Metformin again? Atheroprotection mediated by macrophage AMPK and ATF1

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Metformin is a biguanide that has been used as a frontline treatment for type 2 diabetes (T2DM) over the past 60 years, though its medicinal roots date back to medieval times. While new medications have emerged in recent years, metformin remains the most widely prescribed anti-diabetic drug. Despite this, the molecular mechanisms by which metformin acts remain incompletely understood [1]. Twenty years ago, metformin was demonstrated to activate hepatic AMP-activated protein kinase (AMPK) [2], a master regulator of energy homeostasis that exerts control over lipid and carbohydrate metabolism. Although metformin-mediated suppression of hepatic glucose production was subsequently shown to be AMPK-independent [3], others reported that AMPK activation underlies metformin-mediated improvements in insulin action by maintaining hepatic lipid homeostasis [4]. Given its low cost, well-known tolerance and broad metabolic benefits, there are efforts currently underway to "repurpose" metformin toward conditions such as cancers, ageing and cardiovascular disease. Atherosclerosis is characterized by chronic- low-grade inflammation that is driven by hyperlipidaemia and immune-infiltration that results in the formation of lipid-rich plaque in the arterial intima. Atherosclerotic macrovascular disease is a leading cause of mortality in people with T2DM and growing evidence suggests that metformin has a direct anti-atherogenic action that may be independent of its effect on glycaemia [5]. AMPK activation in vascular tissues has also been demonstrated to exhibit anti-inflammatory and anti-atherogenic actions [6]. Therefore, while potentially not independent, or mutually exclusive of any effect on glycaemia, stimulation of AMPK

by metformin may still hold therapeutic promise against atherosclerosis given its role in lipid metabolism and inflammation.

In the present study, Seneviratne *et al*. sought to add to the limited preclinical understanding we have and examine whether metformin treatment had anti-atherogenic actions in normoglycemic but atherogenic mice and whether this potential benefit was AMPK-mediated. Their studies support a novel mechanism by which metformin suppresses atherogenesis by promoting a protective, pro-resolving macrophage M2-like phenotype that overlaps with a [Mhem] phenotype in an AMPK- and activating transcription factor 1 (ATF1)-dependent manner [7].

Their study made use of low-density lipoprotein receptor (*Ldlr*) knockout (KO) mice fed a regular chow diet to avoid the hyperglycaemia that accompanies Western diet feeding of *Ldlr* KO mice. A preventative and clinically relevant metformin regime saw a significant reduction in early, macrophage-rich atherosclerotic lesions in these mice without altering glucose or lipid profiles. Given this, the authors focussed on the hematopoietic compartment. Transplantation of bone marrow from AMPKβ1-deficient (*Prkab1*$^{-/-}$) mice into *Ldlr* KO recipients abrogated the atheroprotective action of metformin, whereas metformin was still effective in mice transplantedwith bone marrow from wild type littermate controls. The same group has previously demonstrated that heme and metformin increase ATF1 phosphorylation in an AMPK-dependent manner in human blood-derived macrophages, leading to the atheroprotective, [Mhem] phenotype [8]. In the current study, transcriptional analysis of primary macrophages showed that metformin promoted the expression of atheroprotective genes in an AMPK- and ATF1-dependent manner. These experiments were also extended in human blood macrophages where Mhem-associated atheroprotective genes required the transcriptional activity of ATF1 with metformin treatment. The importance of this pathway was further validated *in vivo*, where metformin stimulated phosphorylation of AMPK, ATF1 and suppressed markers of inflammation in lesional cells. Finally, in contrast to plaque reduction seen in preventative treatment of early atherosclerosis, metformin treatment of the same mice/model with more established atherosclerosis increased the extent of the layer of vascular smooth muscle cells (VSMCs) between the endothelium and the macrophages. A scheme of the proposed mechanism is shown in Figure 1.

This work adds to previous studies that have shown a protective effect of metformin in mice and points to a mechanistic role for haematopoietic AMPK. While there was a clear rationale for the use of chow fed *Ldlr* KO mice, thereby removing potential influence of hyperglycaemia from the model system, this also removed an important variable and driver of atherogenesis, hyperlipidaemia. There are, however, no mouse models that perfectly model the progression of human atherosclerosis. The beneficial effects of metformin described here should be considered in the context of the earliest stages of atherosclerosis. It is, however, also intriguing that metformin shows evidence of increasing plaque stability in more established atherosclerosis, albeit in a model without the potential for advanced plaque. Although the AMPK- or ATF1-dependence of this was not tested in the study of Seneviratne *et al*., metformin has been reported to increase indices of plaque stability via mechanisms that require AMPK in vascular smooth muscle cells [9] and several atherosclerosis-prone AMPK knockout mouse models have been reported to exhibit more advanced, less stable plaques [6]. It will indeed be interesting to test the preventative and therapeutic potential of metformin (and potentially other AMPK activators) in pre-clinical models of more advanced and aggressive atherogenesis.

Overall, Seneviratne *et al.* provide evidence for an atheroprotective effect of metformin involving activation of haematopoietic AMPK and ATF1. The significance of this work lies in the translational appeal for people without diabetes. The epidemiological evidence suggesting that metformin use protects against adverse cardiovascular outcomes in people is not overwhelming, yet there is a therapeutic benefit [5]. However, from a translational perspective, the potential that metformin treatment has to initiate anti-inflammatory and atheroprotective gene programs in monocytes and macrophages of people both with and without diabetes is a testable hypothesis. Do human immune cells experience the same immune programming upon oral metformin administration? How well does this metformin-induced polarization influence plaque macrophage dynamics? While both are important questions, a final consideration may relate to the appropriate therapeutic window. Could metformin be used to prevent atherogenesis in those at risk regardless of whether they have diabetes? Furthermore, given the finding that a clinically relevant dose of metformin shows evidence of increasing plaque stability, does metformin have the potential to stabilize vulnerable

plaque in advanced human lesions, while at the same time favourably altering immunometabolism? Metformin is cost effective and has a long-standing reputation for being safe. It is also worth noting that selective AMPK activators are also currently in clinical trials for the treatment of fatty liver disease and type 2 diabetes [10,11]. There may, therefore, also be the potential for targeting macrophage AMPK directly to prevent atherogenesis or stabilize plaques. Addressing these questions could unravel the full potential of metformin for the treatment and management of cardiovascular disease as well as other disorders with an inflammation component, independent of diabetes.

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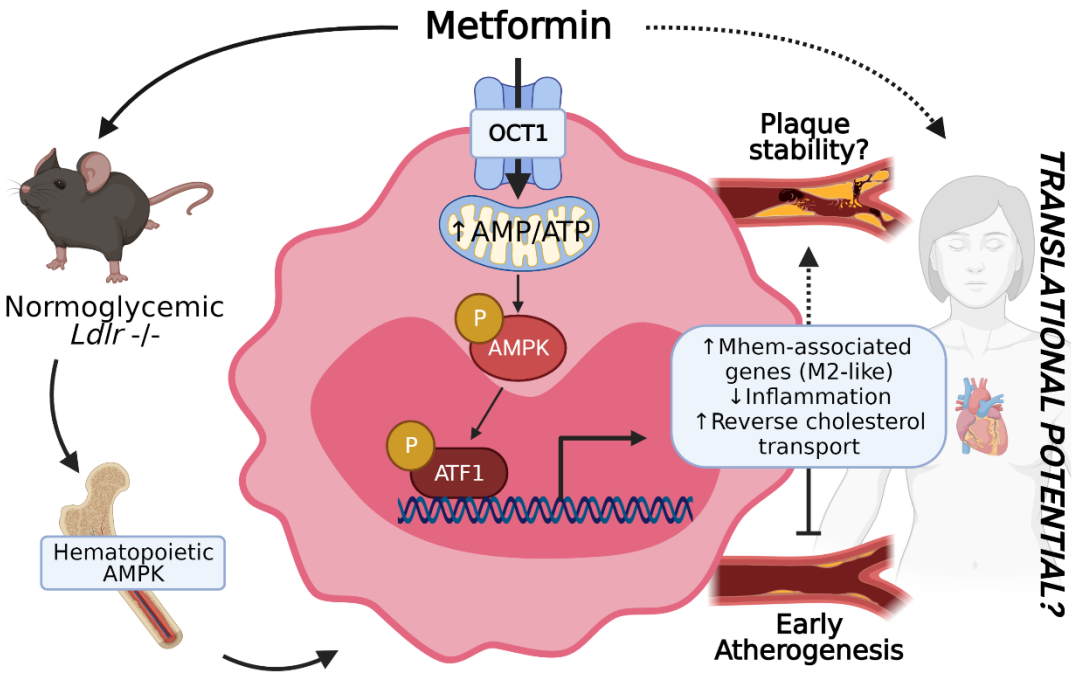

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158 **Figure 1: Model of attenuated atherogenesis in response to metformin in**
159 **macrophages**

160 Metformin is transported into macrophages, likely via the organic cation transporter-1
161 (OCT1), where it is activates AMPK through inhibition of mitochondrial ATP synthesis,
162 leading to activation of AMPK. AMPK phosphorylates and activates ATF1-mediated
163 transcription of genes concerned with reverse cholesterol transport and resolution of
164 inflammation, leading to a phenotype resembling Mhem. In normoglycemic *Lldr*$^{-/-}$ mice,
165 via haematopoietic AMPK, this subsequently suppresses atherogenesis and may
166 increase stability of established plaques. Created with BioRender.com.

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