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Title: Rationale For the Use of Metformin and Exercise to Counteract Statin-associated Side Effects

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Abstract

Statins are the most widely prescribed drugs for lowering low-density lipoprotein cholesterol (LDL-C) and reducing cardiovascular morbidity and mortality. They are usually well-tolerated, but have two main safety concerns: statin-associated muscle symptoms (SAMS) and new-onset type 2 diabetes (NOD). Mitochondrial damage and muscle atrophy are likely the central mechanisms producing SAMS, whereas decreased glucose transport, fatty acid oxidation and insulin secretion are likely involved in the development of NOD. Metformin and exercise training share many pathways that could potentially contrast SAMS and NOD. Clinical evidence also supports the combination of statins with metformin and exercise. This combination appears attractive both from a clinical and an economical viewpoint, since all three therapies are highly cost-effective and their combination could result in diabetes and cardiovascular disease prevention.

Keywords: statin-associated muscle symptoms; new-onset diabetes; metformin; exercise training; cardiovascular.

Abbreviation: *PCSK9i*: proprotein convertase subtilisin/kexin type 9 inhibitors; *SAMS*: statin-associated muscle symptoms; *NOD*: new-onset type 2 diabetes; *CVD*: cardiovascular disease; *HMG-CoA*: 3-hydroxy-3-methyl-glutaryl-CoA; *OATP2B1*: Organic Anion Transporting Polypeptide 2 B1; *CoQ10*: ubiquinone; *GGPP*: garanygeranyl pyrophosphate ; *FPP*: farnesyl pyrophosphate; *AMPK*: adenosine monophosphate kinase; *FoXO3a*: Forkhead box O3a; *MURF1*: Muscle Ring Finger 1; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator 1α; *mTORC*: mammalian target of rapamycin complex; *PI3K*: phosphatidylinositol 3-kinase; *Akt*: protein kinase B; *GLUT4*: glucose transporter 4; *IRS-1*: insulin receptor substrate 1; *IGF1:* insulin-like growth factor 1; *ACC*: acetyl-CoA carboxylase; *TSC2*: tumor sclerosis complex 2; *GSK-3*: glycogen synthase kinase 3; *MAPK*: mitogen-activated protein kinase; *SIRT1*: sirtuin 1; *GLP-1*: glucagon-like peptide-1; *DPP-4*: dipeptydil-peptidase-4.

Introduction

Statins are the most widely prescribed drugs for lowering low-density lipoprotein cholesterol (LDL-c) and reducing cardiovascular morbidity and mortality[1]. The benefits of statin therapy are supported by high-quality research studies indicating that every 1 mmol/L (38.7 mg/dL) reduction in LDL-c is associated with a 22% relative risk reduction in cardiovascular disease (CVD) events[2]. It is now possible, with the development of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, to produce very large reductions in LDL-c[3,4]. Median LDL-c levels as low as 30 mg/dL in the FOURIER Study were associated with reductions in CVD events, with no evidence of safety issues over the 2.2 years of the study duration[5]. Statins are well-tolerated, but have two main safety concerns: statin-associated muscle symptoms (SAMS) and new-onset type 2 diabetes (NOD)[6].

This review focuses on these two statin side-effects. We will discuss the responsible mechanisms of statin-associated SAMS and NOD. Then we will provide a rationale for the potential role of Metformin and exercise in the treatment/prevention of SAMS and NOD (Figure 1).

Methods

A PubMed (https://pubmed.ncbi.nlm.nih.gov/) search was carried out to identify the relevant literature. The following key words were used: statins, statin-associated muscle symptoms, statin myalgia, statin-associated diabetes, metformin and statins, exercise and statins. We included human, as well as animal and in vitro mechanistic studies. Articles published in the last 20 years in English language were included. Additional relevant articles were identified from the reference lists of selected articles and from a hand search of pertinent journals.

SAMS and diabetes

SAMS are defined as "muscle pain, weakness and aches, usually symmetrical and proximal, affecting the thighs, buttocks, calves and back muscles, not normally associated with marked creatine kinase (CK) elevation"[7]. SAMS are statins' most prevalent adverse event, and they have been estimated to comprise about 72% of all statin adverse events[8]. Up to 60% of patients who discontinue statins cite SAMS as a contributing factor[9]. The frequency of SAMS varies from 0.1-0.2% in Randomized Controlled Trials to 29% in non-blinded observational studies[7]. One reason for such a wide variation may depend on the "nocebo effect" (effect of negative expectations)[10] or the "drucebo effect" (beneficial or adverse effects of a drug, which result from expectation and are not pharmacologically caused by the drug)[3,11,12]. Another factor might be the lack of standardized diagnostic tests, which makes the diagnosis of SAMS difficult and can lead to labeling every muscle complaint as SAMS producing an, excessive diagnosis in clinical practice[13].

The risk of new-onset type 2 diabetes (NOD) has been estimated to increase 10-12 % with statin use[14]. Different doses and types of statins demonstrated different potential to increase the incidence of diabetes[15]. NOD risk is higher in patients with impaired fasting glucose and elements of the metabolic syndrome (hypertension, triglycerides >150 mg/dl and Body Mass Index >30 kg/m^2)[16]. Metabolic Syndrome (MS) patients are also at increased risk of type 2 diabetes without statin use.[17,18]. MS patients may be more likely to experience SAMS [19], which may put them at risk to discontinue statins and reduce their physical activity[20]. Both, statin discontinuation and physical inactivity, further increase the risk for future cardiovascular events and death[21–23]. Although statins increase the risk of NOD, its long-term clinical outcomes are not clear. Statins have not been shown to increase microvascular complications of diabetes[24]. High adherence to statins decreases macrovascular complications in diabetic patients, thus making statin-associated diabetes possibly less deleterious than diabetes from other etiologies[25].

The primary site of action of statins is the liver. 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibition in the hepatocyte increases LDL receptor activity and reduces blood LDL-C. However, other tissues are also sensitive to statin action. Among these, the skeletal muscle expresses the Organic Anion Transporting Polypeptide 2 B1 (OATP2B1), which transports statins into cells[26,27]. Compared to hepatocytes, HMG-CoA reductase in

skeletal muscle is 40 times more sensitive to inhibition by statins [28,29], and such effects increase in situations that increase statin plasma concentrations[30].

Inhibition of the mevalonate pathway decreases the concentration of downstream products, including sterol isoprenoids, non-sterol isoprenoids and prenylated proteins and increases HMG-CoA precursors, including acetyl CoA used to synthesize acyl CoA and triglycerides[6]. Other products of the mevalonate pathway such as dolichol, ubiquinone (CoQ10), heme A, garanygeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP), are involved in chromatin organization and gene expression[31], intra-cellular signaling and trafficking[32], mitochondrial electron transport[31], protein synthesis, cell growth and differentiation[33], cytoskeletal assembly, membrane function, and apoptosis[28,34]. Reductions in these intermediates in skeletal muscle and the pancreatic β-cell have been linked to SAMS and NOD (see[6,35]for review). For the purpose of this review we will only discuss the major mechanisms involved in SAMS and NOD.

HMG-CoA reductase inhibition has been associated with dysfunctional electron transport chain (ETC) in the mitochondrial inner membrane[35]. This decreases ATP production and increases reactive oxygen species (ROS) from the interaction of oxygen with the electrons escaped from the ETC[36]. The excess in oxidative stress initiates a cascade of events, such as Ca++ influx into the mitochondrial inter-membrane space, rupture of the outer membrane and mitochondrial damage[35]. Cytochrome C is released from the mitochondria to the cytoplasm, where it forms a complex called an "apoptosome" inducing mitophagy and cell apoptosis[35,37]. In tissues where ROS are normally present in high amounts, like oxidative muscle fibers, cellular antioxidant systems neutralize ROS, making the cell less susceptible to damage[35]. In glycolytic (type II) muscle fibers, which normally have fewer mitochondria and are less exposed to ROS, antioxidant defense systems are lacking, making them more prone to damage. Damage of the mitochondrial network activates the adenosine monophosphate kinase-Forkhead box O3a (AMPK-FoXO3a) pathway that induces muscle atrophy genes (atrogin-1 and Muscle Ring Finger 1) and muscle loss[38]. A decrease in peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) has been observed with HMG-CoA reductase inhibition[35]. PGC-1α is a key regulator of mitochondrial biogenesis and increases mitochondrial content and function[39,40]. The combination of

mitochondrial damage with the reduction of PGC-1α results in net reduction of normally functioning mitochondria[41]. Therefore, mitochondrial loss predominates over mitochondrial genesis and disrupts cellular energetics.

Reduced cellular energy levels, activate AMPK and inhibit mammalian target of rapamycin complex 2 (mTORC2)[35,42]. The latter is one of the two distinct mTOR complexes (mTORC1 and mTORC2), which integrate signals concerning the availability of cellular energy, nutrients and growth factors to affect protein and lipid metabolism, cell proliferation and autophagy through the phosphorylation of distinct effectors[43]. Inhibition of mTORC2 interferes with the PI3K/Akt/mTOR pathway (phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin) [42,44] This pathway regulates protein synthesis and degradation, apoptosis and insulin action[35,44]. As a result, there is an inhibition of the mTORC2 signaling involved in protein synthesis and an increase in FoxO3a-atrogin-1 and MURF1 (Muscle Ring Finger 1) expression. These mediate a decrease in protein synthesis, an increase in protein degradation, and subsequent muscle atrophy[44–46]. In addition, the PI3K/Akt pathway is involved in regulating glucose uptake and insulin action[35]. Statins inhibit glucose transporter 4 (GLUT4) vesicle translocation, as well as insulin receptor (IR), insulin receptor substrate 1 (IRS-1) and glycogen synthesis[47–49]. Inhibition of GLUT4 translocation by statins has also been demonstrated in adipose tissue[50]. GLUT4 is expressed in skeletal and cardiac muscles as well as in both brown and white adipocytes, cells which are important in insulin-sensitive glucose transport [51]. Therefore, Akt inhibition by statins is associated with insulin resistance and may favor the development of type 2 diabetes. This hypothesis is supported by the reversal/prevention of such effects in vitro with exposure of muscle cells to insulin, insulin-like growth factor 1 (IGF1)[44], mevalonate[46] and GGPP[35].

Long-term statin therapy is also associated with reduced triglyceride synthesis from fatty acids (FA) and diacylglycerol (DAG)[52]. On the other hand, mitochondrial dysfunction may lead to impaired β-oxidation[53]. These conditions favor the accumulation of non-esterified intermediates of lipid metabolism, such as FA, DAG and ceramides, producing "lipotoxicity"[54,55]. Lipotoxicity affects non-adipose tissues including skeletal and heart muscle, the liver and β-cells where it interferes with insulin signaling, insulin secretion and

glucose metabolism[56]. The ultimate result is insulin resistance, muscle atrophy[55], β-cell failure, hepatic steatosis, and eventually type 2 diabetes[56].

Similar to muscle, HMG-CoA Reductase inhibition in the pancreatic β cell reduces intracellular isoprenoids and CoQ10, altering ATP synthesis[57,58]. Low intracellular ATP levels prevent the closure of the ATP-dependent potassium channel, necessary for the activation of the metabolic cascade ensuing exocytosis of insulin-containing granules[59]. When intracellular LDL-C is lowered by statins, the β-cell up-regulates LDL receptors and increases LDL-C uptake from the plasma to replenish intracellular LDL-C[58]. LDL-C overload could contribute to β-cell toxicity through at least two possible mechanisms. First, LDL-C accumulation inhibits glucokinase, the rate-limiting enzyme for intracellular glucose metabolism, which lowers β-cell ATP levels and further reduces glucose-mediated insulin secretion[60]. Second, plasma-derived LDL-C may undergo oxidative modification, initiating inflammation[58], which can induce β-cell apoptosis[61]. Hence, statin-induced β-cell failure could contribute to the development of overt type 2 diabetes in the presence of insulin resistance/impaired fasting glucose and other risk factors for NOD.

In summary, HMG-CoA reductase inhibition in skeletal muscle and pancreatic β-cells, leads to a series of changes that increase the risk of muscle damage and NOD. Mitochondrial damage and muscle atrophy are likely the central mechanisms producing SAMS, whereas decreased glucose transport, fatty acid oxidation and insulin secretion are likely involved in the development of NOD. Physiological and pharmacological interventions able to restore cellular energetic pathways and stimulate mitochondrial biogenesis could counteract statin side-effects. Even though these side effects occur in a minority of patients, their occurrence may impact adherence to statin therapy. Nearly 10% of patients stop taking a statin because of drug related symptoms[62], thus affecting future prevention of cardiovascular events. Every attempt should be made to increase treatment adherence in high risk populations for whom statins are highly recommended because benefits of therapy by far outweigh the risks[62].

Exercise and statins

Role of exercise in statin-associated diabetes and muscle symptoms

Approximately 80% of insulin-stimulated glucose disposal occurs in skeletal muscle[63,64]. During contraction, skeletal muscle can increase ATP turnover >100 fold to provide energy for exercise[65]. ATP consumption by the contracting muscle, leads to energy depletion and decreases the ATP:AMP ratio. This causes phosphorylation and subsequent activation of AMPK, in an intensity-dependent manner[64]. AMPK is known as the master metabolic regulator[66] because it increases in low energy/nutrient states and stimulates ATP-generating processes, thus restoring normal energy levels[67]. Active AMPK, phosphorylates downstream targets, such as acetyl-CoA carboxylase (ACC), tumor sclerosis complex 2 (TSC2), mTORC1, HMG-CoA reductase, PGC-1α, and glycogen synthase kinase 3 (GSK-3)[66,68]. In addition, AMPK acts at different levels of the IRS1/PI3K/Akt pathway, increasing GLUT4 translocation and insulin signal transduction, thus potentiating insulin effects[64]. These changes favor glucose uptake, glycolysis, glucose and fatty acid (FA) oxidation, and mitochondrial biogenesis[68]. At the same time, ATP-consuming processes such as gluconeogenesis, glycogen synthesis, lipogenesis, cholesterol and protein synthesis are suppressed[68,69]. Therefore, the activation of AMPK shifts lipid metabolism towards FA-CoA oxidation and ATP production rather than lipid peroxidation or ceramide and diacylglycerol synthesis[66,70]. Such effects may prevent the tissue lipotoxicity, associated with insulin resistance, β-cell dysfunction and apoptosis[71]. AMPK may play a central role in regulating muscle insulin sensitivity and glucose metabolism after exercise[72,73], Glycogen depletion, leads to an insulin-mediated glucose uptake and stimulates glycogen synthase that replenishes glycogen deposits[64], thus contributing to post-exercise insulin sensitivity and glucose homeostasis.

Type 2 diabetes is associated with AMPK dysregulation[68] and decreased mitochondrial content and function[74,75]. AMPK plays an important role in the development of insulin resistance and type 2 diabetes[67] and evidence supports that AMPK activation by exercise could reverse muscle insulin resistance [76].

Exercise is a powerful stimulus for mitochondrial biogenesis[77,78]. Muscle contraction triggers signaling events including calcium ion release, changes in the AMP:ATP ratio and in the production of ROS[75,79]. These lead to activation of transcription factors, coactivators and

regulators, among these AMPK, P38 mitogen-activated protein kinase (MAPK) and sirtuin 1 (SIRT1), all of which activate PGC-1α[80–82]. PGC-1α may translocate from the nucleus to the mitochondria to coordinate mitochondrial DNA transcription and to generate proteins that contribute to mitochondrial biogenesis[75]. Human studies have shown that mitochondrial function and capacity increase shortly after endurance exercise [83,84]. Russel and colleagues suggest that exercise intensity is the best determinant of increases in mitochondrial function, whereas exercise volume is the best predictor of mitochondrial content[79].

Muscle insulin resistance is associated with reduced mitochondrial function[74] and content[85]. Endurance exercise increases insulin sensitivity and skeletal muscle mitochondrial proteins in type 2 diabetes[79,86]. Similar effects have been demonstrated after resistance exercise in the muscle of aged men[80,87]. Reduction or cessation of physical activity quickly reverses mitochondrial adaptations[84,88], and shifts the muscle contractile characteristics towards type II (glycolytic), often with muscle atrophy [75,89].

An increase in mitochondrial content and oxidative capacity means that more ROS will be generated in the respiratory chain, but lower ROS levels have been reported with exercise[79]. This is likely due to increased mitochondrial content per unit of tissue which increases respiration efficiency [79]. Exercise-stimulated mitochondrial biogenesis also prevents the ROS excess associated with conditions such as insulin resistance, hyperglycemia[90] and myopathy[91]. In addition to these effects on mitochondrial function Induced expression of PGC-1 α reduces muscle protein degradation and atrophy by directly interfering with a FoxO3/atrogin-1/MURF1 pathway[92].

This effect is probably augmented by an increase in mTORC2 from endurance exercise[93]. mTORC2 is necessary for the activation of Akt, which inhibits FoxO3, thereby preventing muscle atrophy[94]. Akt activation, on the other hand, stimulates the translocation of GLUT4, increasing glucose uptake[35,95]. Endurance exercise also synergically increases insulin sensitivity via reducing another component of the mTOR family, mTORC1[64], which reduces the inhibition of IRS1 and improves insulin signaling[64].

The roles of mTORC1 and mTORC2 in resistance exercise-induced hypertrophy have been reviewed[96]. Resistance exercise increases mTORC1 and mTORC2, thereby increasing

skeletal muscle protein synthesis (mTORC1) and decreasing protein degradation (mTORC2/Akt/FoxO3 pathway)[96], thus augmenting muscle hypertrophy[97].

In summary, aerobic and resistance exercise training can improve fat oxidation, glucose transport, insulin sensitivity, mitochondrial biogenesis, ROS generation and increase muscle mass. These effects offer a theoretical basis to support the hypothesis that exercise training may prevent statin-associated muscle damage and statin-related type 2 diabetes.

Statin therapy and exercise training

The association between statins and exercise training (ET) has not been extensively studied, although there are reports that muscle complaints are more frequent in physically active individuals[98]. A double-blind, placebo-controlled trial, found that subjects treated with lovastatin had higher creatine kinase (CK) levels after eccentric exercise than did placebo-treated controls, suggesting that statins increase sarcolemma injury in response to an acute exercise challenge[99]. A study of Boston Marathoners found higher CK levels after the race in statin users compared to those not using statins[100].

Statin use has been associated with increased muscle fatigability[101], decreased muscle strength[102] and decreased oxidative capacity[101]. Others have found no consistent adverse effects of statin therapy on cardiorespiratory fitness, muscle strength, athletic performance, or physical activity patterns[98,103]. It has been suggested that lower muscle performance seen with statins, may not be related to the statins, but to pre-existing low exercise levels, which produce hypercholesterolemia prompting statin therapy[104]. Starting statin therapy does appear to reduce physical activity levels [105]. Also, the Lifestyle Interventions and Independence for Elders (LIFE) Study found similar exercise training adherence between subjects on and off statins[106], suggesting that statins *per se* do not affect exercise performance. SAMS, however, can be associated to less engagement in endurance and muscle-strengthening activity[20] and thus, a decrease in cardiovascular and muscular fitness[107]. Studies in mice suggest that statin-myopathy may reduce activity in a manner that prevents muscle from mounting a beneficial adaptive response to training[108]. Taken together, this evidence suggests the presence of SAMS is to be held responsible for the reduced exercise adherence and decreased fitness, rather than statin use *per se*.

Combining statin therapy with exercise training (ET), could produce multiple benefits in specific populations. Statins reduce all-cause mortality in patients with hyperlipidemia, diabetes mellitus, and/or hypertension[109]. ET reduces the risk of cardiovascular disease, obesity and type 2 diabetes, osteoporosis and loss of function with age[110]. No longitudinal randomized controlled trials have directly evaluated the combination of statins with ET in high-risk populations. A meta-analysis of 17 studies concluded that chronic exercise training prior to statin treatment could prevent SAMS[111]. Cardiorespiratory fitness (CRF) shows a linear inverse relationship with the risk of diabetes, with each MET (metabolic equivalent) increase in CRF producing approximately an 8% lower relative risk RR of type 2 diabetes in the general population[112]. Similar risk reduction is seen in statin users[113]. Furthermore, the risk of diabetes in statin-treated patients is inversely related to CRF, and diabetes risk was evident only in the Least-fit (peak VO_2 4.8 ± 1.2 METs) and Low-fit (peak VO_2 6.5 ± 1.1 METs) patients[114]. In statin-treated patients, high levels of CRF (peak VO_2 > 9 METs) were associated with significantly lower mortality risk (HR 0.3) compared to the least fit population (peak VO_2 < 5 METs)[115].

Combined aerobic and resistance exercise, and metformin are first line therapies for preventing and treating type 2 diabetes[116]**Metformin and Statins**

Metformin has multiple molecular mechanisms of action, which are not entirely understood. Metformin alters hepatic glucose metabolism by enhancing the action of insulin and opposing the effect of glucagon, thus reducing hepatic glucose production[117-119]. The liver had long been considered metformin's main site of action, but studies of gut-restricted, delayed-release metformin has demonstrated that metformin's predominant glucose-lowering effect occurs in the distal part of the small intestine[120]. Metformin increases glucose uptake and anaerobic metabolism by enterocytes, leading to lower systemic glucose absorption and increases delivery of the lactate generated to the liver[117,121]. Colonic enterocytes secrete glucose into the blood stream even when no glucose is present in the colon, suggesting that colonic enterocytes take up and metabolized glucose from the systemic circulation[122]. Metformin also enhances glucagon-like peptide-1 (GLP-1) action, either by reducing dipeptydil-peptidase-4 (DPP-4) activity or by increasing intestinal GLP-1 production[117,123,124]. GLP-1 acts on the pancreatic β-cells to enhance/restore early-phase

insulin secretion[125], and indirectly suppresses hepatic gluconeogenesis through central hypothalamic signaling [126].

Metformin reaches concentrations inside the mitochondria that are nearly 1000 times higher than in the extracellular medium[127]. Metformin inhibits complex I of the mitochondrial electron transport chain increasing the production of mitochondrial-derived reactive nitrogen species[128], and the AMP:ATP and NAD+/NADH ratios[117]. This metformin-induced mitochondrial dysfunction leads to activation of cellular AMPK[117,129]. Other cellular organelles, such as lisosomes, may also play a role in AMPK activation[130].

AMPK activation affects several other enzymes involved in lipid metabolism. AMPK phosphorylates ACC1/ACC2, which inhibits the conversion of acetyl CoA to malonyl CoA, thus decreasing lipogenesis and promoting lipid oxidation[131]. These increase muscle and liver insulin sensitivity[118,132,133] and attenuate organ and endothelial lipo-toxicity[132,134]. Reductions in liver lipid content further reduce insulin resistance and hepatic glucose production[70]. The effects of metformin on lipid oxidation and glucose metabolism may also have a role in regulating insulin secretion by protecting the pancreatic β-cell from the lipo – and gluco-toxicity associated with insulin resistance and diabetes[132]. Metformin also decreases cyclic AMP (cAMP), which suppresses glucagon-induced gluconeogenesis, further aiding glycemic control[117–119].

Metformin also reduces HMG-CoA reductase activity, which inhibits the mevalonate pathway[68,135], giving metformin statin-like properties. Inhibition of the mevalonate pathway, reduces isoprenoids, prenylated proteins and cholesterol, which could potentiate the cholesterol-lowering effect of statins[136]. Similar to the glucose-lowering effect, the intestine rather than the liver may play a central role in this effect of metformin[132]. Metformin inhibits intestinal bile acid absorption[137]. This forces the liver to use cholesterol to increase bile acid production[138]. On the other hand, by acting as a statin, metformin could potentially worsen muscle symptoms[139]. AMPK activation is central to the hypothetical mechanisms by which metformin might produce SAMS. As mentioned above, AMPK phosphorylation stimulates pathways that generate ATP and inhibits pathways that consume ATP[67]. As a result, synthesis of non-essential proteins is decreased and protein degradation is favored by stimulation of the FoxO pathway and inhibition of mTORC1[140]. These pathways could up-

regulate the expression of atrogin-1 and MURF1 similar to statins [141], thus exacerbating muscle degradation. In contrast to statins, metformin increases PGC-1 α, which increases mitochondrial content and function[90,118,142,143] and reduces atrogin-1 expression and muscle atrophy[144]. Studies in mice show that metformin decreases tumor-associated skeletal muscle myopathy through insulin-mediated activation of PI3k/Akt signaling and by decreasing of atrogin-1[145]. Similarly, metformin counteracts muscle atrophy induced by obesity, partly through the PGC-1α-FoxO3 pathway[92]. Statin-induced over expression of atrogin-1 can be prevented by over-expression of PGC-1α in Zebra fish[45]. Therefore, metformin, by activating AMPK and inducing the expression of PGC-1 α, could counteract the atrogin-1 expression with statins, thus reducing SAMS and allowing preservation of skeletal muscle.

There are no randomized controlled trials testing the effect of metformin on SAMS in humans. The ACCORD study randomized diabetics who were on statins to fenofibrate or placebo. An exploratory analysis of ACCORD found that metformin users had fewer SAMS than subjects not treated with metformin. [146]. The effectiveness of metformin in preventing type 2 diabetes in high-risk populations has been demonstrated by randomized-controlled clinical trials[147–149]. Metformin has a "Grade A" recommendation from the American Diabetes Association for the prevention of Type 2 Diabetes[116]. Nevertheless, it is not frequently used for this purpose[150,151]. If we were to consider statin use as a risk factor for diabetes, it is possible that metformin could also delay/prevent statin-associated diabetes. This possibility has not been studied to our knowledge.

Metabolic syndrome is associated with increased risk of cardiovascular morbidity and mortality[152]. Most patients with metabolic syndrome would benefit from both statin therapy and metformin to prevent cardiovascular disease and type 2 diabetes. Brinton and colleagues found that metabolic syndrome patients were at increased risk of developing SAMS while on statins[19]. In a hypothetical scenario, the patient who is experiencing side effects from statins, may initially avoid physical activity due to muscle aches, which leads to decreased fitness and increased risk of developing cardio-metabolic disease. If muscle symptoms impact greatly the patient's life, the patient will probably stop taking statins altogether, thus escalating the risk of cardiovascular events. Any strategy that reduced SAMS would increase the number of patients able to adhere to statin therapy[9] and engage in

physical exercise[20]. This could reduce all-cause mortality and recurrence of cardiovascular disease[153] and type 2 diabetes[154].

However, the prescription of metformin to prevent statin side effects would be off-label and careful risk-benefit evaluations should be made considering metformin itself may lead to more side effects likediarrhoea, vitamin B12 malabsorption, lactic acidosis ect[155]. Some of these side effects, especially gastrointestinal disturbances, may become very bothersome to patients and reduce their adherence to therapy altogether. The appearance of side effects, as well as the pharmacologic response to metformin, are influenced by genetic determinants[156]. Being aware of these pharmacogenetic determinants would help predict the responses to the drug and identify patients who could benefit the most, with fewer side effects.

Conclusions

The idea of combining statins with metformin and exercise appears attractive, both from a clinical and economical viewpoint. All three therapies are highly cost-effective [157–159] and would have major public health implications if their combination resulted in additional diabetes and cardiovascular disease prevention. This combination is strongly supported by molecular pathways, but relevant clinical evidence remains scarce. Prospective, randomized-controlled trials are warranted to assess the effect of metformin and exercise on SAMS and diabetes prevention among statin users, particularly those at higher risk of diabetes.

REFERENCES:

1. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: Prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet*. 2005;366(9493):1267-1278. doi:10.1016/S0140-6736(05)67394-1

2. Silverman MG, Ference BA, Im K, et al. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: A systematic review and meta-analysis. *JAMA - J Am Med Assoc*. 2016;316:1289– 1297. doi:10.1001/jama.2016.13985

3. Moriarty PM, Thompson PD, Cannon CP, et al. Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: The ODYSSEY ALTERNATIVE randomized trial. *J Clin Lipidol*. 2015. doi:10.1016/j.jacl.2015.08.006

4. Nissen SE, Stroes E, Dent-Acosta RE, et al. Efficacy and tolerability of evolocumab vs ezetimibe in patients with muscle-related statin intolerance: The GAUSS-3 randomized clinical trial. *JAMA - J Am Med Assoc*. 2016;315(15):1580-1590. doi:10.1001/jama.2016.3608

5. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376(18):1713-1722. doi:10.1056/NEJMoa1615664

6. Ward NC, Watts GF, Eckel RH. Statin Toxicity. *Circ Res*. 2019;124(2):328-350. doi:10.1161/circresaha.118.312782

7. Mach F, Ray KK, Wiklund O, et al. Adverse effects of statin therapy: perception vs. the evidence – focus on glucose homeostasis, cognitive, renal and hepatic function, haemorrhagic stroke and cataract. *Eur Heart J*. 2018;39(27):2526-2539. doi:10.1093/eurheartj/ehy182

8. Backes JM, Ruisinger JF, Gibson CA, Moriarty PM. Statin-associated muscle symptoms—Managing the highly intolerant. *J Clin Lipidol*. 2017;11(1):24-33. doi:10.1016/j.jacl.2017.01.006

9. Cohen JD, Brinton EA, Ito MK, Jacobson TA. Understanding Statin Use in America and Gaps in Patient Education (USAGE): An internet-based survey of 10,138 current and former statin users. *J Clin Lipidol*. 2012;6(3):208-215. doi:10.1016/j.jacl.2012.03.003

10. Colloca L. Nocebo effects can make you feel pain: Negative expectancies derived from features of commercial drugs elicit nocebo effects. *Science*. 2017;358(6359):44. doi:10.1126/science.aap8488

11. Penson PE, Mancini GBJ, Toth PP, et al. Introducing the 'Drucebo' effect in statin therapy: a systematic review of studies comparing reported rates of statin-associated muscle symptoms, under blinded and open-label conditions. *J Cachexia Sarcopenia Muscle*. 2018. doi:10.1002/jcsm.12344

12. Gupta A, Thompson D, Whitehouse A, et al. Adverse events associated with unblinded, but not with blinded, statin therapy in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid-Lowering Arm (ASCOT-LLA): a randomised double-blind placebo-controlled trial and its non-randomised non-blind extension p. *Lancet*. 2017;389(10088):2473-2481. doi:10.1016/S0140-6736(17)31075-9

13. Thompson PD, Panza G, Zaleski A, Taylor B. Statin-associated side effects. *J Am Coll Cardiol*. 2016;67(20):2395-2410. doi:10.1016/j.jacc.2016.02.071

14. Carmena R, Betteridge DJ. Diabetogenic Action of Statins : Mechanisms. *Curr Atheroscler Rep*. 2019;21(23):1-9.

15. Navarese EP, Buffon A, Andreotti F, et al. Meta-Analysis of Impact of Different Types and Doses of Statins on New-Onset Diabetes Mellitus. *Am J Cardiol*. 2013;111:1123-1130.

16. Waters DD, Ho JE, DeMicco DA, et al. Predictors of New-Onset Diabetes in Patients Treated With Atorvastatin Results From 3 Large Randomized Clinical Trials. *J Am Coll Cardiol*. 2011;57(14):1535-1545.

17. Robert H Eckel, Scott M Grundy PZZ. The metabolic syndrome. *Lancet*. 2005;365:1415–28. doi:10.1093/alcalc/agx075

18. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk: A systematic review and meta-analysis. *J Am Coll Cardiol*. 2010. doi:10.1016/j.jacc.2010.05.034

19. Brinton EA, Maki KC, Jacobson TA, Sponseller CA, Cohen JD. Metabolic syndrome is associated with muscle symptoms among statin users. *J Clin Lipidol*. 2016;10(4):1022-1029. doi:10.1016/j.jacl.2016.05.003

20. Loenneke JP, Loprinzi PD. Statin use may reduce lower extremity peak force via reduced engagement in muscle-strengthening activities. *Clin Physiol Funct Imaging*. 2018;38(1):151-154. doi:10.1111/cpf.12375

21. Chowdhury R, Khan H, Heydon E, et al. Adherence to cardiovascular therapy: A meta-analysis of

prevalence and clinical consequences. *Eur Heart J*. 2013. doi:10.1093/eurheartj/eht295

22. Ding D, Lawson KD, Kolbe-Alexander TL, et al. The economic burden of physical inactivity: a global analysis of major non-communicable diseases. *Lancet*. 2016;388(10051):1311-1324. doi:10.1016/S0140-6736(16)30383-X

23. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet*. 2012;380(9838):219-229. doi:10.1016/S0140-6736(12)61031-9

24. Bell DSH, DiNicolantonio JJ, O'Keefe JH. Is statin-induced diabetes clinically relevant? A comprehensive review of the literature. *Diabetes, Obes Metab*. 2014;16(8):689-694. doi:10.1111/dom.12254

25. Corrao G, Monzio Compagnoni M, Rea F, Merlino L, Catapano AL, Mancia G. Clinical significance of diabetes likely induced by statins: Evidence from a large population-based cohort. *Diabetes Res Clin Pract*. 2017;133:60-68. doi:10.1016/j.diabres.2017.08.008

26. Kikutani Y, Kobayashi M, Konishi T, et al. Involvement of Monocarboxylate Transporter 4 Expression in Statin-Induced Cytotoxicity. *J Pharm Sci*. 2016;105(4):1544–1549. doi:10.1016/j.xphs.2016.01.014

27. Rocha KC e., Pereira BMV, Rodrigues AC. An update on efflux and uptake transporters as determinants of statin response. *Expert Opin Drug Metab Toxicol*. 2018:613-624. doi:10.1080/17425255.2018.1482276

28. Muntean DM, Thompson PD, Catapano AL, et al. Statin-associated myopathy and the quest for biomarkers: can we effectively predict statin-associated muscle symptoms? *Drug Discov Today*. 2017:85–96. doi:10.1016/j.drudis.2016.09.001

29. Bouitbir J, Charles A-L, Echaniz-Laguna A, et al. Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a 'mitohormesis' mechanism involving reactive oxygen species and PGC-1. *Eur Heart J*. 2012;33:1397-1407. doi:10.1093/eurheartj/ehr287

30. Mancini GBJ, Baker S, Bergeron J, et al. Diagnosis, Prevention, and Management of Statin Adverse Effects and Intolerance: Canadian Consensus Working Group Update (2016). *Can J Cardiol*. 2016;32(7):S35-S65. doi:10.1016/j.cjca.2016.01.003

31. Apostolopoulou M, Corsini A, Roden M. The role of mitochondria in statin-induced myopathy. *Eur J Clin Invest*. 2015;45(7):745-754. doi:10.1111/eci.12461

32. Resh MD. Trafficking and signaling by fatty-acylated and prenylated proteins. *Nat Chem Biol*. 2006:584–590. doi:10.1038/nchembio834

33. Hashemi M, Hoshyar R, Ande SR, et al. Mevalonate Cascade and its Regulation in Cholesterol Metabolism in Different Tissues in Health and Disease. *Curr Mol Pharmacol*. 2017;10:13-26.

34. Bhandari S, Gupta P, Quinn P, et al. Pleiotropic effects of statins in hypercholesterolaemia: a prospective observational study using a lipoproteomic based approach. *Lancet*. 2015;385:S21. doi:10.1016/s0140-6736(15)60336-1

35. Bouitbir J, Sanvee GM, Panajatovic M V., Singh F, Krähenbühl S. Mechanisms of statin-associated skeletal muscle-associated symptoms. *Pharmacol Res*. 2019. doi:10.1016/j.phrs.2019.03.010

36. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell*. 2005:483–495. doi:10.1016/j.cell.2005.02.001

37. Bouitbir J, Singh F, Charles A-L, et al. Statins Trigger Mitochondrial Reactive Oxygen Species-Induced Apoptosis in Glycolytic Skeletal Muscle. *Antioxid Redox Signal*. 2015;24(2):84-98. doi:10.1089/ars.2014.6190

38. Romanello V, Guadagnin E, Gomes L, et al. Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J*. 2010;29:1774–1785. doi:10.1038/emboj.2010.60

39. Lin J, Wu H, Tarr PT, et al. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature*. 2002;418(6899):797–801. doi:10.1038/nature00904

40. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): Transcriptional coactivator and metabolic regulator. *Endocr Rev*. 2003;24(1):78-90. doi:10.1210/er.2002-0012

41. Bouitbir J, Daussin F, Charles AL, et al. Mitochondria of trained skeletal muscle are protected from deleterious effects of statins. *Muscle and Nerve*. 2012;46(3):367-373. doi:10.1002/mus.23309

42. Bonifacio A, Sanvee GM, Bouitbir J, Krähenbühl S. The AKT/mTOR signaling pathway plays a key role in statin-induced myotoxicity. *Biochim Biophys Acta - Mol Cell Res*. 2015.

doi:10.1016/j.bbamcr.2015.04.010

43.   Murray ER. Towards specific inhibition of mTORC2. *Aging (Albany NY)*. 2017;9(12):2461-2462.

44.   Bonifacio A, Sanvee GM, Brecht K, et al. IGF-1 prevents simvastatin-induced myotoxicity in C2C12 myotubes. *Arch Toxicol*. 2017;91(5):2223-2234. doi:10.1007/s00204-016-1871-z

45.   Hanai J, Sukhatme VP, Lecker SH, et al. The muscle-specific ubiquitin ligase atrogin-1 / MAFbx mediates statin-induced muscle toxicity. *J Clin Invest*. 2007;117(12):3940-3951. doi:10.1172/JCI32741.3940

46.   Tuckow AP, Jefferson SJ, Kimball SR, Jefferson LS. Simvastatin represses protein synthesis in the muscle-derived C 2C12 cell line with a concomitant reduction in eukaryotic initiation factor 2B expression. *Am J Physiol - Endocrinol Metab*. 2011;30(3):E564–70. doi:10.1152/ajpendo.00383.2010

47.   Sun B, Zhong Z, Wang F, et al. Atorvastatin impaired glucose metabolism in C2C12 cells partly via inhibiting cholesterol-dependent glucose transporter 4 translocation. *Biochem Pharmacol*. 2018;150:108–119. doi:10.1016/j.bcp.2018.01.021

48.   Li W, Liang X, Zeng Z, et al. Simvastatin inhibits glucose uptake activity and GLUT4 translocation through suppression of the IR/IRS-1/Akt signaling in C2C12 myotubes. *Biomed Pharmacother*. 2016;83:194–200. doi:10.1016/j.biopha.2016.06.029

49.   Yaluri N, Modi S, Kokkola T. Simvastatin induces insulin resistance in L6 skeletal muscle myotubes by suppressing insulin signaling, GLUT4 expression and GSK-3β phosphorylation. *Biochem Biophys Res Commun*. 2016;480(2):194–200. doi:10.1016/j.bbrc.2016.10.026

50.   Nakata M, Nagasaka S, Kusaka I, Matsuoka H, Ishibashi S, Yada T. Effects of statins on the adipocyte maturation and expression of glucose transporter 4 (SLC2A4): Implications in glycaemic control. *Diabetologia*. 2006;49:1881–1892.

51.   Klip A, McGraw TE, James DE. Thirty sweet years of GLUT4. *J Biol Chem*. 2019;294(30):11369-11381. doi:10.1074/jbc.REV119.008351

52.   Larsen S, Vigelsø A, Dandanell S, Prats C, Dela F, Helge JW. Simvastatin-Induced Insulin Resistance May Be Linked to Decreased Lipid Uptake and Lipid Synthesis in Human Skeletal Muscle: the LIFESTAT Study. *J Diabetes Res*. 2018;2018:1-7. doi:10.1155/2018/9257874

53.   Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes. *N Engl J Med*. 2004;350(7):664–671. doi:10.1056/NEJMoa031314

54.   Defronzo RA. Dysfunctional fat cells, lipotoxicity and type 2 diabetes. In: *International Journal of Clinical Practice, Supplement*. ; 2004:9–21.

55.   Meex RCR, Blaak EE, van Loon LJC. Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes. *Obes Rev*. 2019;(November 2018):1-13. doi:10.1111/obr.12862

56.   Ye R, Onodera T, Scherer PE. Lipotoxicity and β Cell Maintenance in Obesity and Type 2 Diabetes. *J Endocr Soc*. 2019;3(3):617-631. doi:10.1210/js.2018-00372

57.   Sattar N, Taskinen M. Statins are diabetogenic e Myth or reality ? *Atheroscler Suppl*. 2012;13:1-10.

58.   Sampson UK, MacRae, Linton F FS. Are statins diabetogenic ? *Curr Opin Cardiol*. 2011;26:342-347. doi:10.1097/HCO.0b013e3283470359

59.   Kruit JK, Brunham LR, Verchere CB, Hayden MR. HDL and LDL cholesterol significantly influence β-cell function in type 2 diabetes mellitus. *Curr Opin Lipidol*. 2010:178–185. doi:10.1097/MOL.0b013e328339387b

60.   Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW. Direct effect of cholesterol on insulin secretion: A novel mechanism for pancreatic β-cell dysfunction. *Diabetes*. 2007;56:2328–2338. doi:10.2337/db07-0056

61.   Donath MY, Böni-Schnetzler M, Ellingsgaard H, Ehses JA. Islet inflammation impairs the pancreatic B-cell in type 2 diabetes. *Physiology*. 2009;24(6):325-331. doi:10.1152/physiol.00032.2009

62.   Newman CB, Preiss D, Tobert JA, et al. Statin Safety and Associated Adverse Events: A Scientific Statement From the American Heart Association. *Arterioscler Thromb Vasc Biol*. 2019;39(2):e38-e81. doi:10.1161/ATV.0000000000000073

63.   DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*. 2009;32 Suppl 2. doi:10.2337/dc09-S302

64.   O'Neill HM. AMPK and exercise: Glucose uptake and insulin sensitivity. *Diabetes Metab J*.

2013;37(1):1-21. doi:10.4093/dmj.2013.37.1.1

65. Sahlin K, Tonkonogi M, Söderlund K. Energy supply and muscle fatigue in humans. *Acta Physiol Scand*. 1998;162(3):261-266. doi:10.1046/j.1365-201X.1998.0298f.x

66. Ruderman N, Prentki M. AMP kinase and malonyl-CoA: Targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov*. 2004;3(4):340-351. doi:10.1038/nrd1344

67. Ruderman NB, Carling D, Prentki M, Cacicedo JM. Insulin resistance and the metabolic syndrome. *J Clin Invest*. 2013;123(7):2764-2772. doi:10.1007/978-0-387-09841-8-34

68. Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. AMPK activation: A therapeutic target for type 2 diabetes? *Diabetes, Metab Syndr Obes Targets Ther*. 2014;7:241-253. doi:10.2147/DMSO.S43731

69. Srivastava RAK, Pinkosky SL, Filippov S, Hanselman JC, Cramer CT, Newton RS. AMP-activated protein kinase: An emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. *J Lipid Res*. 2012;53(12):2490–2514. doi:10.1194/jlr.R025882

70. Day EA, Ford RJ, Steinberg GR. AMPK as a Therapeutic Target for Treating Metabolic Diseases. *Trends Endocrinol Metab*. 2017;28(8):545-560. doi:10.1016/j.tem.2017.05.004

71. El-Assaad W, Buteau J, Peyot ML, et al. Saturated fatty acids synergize with elevated glucose to cause pancreatic β-cell death. *Endocrinology*. 2003;144:4154–4163. doi:10.1210/en.2003-0410

72. Kjøbsted R, Roll JLW, Jørgensen NO, et al. AMPK and TBC1D1 regulate muscle glucose uptake after, but not during, exercise and contraction. *Diabetes*. 2019;68(7):1427-1440. doi:10.2337/db19-0050

73. Kjøbsted R, Munk-Hansen N, Birk JB, et al. Enhanced muscle insulin sensitivity after contraction/exercise is mediated by AMPK. *Diabetes*. 2017;66(3):598-612. doi:10.2337/db16-0530

74. Kelley DE, He J, Menshikova E V., Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*. 2002;51:2944–2950. doi:10.2337/diabetes.51.10.2944

75. Bishop DJ, Granata C, Eynon N. Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? *Biochim Biophys Acta - Gen Subj*. 2014;1840(4):1266-1275. doi:10.1016/j.bbagen.2013.10.012

76. Kjøbsted R, Treebak JT, Fentz J, et al. Prior AICAR stimulation increases insulin sensitivity in mouse

skeletal muscle in an AMPK-dependent manner. *Diabetes*. 2015;64(6):2042-2055. doi:10.2337/db14-1402

77. Little JP, Safdar A, Bishop D, Tarnopolsky MA, Gibala MJ. An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1α and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol - Regul Integr Comp Physiol*. 2011;300:R1303–R1310. doi:10.1152/ajpregu.00538.2010

78. Serpiello FR, McKenna MJ, Bishop DJ, et al. Repeated sprints alter signaling related to mitochondrial biogenesis in humans. *Med Sci Sports Exerc*. 2012;44:827–834. doi:10.1249/MSS.0b013e318240067e

79. Russell AP, Foletta VC, Snow RJ, Wadley GD. Skeletal muscle mitochondria: A major player in exercise, health and disease. *Biochim Biophys Acta - Gen Subj*. 2014;1840(4):1276-1284. doi:10.1016/j.bbagen.2013.11.016

80. Kim Y, Triolo M, Hood DA. Impact of Aging and Exercise on Mitochondrial Quality Control in Skeletal Muscle. *Oxid Med Cell Longev*. 2017;2017:1-16. doi:10.1155/2017/3165396

81. Koltai E, Hart N, Taylor AW, et al. Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. *Am J Physiol - Regul Integr Comp Physiol*. 2012;303(2):R127–R134. doi:10.1152/ajpregu.00337.2011

82. Kang C, Chung E, Diffee G, Ji LL. Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: Role of PGC-1α. *Exp Gerontol*. 2013;48(11):1343–1350. doi:10.1016/j.exger.2013.08.004

83. Kras KA, Hoffman N, Roust LR, Benjamin TR, De Filippis EA, Katsanos CS. Adenosine Triphosphate Production of Muscle Mitochondria after Acute Exercise in Lean and Obese Humans. *Med Sci Sports Exerc*. 2019;51(3):445-453. doi:10.1249/MSS.0000000000001812

84. Ryan TE, Southern WM, Brizendine JT, Mccully KK. Activity-induced changes in skeletal muscle metabolism measured with optical spectroscopy. *Med Sci Sports Exerc*. 2013;45(12):2346-2352. doi:10.1249/MSS.0b013e31829a726a

85. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsøe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia*. 2007;50:790–796.

doi:10.1007/s00125-007-0594-3

86.   Van Tienen FHJ, Praet SFE, De Feyter HM, et al. Physical activity is the key determinant of skeletal muscle mitochondrial function in type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97:3261–3269. doi:10.1210/jc.2011-3454

87.   Ogborn DI, McKay BR, Crane JD, et al. Effects of age and unaccustomed resistance exercise on mitochondrial transcript and protein abundance in skeletal muscle of men. *Am J Physiol - Regul Integr Comp Physiol*. 2015;308(8):R734–R741. doi:10.1152/ajpregu.00005.2014

88.   Kenny HC, Rudwill F, Breen L, et al. Bed rest and resistive vibration exercise unveil novel links between skeletal muscle mitochondrial function and insulin resistance. *Diabetologia*. 2017. doi:10.1007/s00125-017-4298-z

89.   Ross A, Leveritt M. Long-term metabolic and skeletal muscle adaptations to short-sprint training: Implications for sprint training and tapering. *Sport Med*. 2001;31(15):1063–1082. doi:10.2165/00007256-200131150-00003

90.   Kukidome D, Nishikawa T, Sonoda K, et al. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes*. 2006;55(1):120-127. doi:10.2337/diabetes.55.01.06.db05-0943

91.   Siciliano G, Simoncini C, Gerfo A Lo, Orsucci D, Ricci G, Mancuso M. Effects of aerobic training on exercise-related oxidative stress in mitochondrial myopathies. *Neuromuscul Disord*. 2012;22(SUPPL. 3):S172-S177. doi:10.1016/j.nmd.2012.10.005

92.   Hasan MM, Shalaby SM, El-Gendy J, Abdelghany EMA. Beneficial effects of metformin on muscle atrophy induced by obesity in rats. *J Cell Biochem*. 2019;120(4):5677-5686. doi:10.1002/jcb.27852

93.   Kleinert M, Parker BL, Fritzen AM, et al. Mammalian target of rapamycin complex 2 regulates muscle glucose uptake during exercise in mice. *J Physiol*. 2017;595(14):4845-4855. doi:10.1113/JP274203

94.   Mallinson JE, Constantin-teodosiu D, Sidaway J, Westwood FR, Greenhaff PL. Blunted Akt / FOXO signalling and activation of genes controlling atrophy and fuel use in statin myopathy. *J phy*. 2009;1:219-230. doi:10.1113/jphysiol.2008.164699

95.   Brault M, Ray J, Gomez Y, Mantzoros CS, Daskalopoulou SS. Statin treatment and new-onset diabetes: A review of proposed mechanisms. *Metabolism*. 2014;63(6):735-745. doi:10.1016/j.metabol.2014.02.014

96.   Ogasawara R, Jensen TE, Goodman CA, Hornberger TA. Resistance Exercise-Induced Hypertrophy: A Potential Role for Rapamycin-Insensitive mTOR. *Exerc Sport Sci Rev*. 2019;47(3):188-194. doi:10.1249/JES.0000000000000189

97.   Gonzalez AM, Hoffman JR, Stout JR, Fukuda DH, Willoughby DS. Intramuscular Anabolic Signaling and Endocrine Response Following Resistance Exercise: Implications for Muscle Hypertrophy. *Sport Med*. 2016;46(5):671-685. doi:10.1007/s40279-015-0450-4

98.   Noyes AM, Thompson PD. The effects of statins on exercise and physical activity. *J Clin Lipidol*. 2017;11(5):1134-1144. doi:10.1016/j.jacl.2017.07.003

99.   Thompson PD, Zmuda JM, Domalik LJ, Zimet RJ, Staggers J, Guyton JR. Lovastatin increases exercise-induced skeletal muscle injury. *Metabolism*. 1997;46(10):1206-1210. doi:10.1016/S0026-0495(97)90218-3

100.   Parker BA, Augeri AL, Capizzi JA, et al. Effect of statins on creatine kinase levels before and after a marathon run. *Am J Cardiol*. 2012;109(2):282-287. doi:10.1016/j.amjcard.2011.08.045

101.   Allard NAE, Schirris TJJ, Verheggen RJ, et al. Statins affect skeletal muscle performance: Evidence for disturbances in energy metabolism. *J Clin Endocrinol Metab*. 2018;103(1):75-84. doi:10.1210/jc.2017-01561

102.   Scott D, Blizzard L, Fell J, Jones G. Statin therapy, muscle function and falls risk in community-dwelling older adults. *QJM*. 2009;102(9):625-633. doi:10.1093/qjmed/hcp093

103.   Panza GA, Taylor BA, Thompson PD. An update on the relationship between statins and physical activity. *Curr Opin Cardiol*. 2016;31(5):572-579. doi:10.1097/HCO.0000000000000317

104.   Williams PT, Thompson PD. Effects of Statin Therapy on Exercise Levels in Participants in the National Runners' and Walkers' Health Study. *Mayo Clin Proc*. 2015;90(10):1338-1347. doi:10.1016/j.mayocp.2015.06.019

105.   Panza GA, Taylor BA, Thompson PD, et al. The effect of atorvastatin on habitual physical activity among healthy adults. *Med Sci Sports Exerc*. 2016;48(1):1-6. doi:10.1249/MSS.0000000000000740

106. Henderson RM, Lovato L, Miller ME, et al. Effect of Statin Use on Mobility Disability and its Prevention in At-risk Older Adults: The LIFE Study. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2016;71(11):1519-1524. doi:10.1093/gerona/glw057

107. Murlasits Z, Radák Z. The Effects of Statin Medications on Aerobic Exercise Capacity and Training Adaptations. *Sport Med*. 2014. doi:10.1007/s40279-014-0224-4

108. Chung HR, Vakil M, Munroe M, et al. The impact of exercise on statin-associated skeletal muscle myopathy. *PLoS One*. 2016;11(12):1-18. doi:10.1371/journal.pone.0168065

109. Taylor F, Huffman MD, Macedo AF, Moore THM, Burke M, Davey Smith G, Ward K ES. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2013;(1):CD004816. doi:10.1136/bmjopen-2018-023085

110. Blair SN, Sallis RE, Hutber a, Archer E. Exercise therapy - the public health message. *Scand J Med Sci Sports*. 2012;22(4):e24-8. doi:10.1111/j.1600-0838.2012.01462.x

111. Gui YJ, Liao CX, Liu Q, et al. Efficacy and safety of statins and exercise combination therapy compared to statin monotherapy in patients with dyslipidaemia: A systematic review and meta-analysis. *Eur J Prev Cardiol*. 2017;24(9):907-916. doi:10.1177/2047487317691874

112. Tarp J, Støle AP, Blond K, Grøntved A. Cardiorespiratory fitness, muscular strength and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetologia*. 2019. doi:10.1007/s00125-019-4867-4

113. Shaya GE, Juraschek SP, Feldman DI, et al. Relation of Exercise Capacity to Risk of Development of Diabetes in Patients on Statin Therapy (the Henry Ford Exercise Testing Project). *Am J Cardiol*. 2017;120(5):769-773. doi:10.1016/j.amjcard.2017.05.048

114. Kokkinos P, Faselis C, Narayan P, et al. Cardiorespiratory Fitness and Incidence of Type 2 Diabetes in United States Veterans on Statin Therapy. *Am J Med*. 2017;130(10):1192-1198. doi:10.1016/j.amjmed.2017.04.042

115. Kokkinos PF, Faselis C, Myers J, Panagiotakos D, Doumas M. Interactive effects of fitness and statin treatment on mortality risk in veterans with dyslipidaemia: A cohort study. *Lancet*. 2013;381(9864):394-399. doi:10.1016/S0140-6736(12)61426-3

116. American Diabetes Association. 3. Prevention or Delay of Type 2 Diabetes: Standards of Medical

Care in Diabetes-2020. *Diabetes Care*. 2020;43(Suppl. 1):S32-S36. doi:10.2337/dc20-S003

117. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia*. 2017;60(9):1577-1585. doi:10.1007/s00125-017-4342-z

118. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: An overview. *Clin Sci*. 2012;122:253–70. doi:10.1042/CS20110386

119. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature*. 2013;494:256–60. doi:10.1038/nature11808

120. Buse JB, DeFronzo RA, Rosenstock J, et al. The primary glucose-lowering effect of metformin resides in the gut, not the circulation: Results from short-term pharmacokinetic and 12-week dose-ranging studies. *Diabetes Care*. 2016;39(2):198-205. doi:10.2337/dc15-0488

121. Bailey CJ, Wilcock C, Scarpello JHB. Metformin and the intestine. *Diabetologia*. 2008;51:1552–1553. doi:10.1007/s00125-008-1053-5

122. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia*. 2016;59(3):426-435. doi:10.1007/s00125-015-3844-9

123. Preiss D, Dawed A, Welsh P, et al. Sustained influence of metformin therapy on circulating glucagon-like peptide-1 levels in individuals with and without type 2 diabetes. *Diabetes, Obes Metab*. 2017;19(3):356-363. doi:10.1111/dom.12826

124. DeFronzo RA, Buse JB, Kim T, et al. Once-daily delayed-release metformin lowers plasma glucose and enhances fasting and postprandial GLP-1 and PYY: results from two randomised trials. *Diabetologia*. 2016;59(8):1645-1654. doi:10.1007/s00125-016-3992-6

125. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007;87(4):1409-1439. doi:10.1152/physrev.00034.2006

126. Soty M, Penhoat A, Amigo-Correig M, et al. A gut-brain neural circuit controlled by intestinal gluconeogenesis is crucial in metabolic health. *Mol Metab*. 2015;4(2):106–17. doi:10.1016/j.molmet.2014.12.009

127. Bridges HR, Jones AJY, Pollak MN, Hirst J. Effects of metformin and other biguanides on oxidative

phosphorylation in mitochondria. *Biochem J*. 2014;462(3):475-487. doi:10.1042/BJ20140620

128.   Nystrom GJ, Lang CH. Sepsis and AMPK Activation by AICAR Differentially Regulate FoxO-1, -3 and -4 mRNA in Striated Muscle. *Int J Clin Exp Med*. 2008;1(1):50-63.

129.   Carling D, Viollet B. Beyond energy homeostasis: The expanding role of AMP-activated protein kinase in regulating metabolism. In: *Cell Metabolism*. Vol 21. Cell Press; 2015:799-804. doi:10.1016/j.cmet.2015.05.005

130.   Zhang CS, Li M, Ma T, et al. Metformin Activates AMPK through the Lysosomal Pathway. *Cell Metab*. 2016;24(4):521-522. doi:10.1016/j.cmet.2016.09.003

131.   Fullerton MD, Galic S, Marcinko K, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat Med*. 2013;19(12):1649-1654. doi:10.1038/nm.3372

132.   van Stee MF, de Graaf AA, Groen AK. Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. *Cardiovasc Diabetol*. 2018;17(1). doi:10.1186/s12933-018-0738-4

133.   Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: A systematic review. *Diabetologia*. 2006;49(3):434-441. doi:10.1007/s00125-006-0141-7

134.   Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes*. 2006;55:496–505. doi:10.2337/diabetes.55.02.06.db05-1064

135.   Viollet B, Guigas B, Leclerc J, et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: From physiology to therapeutic perspectives. *Acta Physiol*. 2009;196(1):81–98. doi:10.1111/j.1748-1716.2009.01970.x

136.   Xu T, Brandmaier S, Messias AC, et al. Effects of metformin on metabolite profiles and LDL cholesterol in patients with type 2 diabetes. *Diabetes Care*. 2015. doi:10.2337/dc15-0658

137.   Garzel B, Hu T, Li L, et al. Metformin Disrupts Bile Acid Efflux by Repressing Bile Salt Export Pump Expression. *Pharm Res*. 2020;37(2):1-10. doi:10.1007/s11095-019-2753-x

138. Hofmann AF, Hagey LR. Key discoveries in bile acid chemistry and biology and their clinical applications: History of the last eight decades. *J Lipid Res*. 2014;55(8):1553–95. doi:10.1194/jlr.R049437

139. Elsaid O, Taylor B, Zaleski A, Panza G, Thompson PD. Rationale for investigating metformin as a protectant against statin-associated muscle symptoms. *J Clin Lipidol*. 2017;11(5):1145-1151. doi:10.1016/j.jacl.2017.06.019

140. Li J, Zhong L, Wang F, Zhu H. Dissecting the role of AMP-activated protein kinase in human diseases. *Acta Pharm Sin B*. 2017;7(3):249-259. doi:10.1016/j.apsb.2016.12.003

141. Nakashima K, Yakabe Y. AMPK activation stimulates myofibrillar protein degradation and expression of atrophy-related ubiquitin ligases by increasing FOXO transcription factors in C2C12 myotubes. *Biosci Biotechnol Biochem*. 2007;71(7):1650–1656. doi:10.1271/bbb.70057

142. Gerstein HC, Colhoun HM, Dagenais GR, et al. Dulaglutide and renal outcomes in type 2 diabetes: an exploratory analysis of the REWIND randomised, placebo-controlled trial. *Lancet*. 2019;6736(19):1-8. doi:10.1016/S0140-6736(19)31150-X

143. Adhihetty PJ, Uguccioni G, Leick L, Hidalgo J, Pilegaard H, Hood DA. The role of PGC-1α on mitochondrial function and apoptotic susceptibility in muscle. *Am J Physiol - Cell Physiol*. 2009;297(1):C217–C225. doi:10.1152/ajpcell.00070.2009

144. Sandri M, Lin J, Handschin C, et al. PGC-1α protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc Natl Acad Sci U S A*. 2006;103(44):16260–16265. doi:10.1073/pnas.0607795103

145. Oliveira AG, Gomes-Marcondes MCC. Metformin treatment modulates the tumour-induced wasting effects in muscle protein metabolism minimising the cachexia in tumour-bearing rats. *BMC Cancer*. 2016;16(418):1-10. doi:10.1186/s12885-016-2424-9

146. Carris NW, Tsalatsanis A, Tipparaju SM, Cheng F, Magness RR, Kumar A. Metformin's impact on statin-associated muscle symptoms: An analysis of ACCORD study data and research materials from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center. *Diabetes, Obes Metab*. 2018;20(8):1994-1999. doi:10.1111/dom.13302

147. Doggrell SA. Metformin & lifestyle intervention prevent Type 2 diabetes: lifestyle intervention has

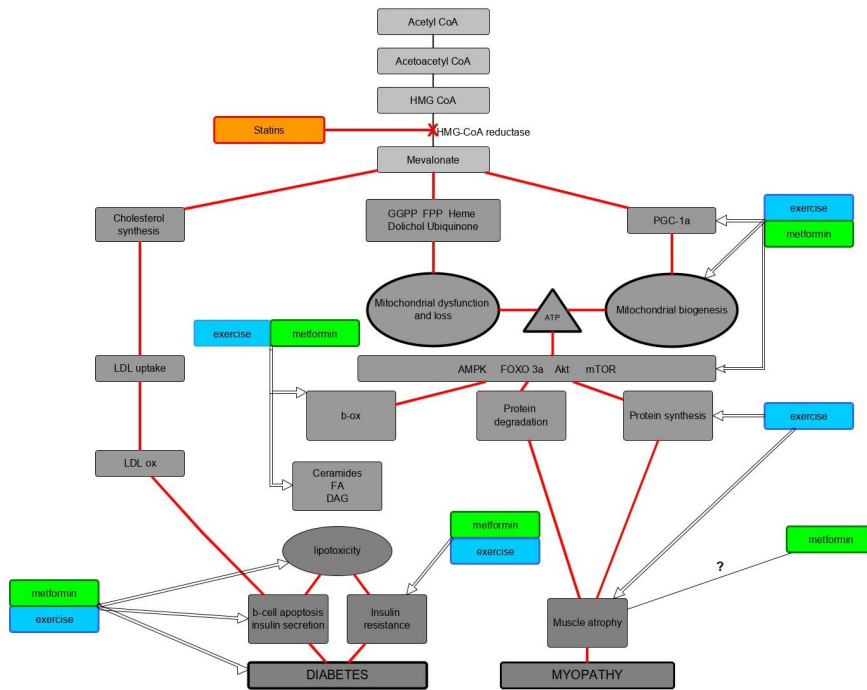the greater effect. *Expert Opin Pharmacother*. 2005;3(7):1011-1013. doi:10.1517/14656566.3.7.1011

148. Ratner R, Goldberg R, Haffner S, et al. Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the diabetes prevention program. *Diabetes Care*. 2005;28(4):888-894. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1307521&tool=pmcentrez&rendertype=abstract.

149. Aroda VR, Knowler WC, Crandall JP, et al. Metformin for diabetes prevention: insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. *Diabetologia*. 2017;60(9):1601-1611. doi:10.1007/s00125-017-4361-9

150. Moin T, Li J, Duru OK, et al. Metformin prescription for insured adults with prediabetes from 2010 to 2012: A retrospective cohort study. *Ann Intern Med*. 2015. doi:10.7326/M14-1773

151. Tseng E, Yeh HC, Maruthur NM. Metformin use in prediabetes among U.S. adults, 2005-2012. In: *Diabetes Care*. ; 2017. doi:10.2337/dc16-1509

152. Di Angelantonio E, Kaptoge S, Wormser D, et al. Association of Cardiometabolic Multimorbidity With Mortality. *JAMA*. 2015;314(1):52-60. doi:10.1001/jama.2015.7008

153. Xu WH, Han BS, Ma LL, Guo WJ, Zhang XJ, Feng B. Relationship between statin adherence and long-term clinical consequences in patients with cardiovascular disease: A systematic review and meta-analysis. *Int J Clin Exp Med*. 2016.

154. Colberg SR, Albright AL, Blissmer BJ, et al. Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Med Sci Sports Exerc*. 2010;42(12):2282-2303.

155. Thomas I, Gregg B. Metformin; a review of its history and future: from lilac to longevity. *Pediatr Diabetes*. 2017;18(1):10-16. doi:10.1111/pedi.12473

156. Florez JC. The pharmacogenetics of metformin. *Diabetologia*. 2017;60(9):1648-1655. doi:10.1007/s00125-017-4335-y

157. Pandya A, Sy S, Cho S, Weinstein MC, Gaziano TA. Cost-effectiveness of 10-year risk thresholds for initiation of statin therapy for primary prevention of cardiovascular disease. *JAMA - J Am Med*

Assoc. 2015;314(2):142-150. doi:10.1001/jama.2015.6822

158.   Myers J, de Souza de Silva CG, Doom R, et al. Cardiorespiratory Fitness and Health Care Costs in Diabetes: The Veterans Exercise Testing Study. *Am J Med*. 2019. doi:10.1016/j.amjmed.2019.04.006

159.   Hostalek U, Gwilt M, Hildemann S. Therapeutic Use of Metformin in Prediabetes and Diabetes Prevention. *Drugs*. 2015;75(10):1071-1094. doi:10.1007/s40265-015-0416-8

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