are painfully unclear on this in the written results section and do not have an accompanying written legend for this figure in the paper. Whether statistically significant or not, it is clear that the combination of FO+Met had a greater impact on reducing fasting triglycerides and ApoB-48 compared to the other interventions.*

The Critical Breakdown

Pros: One Pro [that is also a Con, such is research] is the pilot nature of the study, i.e., science has to start somewhere, and the potential combination of omega-3 fish oil as an adjuvant to metformin may hold some promise for dyslipidemia treatment in PCOS. The participants were well matched for baseline characteristics, and fit a high-risk PCOS phenotype in whom management of CVD risk is crucial. The post-prandial test meal and blood sampling for 8hrs afterward was a good addition to the study design, providing insight into potential changes in post-prandial fat metabolism from the intervention. Compliance to the interventions, assessed by documenting capsules consumed, was very high at 98%.

Cons: The Pro that is also the Con: as a pilot, open-label trial the study was small, potentially underpowered, and prone to bias with the open-label design ['Open label' means that both the researchers and the participants know what intervention the participants are receiving, and the study is therefore unblinded]. The authors don' t provide any rationale for their choice of omega-3 supplement [mixed EPA/DHA] and dose. Pilot studies often run the risk of being underpowered [irrespective of what the power calculation arrived at for numbers, a power calculation for a pilot study is still a guesstimate], and the lack of statistical significance for findings that appear to have a relatively large magnitude of effect [e.g., the reduction in insulin] may support this. Given that the study was open-label, therefore the researchers randomly assigned participants to each group, why only 8 were assigned to the FO+Met group but 13 to the FO group makes little sense with the imbalance, if the aim was to investigate the potential efficacy of the combination. It may because they assumed the combination would have more effect, and more numbers in the FO only group may have helped increase power. But this is not explained or justified. This last point is niche, but important: their figure legends and details of significance in findings is woefully lacking.

Key Characteristic

Science has to start somewhere, and it is important to bear in mind that this was a pilot study. A pilot study is generally considered the first step forward in a research area or specific intervention. As a result, they are purposefully small studies, because conducting a massive, expensive study without any prior indication that your treatment could be successful could be a waste of resources. One of the key reasons for conducting a pilot study is to use the preliminary data from the outcomes for more robust power calculation for a bigger trial, so the larger trial has adequate numbers of participants. An important consideration applies to the results of a pilot study, which is that the findings are not treated as confirmatory, but rather as hypothesis-generating. The present study, for example, yielded findings that were largely statistically insignificant, however the absolute differences and magnitude of effect of these same outcomes indicates that the FO+Met combination showed some efficacy, compared to the other treatments. Thus, there would appear to be sufficient preliminary data from the present study to warrant a larger, higher powered intervention on this treatment.

Interesting Finding

The 40% suppression of ApoB-48 from the combination of FO+Met is particularly striking, given that FO alone resulted in a 15% lower circulating ApoB-48 level. Recall that ApoB-48 reflects intestinally-derived lipoproteins. Until very recently, this pathway of fat metabolism has been very difficult to study in detail, because chylomicrons appear rapidly and in waves in the post-prandial period, and have been difficult to distinguish from liver-derived VLDL. In 2019, Björnson et al. [7] published an elegant study using stable isotope tracers to distinguish between ApoB-48 and ApoB-100 following a fat-rich test meal, showing that intestinally-derived ApoB-48 appeared not only in chylomicrons, but directly into VLDL-sized lipoproteins. ApoB48 in chylomicrons returned to near zero at the 24hr time-point in all subjects. In contrast, substantial levels of ApoB48 in VLDL were still present 24hr later, an effect which was greatest in participants with high triglycerides. This means that atherogenic ApoB-containing lipoproteins in the range of VLDL size may be circulating across an entire day in people with high triglycerides, such as the population in the present study. The fact that FO+Met had an additive effect on suppressing ApoB-48, compared to either treatment alone, is an interesting finding with potentially important implications for management of dyslipidemia.

Relevance

With the caveats of the Key Characteristic noted, it is possibly that the overall lack of significance in this pilot study reflected the very small numbers in the study groups and lack of adequate statistical power. Nonetheless, for a hypothesis-generating study that was the first to test the combination of FO+Met in women with PCOS, the following findings are all noteworthy for their magnitude of effect:

- ~7kg reduction in total fat mass
- 29% and 32% reduction in fasting insulin and HOMA-IR, respectively
- 43% and 40% reduction in fasting and postprandial triglycerides, respectively
- 40% and 32% reduction in fasting and postprandial ApoB-48, respectively

The fact that the magnitude of effect in each of the above were greater than either FO or Met-only indicates a potential additive effect of the combination therapy. One would think there is sufficient proof-in-concept from this small pilot trial to warrant a larger intervention of FO+Met in the treatment of dyslipidemia in PCOS.

It remains to be fully elucidated how much of the atherogenic lipoprotein phenotype in PCOS is determined by insulin resistance, disrupted androgen and oestrogen metabolism, or an interaction of these factors. The fact that the participants in this study had very high levels of adiposity should also be factored in, because at that level of body fat we would expect to see dyslipidemia. It could be that the effect of metformin on suppressing liver glucose output, improving insulin sensitivity, and the effects of fish oils on reducing post-prandial circulating fat levels, combine additively to benefit the pathophysiology of PCOS. Further studies will hopefully pick up this ball and run it further.

Application to Practice

There is little application of the findings of any pilot trial. Rather, we are left to principles of what we already know. For PCOS, in the high adiposity/androgen/insulin phenotype, there is no doubt that weight loss improves the hormonal milieu and metabolic landscape. For nutrition professionals, it is important to note that the usual fish oil supplements used for profound reductions in triglycerides are pharmaceutical-grade products. Thus, the prescription of these products will be in the remit of a client' s cardiologist/medical team. As will statins or other lipid-lowering therapies, which will do more for reducing CVD risk than any combination of weight loss or nutritional supplements. That said, there remains plenty that can be done within our remit, from dietary fat modifications, to fibre intake and additional supplements [e.g., psyllium husk].

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