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AMPK activation—protean potential for boosting healthspan

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Abstract AMP-activated kinase (AMPK) is activated when the cellular (AMP+ADP)/ATP ratio rises; it therefore serves as a detector of cellular "fuel deficiency." AMPK activation is suspected to mediate some of the health-protective effects of long-term calorie restriction. Several drugs and nutraceuticals which slightly and safely impede the efficiency of mitochondrial ATP generation—most notably metformin and berberine—can be employed as clinical AMPK activators and, hence, may have potential as calorie restriction mimetics for extending healthspan. Indeed, current evidence indicates that AMPK activators may reduce risk for atherosclerosis, heart attack, and stroke; help to prevent ventricular hypertrophy and manage congestive failure; ameliorate metabolic syndrome, reduce risk for type 2 diabetes, and aid glycemic control in diabetics; reduce risk for weight gain; decrease risk for a number of common cancers while improving prognosis in cancer therapy; decrease risk for dementia and possibly other neurodegenerative disorders; help to preserve the proper structure of bone and cartilage; and possibly aid in the prevention and control of autoimmunity. While metformin and berberine appear to have the greatest utility as clinical AMPK activators—as reflected by their efficacy in diabetes management—regular ingestion of vinegar, as well as moderate alcohol consumption, may also achieve a modest degree of health-protective AMPK activation. The activation of AMPK achievable with any of these measures may be potentiated by clinical doses of the drug salicylate, which can bind to AMPK and activate it allosterically.

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AMPK—cellular monitor of fuel availability

AMP-activated kinase (AMPK) is sometimes described as the "fuel gauge" of the cell, inasmuch as it is activated by an increase in the cellular ratio of AMP+ADP to ATP (Xiao et al. 2011; Carling et al. 2011; Hawley et al. 2010). AMPK is a heterotrimer, consisting of α, β, and γ subunits. Two distinct genes code for α subunits, two for β, and three for γ; hence, seven genes contribute to the various isoforms of this enzyme. The α subunit develops serine/threonine kinase activity when its Thr172 is phosphorylated by various upstream kinases. The γ subunit has a regulatory function; it contains four tandem repeats of a "CBS" sequence (named after the presence of such sites in cystathionine-β-synthase) numbered 1–4. CBS site 2 appears to remain empty, whereas site 4 constitutively binds AMP. The γ subunit's regulatory function is exerted through CBS sites 1 and 3, each of which can reversibly bind either AMP, ADP, or ATP. AMP binding to site 1 allosterically boosts the kinase activity of the activated enzyme. At site 3, binding of either AMP or ADP suppresses the ability of phosphatases to remove the phosphate from Thr172 and hence deactivate the enzyme (Xiao et al. 2011). The chief activating upstream kinases targeting AMPK are LKB1, which is constitutively active and widely expressed, and the calmodulin-dependent kinase kinases (CaMKK), whose activity is stimulated by an increase in free intracellular calcium. Acute activation of AMPK is therefore seen when the (AMP+ADP)/ATP ratio rises, or when intracellular free calcium rises—conditions

which often signal cellular stress. Modulation of the activity of the phosphatases which target Thr172 of the α subunit—PP2A and PP2C—can also influence AMPK activity (Wu et al. 2007).

A number of drugs, phytochemicals, and hormones have the potential to activate AMPK. Many of these agents boost the (AMP+ADP)/ATP ratio by impeding the efficiency of mitochondrial electron transport (metformin, berberine, thiazolidinediones, dinitrophenol), inhibiting mitochondrial ATP synthase (resveratrol, quercetin) or inhibiting glycolysis (2-deoxyglucose) (Hawley et al. 2010). Hormones and drugs which increase intracellular free calcium can also function as AMPK activators; for example, the ability of HDL particles to activate AMPK in endothelial cells reflects, in part, an increase in calcium influx that activates CaMKK (Kimura et al. 2010). The compound 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) is converted intracellularly to an analog of AMP which mimics AMP's activating impact on AMPK. AMPK can also be activated allosterically by certain agents which bind to it at a site distinct from its AMP/ADP binding region. The drug A769662 works in this way, and a recent study reveals that the phytochemical drug salicylate can activate AMPK in a similar manner (Cool et al. 2006; Hawley et al. 2012). A modest degree of AMPK activation can be achieved with clinically relevant concentrations of salicylate, and this effect is complementary to the activating impact of AMP. The ability of the hormone adiponectin to activate AMPK has recently been traced to the ceramidase activity of the activated adiponectin receptor; ceramide suppresses AMPK activity by activating PP2A (Wu et al. 2007; Holland et al. 2011). Analogously, lipoic acid has the potential to support AMPK activity by countering oxidant-mediated activation of neutral sphingomyelinase, whose product is ceramide (Smith et al. 2008; Lee et al. 2005). Additionally, activation of the AdipoR1 receptor in myocytes has been shown to provoke a calcium influx that activates AMPK via calmodulin kinase kinase-β (Iwabu et al. 2010).

Since AMPK detects cellular energy deficit, its *raison d'etre* is to boost the capacity of cells to generate ATP via substrate oxidation while simultaneously suppressing the activity of metabolic pathways which utilize ATP (Hardie 2007). Hence, AMPK boosts mitochondrial biogenesis, aids mitochondrial antioxidant protection, and increases expression and activity of glucose transporters and glycolytic enzymes; concurrently, non-essential synthesis of proteins, lipids, and carbohydrates is decreased (Hardie 2007; Kukidome et al. 2006; Colombo and Moncada 2009). However, some key effects of AMPK—such as activation of the endothelial nitric oxide synthase (eNOS) (Chen et al. 1999, 2009a)—do not readily fit into this paradigm. Perhaps this reflects the fact that, in some instances, a short-term increase in ATP expenditure can pay off in a subsequent increase in capacity for ATP production—as when activation of eNOS induces an increase in tissue perfusion.

Are AMPK activators calorie restriction mimetics?

AMPK has been conserved throughout the evolution of eukaryotes, and there is evidence that activation of AMPK plays an obligate role in the life-extending activity of caloric restriction in lower eukaryotes such as yeast, worms, and flies (Canto and Auwerx 2011). Evidence is currently conflicting as to whether the fuel deficit stress induced by calorie restriction regimens in rodents is sufficient to activate AMPK (Canto and Auwerx 2011). Such activation may however occur indirectly via upregulation of adiponectin production in adipose tissue (Dolinsky et al. 2010). In any case, there is good reason to suspect that chronic activation of AMPK can serve as a calorie restriction mimetic in mammals. AMPK boosts the activity of Sirt1, another evolutionarily conserved enzyme which likewise is a mediator of the life-prolonging impact of calorie restriction in lower eukaryotes; AMPK does so by somehow increasing the expression of nicotinamide phosphoribosyl transferase (NAMPT), which is rate-limiting for the regeneration of Sirt1's obligate cofactor NAD+ (Fulco et al. 2008; Canto et al. 2009). In light of the overexuberant media coverage generated recently by reports that the wine phytochemical resveratrol may exert a prolongevity effect by activating Sirt1, it should be noted that this activation now appears to be indirect, mediated via resveratrol's impact on AMPK (Um et al. 2010; Canto et al. 2010). (Pharmacokinetic studies show that absorbed resveratrol is conjugated so rapidly in humans that it has little clinical potential as a systemic AMPK activator (Walle et al. 2004; Boocock et al. 2007).)

AMPK mimics the impact of the growth factor downregulation associated with calorie restriction by inhibiting activity of the mammalian target of rapamycin complex 1 (mTORC1) (Shaw 2009). This complex, via phosphorylation of its targets p70 ribosomal S6 kinase 1 (S6K1) and

4EBP1, upregulates protein synthesis and plays a key role in cell proliferation; its activity is suppressed by calorie restriction (Chong et al. 2010; Blagosklonny 2010). AMPK can also phosphorylate and thereby boost the transcriptional activity of FOXO3a, which induces expression of a number of antioxidant enzymes and other stress resistance proteins (Colombo and Moncada 2009; Greer et al. 2007; Olmos et al. 2009).

By inhibiting mTORC1 activity, and also via direct phosphorylation, AMPK stimulates ULK1, a kinase which is a key trigger for the process of macroautophagy (Egan et al. 2011). This "cell cleansing" process is also abetted by Sirt1 and FOXO activity (Hariharan et al. 2010). Since macroautophagy rids the cell of aging, potentially pro-oxidative mitochondria, it is homeostatically appropriate that AMPK, Sirt1, and FOXO3a also collaborate in promoting mitochondrial biogenesis, largely by boosting the expression and pro-transcriptional activity of PPAR-γcoactivator-1α (PCG-1α) (Kukidome et al. 2006; Canto et al. 2010). The efficiency of macroautophagy declines with increasing age in mammals, and there is considerable speculation that the upregulation of macroautophagy evoked by calorie restriction in many tissues contributes crucially to the life prolongation and "aging retardation" achieved by such restriction (Bergamini et al. 1998; Cuervo 2008). Indeed, concurrent upregulation of macroautophagy, mitochondrial biogenesis, and expression of mitochondrial antioxidant proteins—as promoted by AMPK activity—could be expected to keep cells structurally and functionally pristine, and is emerging as a central motif in the pro-longevity impact of calorie restriction. Not surprisingly, treatment of mice with the AMPK-activating drug metformin has been shown to replicate a number of the effects of long-term calorie restriction on hepatic gene expression (Dhahbi et al. 2005).

At a systemic level, AMPK activation can modestly lower serum glucose and insulin levels by downregulating hepatic gluconeogenesis (as discussed below) and hence slowing hepatic glucose output; this reduction in insulin can be expected to decrease IGF-I bioactivity by upregulating hepatic production of IGFBP-1 (De Leo et al. 2000; Jakubowicz et al. 2001; McCarty 2004). These effects are similar to the impact of calorie restriction on serum levels of glucose, insulin, and free IGF-I—though more modest in magnitude.

Downregulation of insulin/IGF-I signaling (or the homologous pathways in lower organisms) is thought to be the key mediator of the lifespan extension associated with calorie restriction in eukaryotes (Bartke 2005). Such signaling activates mTORC1 and S6K1, while inhibiting FOXO activity; AMPK has a countervailing impact in these regards. Agents or measures which inhibit mTORC1 or S6K1, or which activate AMPK, have indeed been reported to increase mean and maximal lifespan in certain strains of rodents (Harrison et al. 2009; Selman et al. 2009; Anisimov 2010; Anisimov et al. 2011; Baur et al. 2006). However, the survival-prolonging impact of AMPK activators metformin and resveratrol has not been observed in some healthy rodent strains fed healthful diets, whereas it is more notable in cancer-prone strains or in rodents fed diets that induce obesity and insulin resistance (Smith et al. 2010; Pearson et al. 2008). Nonetheless, an improvement in markers for healthspan has been observed in resveratrol-treated mice even when lifespan has not been influenced (Pearson et al. 2008). Hence, although it seems unlikely that AMPK activation can replicate the full lifespan-lengthening impact of calorie restriction, it may have considerable potential for promoting increased *healthspan*, and arguably might be able to amplify the longevity benefits of modest degrees of daily calorie restriction or of more practical dietary strategies (e.g., modified alternate-day fasting, carbohydrate-concentrated diets) that episodically minimize serum levels of glucose, insulin, and free IGF-I (Varady and Hellerstein 2007; Johnson et al. 2006, 2007; McCarty 2013).

Indeed, independent of any impact of AMPK on the aging process per se, there is considerable reason to suspect that agents which can safely activate AMPK in humans—most notably metformin and berberine (Zhou et al. 2001; Musi et al. 2002; Lee et al. 2006; Turner et al. 2008)—have the potential to boost healthspan via a bewildering variety of protective effects: preventing atherosclerosis, heart attack, and stroke; preventing cardiac hypertrophy and aiding management of congestive failure; ameliorating metabolic syndrome while antagonizing weight gain; reducing risk for type 2 diabetes and aiding its metabolic control; reducing risk for many cancers and improving the outcome of cancer therapies; postponing or preventing onset of dementia and possibly other neurodegenerative disorders; aiding preservation of cartilage and of bone density; and reducing risk for, or aiding control of, autoimmune disorders. This rather audacious claim is supported, in part, by epidemiology and clinical trials focusing on metformin use in diabetics; other pertinent support comes from rodent and cell culture studies.

Vascular protection

The first strong clue that metformin might have unusual utility for promoting vascular health emerged from the United Kingdom Prospective Diabetes Study, a prospective controlled study which examined long-term health outcomes in obese diabetics randomly allocated to therapy with metformin, a sulfonylurea, insulin, or dietary control. Even though patients treated with metformin achieved glycemic control no better than that achieved by patients treated with sulfonylureas or insulin, their mortality over 10 years of follow-up from myocardial infarction, stroke, or all-causes was significantly lower (UK Prospective Diabetes Study (UKPDS) Group 1998). Subsequent epidemiological studies have concluded that, as compared to patients achieving comparable metabolic control with other agents, metformin-treated patients are less likely to die from MI, stroke, or congestive failure (Scarpello 2003; Holman et al. 2008; Tzoulaki et al. 2009; Masoudi et al. 2005; Roussel et al. 2010; Schramm et al. 2011).

Studies with rodents and cultured cells demonstrate that metformin and other AMPK activators can act directly on vascular endothelium, foam cells, and cardiomyocytes in ways that could be expected to diminish risk for atherosclerosis, thrombotic events, and ventricular hypertrophy. Much of this protection stems from the ability of AMPK to activate eNOS via direct phosphorylations of Ser633 and Ser1177; the activating phophorylation of Ser633 is not duplicated by Akt activity (Chen et al. 1999, 2009a). NO, in the modest physiological concentrations produced by eNOS, is well known to exert anti-atherosclerotic, antithrombotic, vasodilatory, and antihypertrophic effects crucial for preserving healthful structure and function of the vascular system (Cooke 2004; Massion and Balligand 2007). Recent studies show that the activation of eNOS induced by HDL and by adiponectin in endothelial cells—thought to play a key role in the vascular protection afforded by these agents—is mediated via activation of AMPK (Kimura et al. 2010; Drew et al. 2004; Deng et al. 2010). Likewise, the ability of exercise-induced shear stress to stimulate eNOS and boost its expression is mediated in part via AMPK; the impact on eNOS expression requires AMPK-mediated induction of the transcription factor kruppel-like factor 2 (Zhang et al. 2006; Young et al. 2009).

Activation of NF-kappaB and increased production of oxidative stress via NAPDH oxidase complexes contribute importantly to the inflammatory endotheliopathy that is conducive to atherogenesis and thrombosis. In many studies, AMPK activation has been shown to downregulate NF-kappaB activation and oxidative stress in various types of cells; the precise mechanisms responsible require further clarification (Salminen et al. 2011a). In part, this effect reflects the fact that Sirt1 activity (as stimulated by AMPK) impairs the transcriptional activity of NF-kappaB by removing an activating acetyl group from p65 (Yeung et al. 2004). A recent study with mouse and human endothelial cell cultures has demonstrated that AMPKα2 can modestly reduce proteasome activity; this likewise results in a downregulation of NF-kappaB activation (Wang et al. 2010). (A similar effect of AMPK on proteasomes has been reported in fibroblasts (Viana et al. 2008).) This downregulation of NF-kappaB, in turn, results in decreased expression of various components of the NAPDH oxidase complex (Wang et al. 2010). The possibility that AMPK may also exert a more rapid inhibitory impact on NADPH oxidase, possibly by suppressing PKC-mediated membrane translocation of p47phox, requires more evaluation (Alba et al. 2004; Ceolotto et al. 2007; McCarty et al. 2009; Piwkowska et al. 2010). Another recent study found that AMPKα2 impedes NF-kappaB activation in endothelial cells via direct phosphorylations of IKK-β; these phophorylations impede IKK-β's ability to phosphorylate IκB and thereby promote nuclear translocation of NF-kappaB (Bess et al. 2011). Whether these findings can be generalized to other types of cells remains to be determined; in any case, it is clear that AMPK can downregulate NF-kappaB activity via several independent mechanisms, the importance of which may vary dependent on cell type. AMPK-mediated proteasomal inhibition can also help to preserve the proper coupling of eNOS (thereby warding off an increase in oxidative stress and a loss of NO production) by slowing proteasomal degradation of GTP cyclohydrolase in oxidatively stressed endothelial cells; this latter enzyme is rate-limiting for the production of eNOS's essential cofactor, tetrahyrobiopterin (Wang et al. 2009).

With respect to the role of foam cells in atherosclerosis, several studies—with one contrary exception (Li et al. 2009)—report that agents which activate AMPK tend to boost cholesterol efflux and prevent lipid accumulation in foam cells exposed to oxidized LDL (Li et al. 2010a; Lee et al. 2010a; Guan et al. 2010). A related report indicates that AICAR-mediated AMPK

activation suppresses the macrophage proliferation induced by exposure to oxidized LDL (Ishii et al. 2009).

AMPK has the potential, like statins, to inhibit HMG-CoA reductase; (Ching et al. 1996) nonetheless, metformin therapy appears at best to have a modest impact on serum levels of LDL. Yet the AMPK activator berberine is notably clinically effective for lowering serum LDL cholesterol (Kong et al. 2004; Abidi et al. 2005; Cicero et al. 2007; Zhang et al. 2008a). This effect appears to be independent of any inhibition of HMG-CoA reductase; rather, it reflects an increase in the half-life of LDL receptor mRNA. Fortunately, this effect is complementary to the stimulatory impact of statins on transcription of this mRNA, such that combined use of statins and berberine can achieve a very marked lowering of LDL levels (Kong et al. 2008). It is not clear whether AMPK has anything to do with this fortuitous benefit of berberine.

Studies examining the impact of AMPK activation on models of ventricular hypertrophy and congestive failure were prompted by epidemiological studies demonstrating that diabetics experiencing heart failure were at lower risk for mortality if they were treated with metformin (Masoudi et al. 2005; Roussel et al. 2010). In rodent studies of cardiac pressure overload, concurrent treatment with AICAR, metformin, or berberine has reduced ventricular hypertrophy; conversely, mice which are genetically deficient in AMPK-α2 or in LKB1 expression are more prone to ventricular hypertrophy in the context of overload (Fu et al. 2011; Beauloye et al. 2011; Benes et al. 2011; Zhang et al. 2011a, 2008b; Li et al. 2007; Hong et al. 2002, 2003). The protection afforded by AMPK in this regard reflects not only an effect on cardiomyocyte hypertrophy, but also an antifibrotic effect (Beauloye et al. 2011; Zhang et al. 2008b). Although the downregulatory impact of AMPK on protein synthesis might play some role in this effect, there is evidence that increased eNOS activity also plays a prominent role; (Zhang et al. 2011a) indeed, it is well established that effective NO activity tends to prevent cardiac hypertrophy (Massion and Balligand 2007).

In a randomized clinical trial enrolling 156 patients with congestive heart failure, patients received berberine or placebo in addition to standard management (Zeng et al. 2003). After 8 weeks, improvements in ventricular ejection fraction, exercise capacity, and frequency of premature ventricular complexes were notably better in the berberine group. At a 2-year follow-up, mortality in the berberine group was about half as high as in the placebo group (7 vs. 13, $p<0.02$). This appears to be the first formal clinical trial which evaluated an AMPK activator in chronic congestive heart failure—in patients most of whom were not diabetic.

However, excessive constitutive activation of AMPK in the heart can have a severely detrimental impact. The Wolff–Parkinson–White syndrome is a genetic cardiomyopathy characterized by ventricular pre-excitation, tachyarrhythmias, and cardiac hypertrophy. This syndrome has been found to reflect various inherited mutations of the γ2 subunit of AMPK which cause constitutive activation of this kinase (Gollob et al. 2001; Arad et al. 2003; Oliveira et al. 2003). This cardiac pathology appears to be largely attributable to an AMPK-driven over-accumulation of glycogen in cardiomyocytes, reflecting increased glucose uptake and glycogen synthase activation; this massive increase in glycogen may induce a feedback inhibition of this otherwise constitutive AMPK activity (Hardie 2011). Since such a syndrome has not been described in patients using pharmaceutical AMPK activators, clinical doses of these agents presumably activate AMPK to a much lesser degree than do the activating y2 mutations that characterize the Wolff–Parkinson–White syndrome; moreover, AMPK is still susceptible to physiological regulation in the presence of these drugs. Much of the favorable influence of AMPK activation on vascular health is likely mediated by its impact on metabolic syndrome, which we now examine.

Controlling metabolic syndrome and diabetes

A key cause of metabolic syndrome is inflammation in visceral adipocytes (Phillips and Prins 2008). As these adipocytes hypertrophy, they tend to become oxidatively stressed and develop an inflammatory phenotype associated with increased production and secretion of cytokines such as IL-6 and TNF-alpha, and decreased secretion of the protective adipokine adiponectin (which, as noted, can activate AMPK). The efficiency of insulin signaling in these adipocytes is impaired, and the resulting upregulation in adipocyte lipolysis floods the body with excessive levels of free fatty acids, most notably during post-absorptive metabolism when free fatty acids are not needed as fuel. This free fatty acid overexposure leads to the accumulation of triglycerides and other types of "ectopic fat" in tissues such as skeletal muscle, vascular endothelium, hepatocytes, and beta cells (Unger et al. 2010; Rasouli et al. 2007).

Certain of these ectopic fat metabolites, such as ceramide and diacyglycerol, activate signaling pathways that promote oxidative stress, insulin resistance, and inflammation in the tissues that harbor them. Hence, metabolic syndrome is often associated with insulin resistance of skeletal muscle fibers, inflammatory dysfunction of vascular endothelium, nonalcoholic steatohepatitis, and, in beta cells, glucolipotoxicity that ultimately may precipitate beta cell "failure" and type 2 diabetes. These effects can be exacerbated by increased exposure to adipose-derived pro-inflammatory cytokines and by diminution of adiponectin activity. The characteristic perturbations of serum lipids seen in metabolic syndrome are largely a consequence of increased free fatty acid flux into hepatocytes, which results in hepatic secretion of an increased number of VLDL particles and apoB molecules (Julius 2003).

The extent to which AMPK can suppress inflammation and oxidative stress in hypertrophied visceral adipocytes, and restore effectiveness of adipocyte insulin signaling, remains unclear. Several reports that serum IL-6 levels decline during metformin or berberine therapy in diabetics are suggestive of an anti-inflammatory impact; (Zhang et al. 2008a; Bulcao et al. 2007; Luque-Ramirez and Escobar-Morreale 2010; Fidan et al. 2011) however, metformin usually does not influence adiponectin levels (Phillips et al. 2003; Sharma et al. 2006; Tarkun et al. 2010). A small amount of evidence suggests that metformin may improve the insulin sensitivity of adipocytes in diabetics (Abbasi et al. 1997, 1998). Whether or not AMPK helps "get to the root" of visceral adipocyte dysfunction, it clearly can mimic the antilipolytic impact of insulin on adipoyctes. Catecholamines and other agonists that boost cAMP in adipocytes stimulate lipolysis owing to a PKA-mediated phophorylation and activation of the hormone-sensitive lipase. Insulin antagonizes this effect by activating a cAMP phosphodiesterase (3B) which opposes the catecholamine-induced rise in cAMP and, hence, prevents activation of PKA and its downstream target hormone-sensitive lipase (Wijkander et al. 1998). AMPK likewise antagonizes catecholamine-mediated activation of hormone-sensitive lipase, but in a different way: by phosphorylating Ser565 on this lipase, it renders the lipase a poor substrate for the activating phosphorylations catalyzed by PKA (Garton and Yeaman 1990; Watt et al. 2006). This likely explains why diurnal levels of serum free fatty acids tend to be suppressed by therapy with metformin or berberine (Abbasi et al. 1997, 1998; Gu et al. 2010). Evidently, since excess free fatty acid exposure is a key mediator of the complications of metabolic syndrome, this downregulatory impact of AMPK activation on serum free fatty acids can be notably protective.

Fortunately, the decrease in adipocyte lipolysis associated with metformin or berberine therapy does not tend to promote weight gain, likely because one of the key effects of AMPK is to boost the efficiency of mitochondrial fatty acid oxidation. AMPK accomplishes this, in the short term, by decreasing malonyl-CoA levels via an inhibitory phosphorylation of the enzyme acetyl-CoA carboxylase and an activating phosphorylation of malonyl-CoA decarboxylase (Park et al. 2002; Dagher et al. 2001). Malonyl-CoA is not only an obligate substrate for fatty acid and cholesterol synthesis, but also functions as an allosteric inhibitor of carnitine palmitoyltransferase-I, rate-limiting for the transfer of fatty acids into the mitochondrial inner matrix and fatty acid oxidation (McGarry et al. 1978). Hence, AMPK activity tends to disinhibit fatty acid oxidation via its impact on malonyl-CoA. In the longer term, AMPK may increase the maximal capacity of tissues for fatty acid oxidation by promoting mitochondrial biogenesis. AMPK also acts to channel free fatty acids towards oxidation by suppressing activity of glycerol-3-phosphate acyltransferase, a key mediator of triglyceride synthesis (Park et al. 2002; Muoio et al. 1999). Hence, when metabolic syndrome exposes tissues to excessive levels of free fatty acids, AMPK tends to route these fatty acids to oxidation, rather than to conversion to triglycerides or the ectopic fat metabolites that are pathogenic.

In short, AMPK activation has the potential to minimize the adverse impact of ectopic fat by slowing adipocyte release of free fatty acids, and also by reducing the propensity of the fatty acids that are released to be converted to pathogenic metabolites. This mechanism may help to explain why metformin can help to prevent or postpone the onset of type 2 diabetes in at-risk subjects, as demonstrated in the Diabetes Prevention Program (Knowler et al. 2002)—it may lessen the exposure of beta cells to the ectopic fat metabolites that promote beta cell failure (El-Assaad et al. 2003; Zhou et al. 2009; Gao et al. 2011).

The most important factor in the improved diabetic glycemic control imparted by metformin appears to be a downregulation of hepatic gluconeogenesis that lessens hepatic glucose output and hence helps to moderate fasting glucose levels (Kirpichnikov et al. 2002). This

reflects the fact that AMPK suppresses transcription of the genes coding for the key gluconeogenic enzymes phophoenolpyruvate carboxykinase and fructose-diphosphatase. AMPK achieves this by phosphorylating, and thereby inhibiting the activity of, two coactivators (CREB binding protein and CRTC2) required for efficient transcription of these genes (He et al. 2009; Koo et al. 2005). Metformin's inhibition of CREB binding protein precisely mimics the impact of insulin in this regard (He et al. 2009). AMPK also boosts the synthesis of the orphan nuclear receptor "small heterodimer partner," which likewise interferes with the transcription of these key genes (Kim et al. 2008; Lee et al. 2010b). And AMPK promotes the phosphorylation and nuclear exclusion of type IIa histone deacetylases (HDAC 4, 5, and 7) which support the contribution of FOXO factors to the transcription of genes coding for gluconeogenic enzymes such as glucose-6-phophatase (Mihaylova et al. 2011). The fact that AMPK works in so many complementary ways to suppress gluconeogenesis may explain why metformin therapy has such a notable clinical effect on this metabolic process. Berberine likewise suppresses gluconeogenesis in diabetic rats; (Xia et al. 2011) its clinical utility for glycemic control appears to be quite comparable to that of metformin (Zhang et al. 2008a; Gu et al. 2010).

A moderate improvement in the efficiency of insulin-mediated glucose uptake in skeletal muscle also contributes to metformin's favorable impact on diabetic control; berberine therapy appears to have a comparable effect (Giannarelli et al. 2003; Wei et al. 2012). Potentially, AMPK activity in skeletal muscle may boost insulin-mediated glucose uptake by various complementary mechanisms. Nuclear HDAC5 suppresses the transcriptional activity of MEF2, a key mediator of the transcription of the GLUT4 gene; by promoting the phosphorylation and nuclear exclusion of HDAC5, AMPK can upregulate GLUT4 expression (McGee et al. 2008). By inhibiting mTORC1, AMPK downregulates the feedback reduction of insulin sensitivity induced by Akt–mTORC1–p70SK6–IRS-1 signaling (Liu et al. 2010). A reduction in membrane cholesterol induced by AMPK may enhance GLUT4 activity (Habegger et al. 2012). And AMPK can promote the membrane translocation of GLUT4 downstream from Akt by phosphorylating AS160, potentially giving AMPK the ability to promote glucose uptake independent of insulin activity (Cartee and Wojtaszewski 2007; Musi and Goodyear 2003). AMPK may also alleviate the adverse impact of fatty acid overexposure on muscle insulin sensitivity by promoting mitochondrial biogenesis and fatty acid oxidation (Hardie 2011; Jager et al. 2007; Attane et al. 2012), by suppressing adipocyte lipolysis, and, in the longer term, promoting a reduction in adipose stores (as discussed below). Which of these effects is most important clinically for metformin's favorable impact on insulin sensitivity is not yet clear.

Weight control

AMPK activators may also help to prevent metabolic syndrome by reducing risk for weight gain. Diabetologists are well aware that metformin therapy tends to promote a modest degree of weight loss, whereas therapy with sulfonylureas or insulin tends to promote weight gain, often exacerbating the underlying problem (Meneghini et al. 2011). Of the newer diabetes therapies, GLP-1 agonists also tend to lower body weight; it is not likely to be accidental that they also activate hepatic AMPK (Meneghini et al. 2011; Ben-Shlomo et al. 2011). Metformin also promotes weight loss in the context of polycystic ovary syndrome and has also been employed with some success to prevent weight gain in patients treated with certain antipsychotic agents (Harborne et al. 2005; Nieuwenhuis-Ruifrok et al. 2009; Praharaj et al. 2011). Hence, there is no reason to suspect that weight control benefits of AMPK activation will be confined to diabetics (Desilets et al. 2008).

Increased hepatic fatty acid oxidation may be a key mediator of metformin's impact on weight control. Efficient hepatic fatty acid oxidation sends a satiety signal to the brain via the vagus nerve; this mechanism likely contributes to the satiety associated with prolonged fasting (Scharrer 1999; Leonhardt and Langhans 2004; St-Onge and Jones 2002). Moreover, it is intriguing to note that expression of a constitutively active form of AMPKα2 in mouse liver induces uncoupling protein-2 (UCP-2)—possibly owing to increased function of Foxo3a and PGC-1alpha, which drive its transcription in endothelial cells (Olmos et al. 2009; Foretz et al. 2005; Xie et al. 2008). Arguably, this effect could be exploited to transform the liver into a thermogenic organ—in which uncoupled oxidation of fatty acids yields CO_2 and heat—when used in conjunction with other strategies that boost hepatic fatty acid oxidation (McCarty 2001a, 2005).

AMPK also suppresses hepatic de novo lipogenesis, via inhibition of the transcription and posttranslational

processing of the key transcription factor for lipogenic enzymes, SREBP-1c (Zhou et al. 2001; Yap et al. 2011; Li et al. 2011). While this effect helps to explain the utility of AMPK activators for preventing obesity in rodents, it is less likely to be of importance in humans, in whom hepatic de novo lipogenesis tends to be of minor significance (Hellerstein 1999).

A recent Japanese study has found that regular vinegar ingestion can promote modest weight loss in obese subjects (Kondo et al. 2009a). This is pertinent in light of the fact—as discussed below—that vinegar (acetic acid) has the potential to activate hepatic AMPK (Kondo et al. 2009b). Vinegar also induced UCP-2 in mouse liver—an effect which was blocked by inhibition of AMPK (Kondo et al. 2009b).

The weight loss associated with metformin therapy is typically modest. This reflects the fact that metformin therapy often has a countervailing positive impact on lean mass (Rodriguez-Moctezuma et al. 2005; Lee et al. 2011; Ibanez et al. 2002). Possibly pertinent to this phenomenon is the fact that type IIa histone deacetylases interact with the key myogenic transcription factor MEF2 to inhibit its transactivational function (Lu et al. 2000). As we have noted, AMPK phosphorylates these deacetylases, promoting their export from the nucleus. Hence, AMPK positively regulates MEF2 activity, boosting the synthesis of myogenin (Fu et al. 2013). Myogenesis from resident pericytes contributes to muscle repair and growth throughout life (Dellavalle et al. 2011). Conversely, AMPK has the potential to promote autophagic degradation of muscle proteins (Sanchez et al. 2012), so the net impact of modest AMPK activation on muscle mass will reflect the balance of countervailing effects. It would be of interest to study the impact of AMPK activators on age-related sarcopenia.

Preventing and controlling cancer

A number of recent epidemiological studies have found that diabetics using metformin, as opposed to alternative therapies, are at lower risk for cancer, including specifically breast, prostate, and colorectal cancer (Evans et al. 2005; Libby et al. 2009; Currie et al. 2009; Wright and Stanford 2009; Landman et al. 2010; Bodmer et al. 2010; Bowker et al. 2010; Zhang et al. 2011b; Decensi et al. 2010). It is likely that AMPK's inhibitory impact on mTORC1 activity plays a major role in this effect. Other circumstances which likewise decrease mTORC1 activity—elevated adiponectin levels, rapamycin therapy, and plant-based diets or superior muscle insulin sensitivity (associated with low serum levels of insulin and free IGF-I)—have also been linked to lower cancer risk in epidemiological analyses (McCarty 2011). This likely is because mTORC1 works in various ways to promote cellular proliferation and inhibit apoptosis—effects which could be expected to promote the accumulation of mutations in, and aid the survival of, pre-cancerous stem cells.

mTORC1 phosphorylates 4EBP-1, thereby freeing the translation initiating factor eIF4E from inhibitory binding (Gingras et al. 2001). eIF4E functions to expedite the translation of a number of "weak" mRNAs which otherwise would be translated to a minimal extent. Some of these mRNAs are handicapped by complex hairpin structures in their 5′ UTRs; others are characterized by a specific nucleotide structure in their 3′ UTRs and require binding to eIF4E to achieve extranuclear transport. Among these weak mRNAs are some which code for proteins that promote proliferation—cyclin D1, c-myc, and ODC—and others that block apoptosis—survivin, Bcl-2, Bcl-xL, Mcl-2, and dad1 (McCarty 2011; Culjkovic et al. 2007; Mamane et al. 2004; De and Graff 2004; Graff et al. 2008). Hence, mTORC1, acting via eIF4E, upregulates the expression of a number of proteins which are conducive to cancerous transformation. Intriguingly, overexpression of eIF4E in fibroblasts and other cell lines has been shown to promote transformation in vitro (Smith et al. 1990). The weak mRNAs whose translation is expedited by mTORC1 also code for a number of proteins that render transformed cells more aggressive in their behavior and more chemoresistant—including HIF-1α, the key driver of the Warburg phenomenon in cancer; (Thomas et al. 2006; Semenza 2007; McFate et al. 2008; Faubert et al. 2013) not surprisingly, constitutive activation of mTORC1, and/or overexpression of eIF4E, is observed in a high proportion of advanced malignancies (De and Graff 2004).

Additionally, there is recent evidence that mTORC1 very rapidly upregulates the activity of Gq-coupled receptors that can promote proliferation in some cell types by somehow activating the Raf–MEK–Erk1/2 pathway (Kisfalvi et al. 2007). Hence, insulin, IGF-I, and various other growth factors have the potential to boost the mitogenic response to various hormones and cytokines that activate such receptors. Metformin, via AMPK, has been shown to antagonize this effect

(Rozengurt et al. 2010; Kisfalvi et al. 2009). Since this effect of mTORC1 is rapid in onset, modulation of protein translation is clearly not involved; the direct target of mTORC1's activity in this regard has not yet been defined.

Another AMPK target which may influence cancer risk and progression is p53. p53 activity is lost during the progression of many cancers, probably reflecting the fact that such a loss promotes genetic lability and cell proliferation, while blunting the apoptotic response to cytotoxic agents (Chari et al. 2009; Athar et al. 2011). In this regard, it is notable that AMPK can confer an activating phosphorylation to p53 at Ser15 (in humans), the same site targeted by the activating kinase ATM; (Jones et al. 2005) this can help to fulfill AMPK's energy conservation mission by slowing cell cycle progression. AMPK interacts at least additively with the DNA damage response in activation of p53 (Sanli et al. 2010). Inasmuch as downregulation of p53 activity promotes cancer progression, it is tempting to speculate that the upregulation of p53 mediated by AMPK could play a role in cancer prevention or control.

The high cancer risk of people afflicted with the genetic disorder Peutz–Jeghers syndrome can be viewed as indirect evidence that AMPK functions as a tumor suppressor. This disorder reflects heterozygosity for loss-of-function mutations of LKB1, whose best-known role is to activate AMPK (Mehenni et al. 1998). People with this syndrome are at greatly increased risk for colonic polyps and a host of cancers; by age 60, 60 % have developed cancer of some sort, and this increases to 85 % at age 70 (Hearle et al. 2006). Intriguingly, in mice that are heterozygous for loss of PTEN, a concurrent genetic deficiency in LKB1 activity markedly accelerates onset of cancer, whereas administration of metformin or other AMPK activators notably slows cancer induction in PTEN-deficient mice (Huang et al. 2008). These findings likely reflect a key role for mTORC1 activity in modulation of cancer risk, as PTEN deficiency upregulates this activity, whereas LKB1-AMPK inhibit it.

The contributions of mTORC1 signaling to cancer aggressiveness, and of subnormal p53 activity to cancer progression, suggest that AMPK activation may be a rational strategy for cancer management, and indeed, there is burgeoning interest in metformin as an adjunctive agent in cancer therapy (Jalving et al. 2010; Micic et al. 2011; Dowling et al. 2011). Much of this interest was prompted by several epidemiological studies which have concluded that diabetic cancer patients receiving metformin therapy tend to have better prognoses than those receiving alternative diabetes therapies; (Jiralerspong et al. 2009; Tan et al. 2011; Lee et al. 2012) this effect may contribute to a reduction in total cancer mortality in diabetics using metformin (Bo et al. 2012). While metformin has obvious potential for slowing cancer growth, there is also new evidence that metformin may specifically target cancer stem cells that are thought to be largely responsible for cancer chemoresistance and recurrence; metformin may change the differentiation state of these cells and/or make them more susceptible to cytotoxic drugs (Hirsch et al. 2009; Iliopoulos et al. 2011; Vazquez-Martin et al. 2011; Vazquez-Martin et al. 2010). Hence, it is hoped that metformin, employed as an adjuvant to chemotherapy regimens, may increase chances for a cure, or at least notably enhance the contribution of such regimens to survival time.

While Western oncologists are focusing on metformin as a cancer therapy adjuvant, quite a number of Chinese studies have demonstrated cancer-retardant or cancer-preventive activity for berberine, in vitro and in mice (Sun et al. 2009; Tang et al. 2009; Diogo et al. 2011). AMPK activation, rather than metformin per se, is emerging as a key strategy for prevention and control of cancer.

The modest reduction in serum insulin and free IGF-I achievable with AMPK activation can also be expected to downregulate mTORC1 activity, complementing the more direct impact of AMPK in this regard (McCarty 2004). Moreover, low insulin and free IGF-I should decrease Akt activity in many tissues; Akt works in a number of ways to suppress apoptosis and thereby raise cancer risk (Song et al. 2005).

AMPK may have a more specific role to play in the prevention of post-menopausal breast cancer. It is now appreciated that the predominant source of the estrogen that drives cancer induction post-menopausally is the aromatase activity of breast stroma (Simpson et al. 2005). AMPK activation has recently been shown to inhibit the transcription of aromatase in breast stroma. In these stromal cells, aromatase transcription is driven by a CREB/CRTC2 complex; AMPK phosphorylates the coactivator CRTC2 in a way that promotes its eviction from the nucleus, thereby preventing transcription of the aromatase gene (Brown et al. 2009; Brown et al. 2010). In this formulation, the elevated serum levels of estrogen in obese post-menopausal women are seen as a red herring; rather, the nexus between obesity, estrogen,

and elevated breast cancer risk is driven by leptin, which suppresses AMPK activity in breast stroma by downregulating LKB1 (Brown et al. 2009; Geisler et al. 2007).

Preventing neurodegenerative disorders

The ability of AMPK to promote macroautophagy suggests that AMPK activation may have potential for preventing or controlling neurodegenerative disorders characterized by intraneuronal or extracellular accumulation of toxic protein aggregates. It stands to reason that autophagy, the "house cleaning" strategy of our cells, should be of particular importance to maintaining the healthful structure and function of long-lived, difficult-to-replace cells such as neurons (Banerjee et al. 2010; Xilouri and Stefanis 2010; Puyal et al. 2011; Jimenez-Sanchez et al. 2012). Indeed, transgenic mice with severely impaired capacity for neuronal autophagy develop neurodegeneration at an early age, and dysfunctional neuronal autophagy is often observed in neurodegenerative disorders (Banerjee et al. 2010; Hara et al. 2006). On the other hand, overwhelmingly intense activation of autophagy can contribute to neuronal death when not properly balanced by biosynthesis (Li et al. 2010b). These considerations have led some researchers to suggest that AMPK-mediated upregulation of autophagy may have greater net utility in the early stages of neurodegenerative disorders, than in their advanced stages (Salminen et al. 2011b). In other words, AMPK activation is best viewed as a preventive rather than therapeutic strategy for addressing neurodegeneration.

With respect to Alzheimer's risk, AMPK activators have been shown to decrease extracellular amyloid-beta accumulation, owing to increased autophagic degradation of this protein (Vingtdeux et al. 2010; Vingtdeux et al. 2011). How AMPK influences amyloid-beta production per se has been the subject of conflicting reports (Marambaud et al. 2005; Chen et al. 2009b; Won et al. 2010), but in any case, upregulation of autophagy can suppress deposition of amyloid-beta aggregates. Indeed, in transgenic APP/PS1mice prone to amyloid deposition, resveratrol administration reduced amyloid deposition in the brain globally; this effect was significant in the cortex though not the hippocampus (Vingtdeux et al. 2010).

With respect to tau phosphorylation, a key factor in the formation of the neurofibrillatory tangles characteristic of Alzheimer's, AMPK appears to have equivocal effects. Sirt1 activity opposes such phosphorylation, so AMPK has the potential to block tau phosphorylation via Sirt1 activation (Salminen et al. 2011b; Greco et al. 2009; Min et al. 2010). On the other hand, AMPK also has the potential to phosphorylate tau directly (Thornton et al. 2011). It is not yet clear which of these mechanisms is predominant during the evolution of Alzheimer's.

The ability of AMPK to boost eNOS activity in the cerebral microvasculature may also aid Alzheimer's prevention, in light of recent evidence that NO of vascular origin acts to impede the synthesis of amyloid-beta by suppressing expression of the BACE1 protease required for its production (Austin et al. 2010). This intriguing finding may help to rationalize the numerous studies demonstrating that people with elevated cardiovascular risk factors—risk factors that would be expected to compromise vascular NO bioactivity—are at increased risk for Alzheimer's as they age (Monsuez et al. 2011). Hence, the favorable impact of AMPK activation on vascular health may tend to reduce risk for dementia, both by limiting stroke risk, and by helping to control cerebral amyloid-beta production.

Activated microglia, via production of peroxynitrite and various pro-inflammatory cytokines and prostanoids, are suspected to contribute to the pathogenesis of many neurodegenerative disorders (Brown 2007). In light of the anti-inflammatory potential of AMPK as noted above, it is reasonable to suspect that AMPK activation might be protective in this regard, and indeed, several studies demonstrate that AMPK activators can exert anti-inflammatory effects on cultured microglia. The ability of berberine-activated AMPK to suppress induction of iNOS and Cox-2 in LPS or interferon-γ-exposed microglia might at least partially reflect the fact that AMPK phosphorylates and triggers the intranuclear transport of HuR, a protein which upregulates the translation of iNOS and Cox-2 mRNAs by binding to AU-rich regions in their 3′ URLs, thereby enhancing the half-life of these mRNAs; loss of cytoplasmic HuR therefore decreases the protein expression of iNOS and Cox-2 (Lu et al. 2010; Wang et al. 2002, 2004; Di et al. 2005; Linker et al. 2005; Sengupta et al. 2003).

(Parenthetically, it should be noted that the ability of AMPK to downregulate iNOS translation suggests a role for AMPK activators in the prevention of septic shock. Indeed, berberine administration has been reported to enhance survival in a murine model of endotoxemia (Li et al. 2006). A further corollary is that

the suppressive impact of AMPK activators on Cox-2 induction may contribute to the cancer-preventive potential of these agents; the marked cancer prevention associated with daily low-dose aspirin use is likely attributable to partial inhibition of Cox-2 (Rothwell et al. 2010).)

Although risks for dementia and cognitive dysfunction are increased in diabetics, so far little epidemiology has focused on the impact of specific diabetic therapies on dementia risk. However, one recent study has found that, as compared to diabetic not receiving drug therapy, those receiving metformin were about 25 % less likely to develop dementia (Hsu et al. 2011). Dementia risk also trended slightly lower in patients receiving sulfonylureas, so it is not clear whether the apparent protection associated with metformin reflected a specific impact on the brain, or simply superior glycemic control. The impact of metformin therapy on risk for Parkinson's disease has not yet been examined.

Preserving cartilage and bone

Diabetics treated with metformin have been found to be at decidedly lower risk for fractures than those treated with thiazolidinediones (Vestergaard et al. 2005; Rejnmark 2008). In part, this reflects an adverse effect of the latter—but there is growing reason to suspect that metformin and AMPK exert a favorable effect on bone density and structure. Metformin exposure in vitro promotes osteoblastic differentiation and behavior—increasing alkaline phosphatase activity and type 1 collagen production (Jang et al. 2011; Cortizo et al. 2006; Kanazawa et al. 2008). These effects appear to be contingent on the AMPK-mediated induction of the short heterodimer partner orphan nuclear receptor cited above. Concurrently, metformin antagonizes osteoclast development by reducing osteoblast production of RANKL, while increasing production of osteoprotegerin (a decoy receptor for RANKL) (Mai et al. 2011). Suppression of RANKL expression and osteoclast development has also been seen with berberine and other AMPK activators (Hu et al. 2008; Lee et al. 2010c). Moreover, both metformin and berberine have demonstrated favorable effects on bone density and structure in ovariectomized rats and in other rodent models of bone loss (Mai et al. 2011; Gao et al. 2010; Li et al. 1999). In light of the fact that the bone protective effects of estrogen are thought to be mediated largely by induction of eNOS in osteoblasts, it is pertinent to recall that AMPK can activate this enzyme directly (Armour et al. 2001; Samuels et al. 2001).

AMPK may also aid in the preservation of cartilage in the context of osteoarthritis. Cartilage loss in osteoarthritis is believed to reflect a catabolic impact of cytokines, most notably IL-1, on chondrocytes, associated with increased production of collagenolytic metalloproteinases and downregulation of the tissue inhibitor of metalloproteinases (Pelletier et al. 1993). Oxidative stress, NF-kappaB activation, and iNOS induction are key mediators of these effects (Lo et al. 1998; Grange et al. 2006; Ahmad et al. 2011; Pelletier et al. 1998, 1999). It is reasonable to suspect that AMPK might act to oppose these effects, and indeed, a recent study has found that AMPK activators notably suppress the catabolic response of chondrocytes to IL-1 or TNF-α exposure; notably, chondrocyte production of MMP-3, MMP-13, and NO was suppressed (Terkeltaub et al. 2011). Conversely, knockout of AMPKa with small interfering RNA exacerbated the catabolic response of chondrocytes to IL-1 and TNF-α. Chinese researchers have reported a very analogous anticatabolic response when IL-1-treated chondrocytes are exposed to berberine; in addition, they report that intra-articular administration of berberine provides protection from cartilage damage in rats concurrently given intra-articular injections of IL-1 (Hu et al. 2011). So far, no epidemiological studies have addressed the impact of metformin therapy on cartilage status in diabetics.

If indeed AMPK activators can protect the healthful structure and function of the vasculature, bones, and cartilage, they may be viewed more generally as beneficial for the health of connective tissues. Such a view would be highly consistent with the thesis that AMPK activation has "anti-aging" potential.

Controlling autoimmunity

AMPK activation may have potential for controlling autoimmune disorders driven by autoreactive Th1 or Th17 lymphocytes. In the murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), metformin, berberine, and AICAR have been shown to be highly protective (Nath et al. 2009a; Qin et al. 2010; Ma et al. 2010; Nath et al. 2005). Some of this protection appears to stem from inhibition of NF-kappaB activation and costimulatory protein

expression in antigen-presenting cells, which lessens the ability of these cells to activate autoreactive Th1 and Th17 helper lymphocytes (Qin et al. 2010). Indeed, when EAE was induced in mice concurrently treated with berberine, adoptive transfer of Th17 cells from these mice to untreated mice completely failed to induce EAE in the recipients; transfer of Th1 cells induced only an attenuated syndrome (Qin et al. 2010). Importantly, berberine treatment did not influence the relative number of CD+4FoxP3+ regulatory T cells. The likelihood that AMPK plays a physiological role in controlling autoimmunity is suggested by the fact that EAE is an especially aggressive syndrome in AMPKα1-null mice (Nath et al. 2009b).

It seems unlikely that EAE will be the only autoimmune disorder in which AMPK activation is protective. Indeed, berberine and AICAR have been shown to ameliorate murine models of colitis, and metformin is protective in a mouse model of autoimmune arthritis (Nath et al. 2009a; Zhou and Mineshita 2000; Lee et al. 2010d; Bai et al. 2010a, b; Kang et al. 2013). Clinically, metformin treatment of diabetes decreases serum levels of IL-17, a trophic factor for Th17 cells (Sumarac-Dumanovic et al. 2013). Further research addressing the immunomodulatory potential of AMPK activation appears indicated.

The Akt–mTORC1 pathway is known to negatively regulate the genesis of Treg cells (Haxhinasto et al. 2008). Other factors being equal, the inhibitory impact of AMPK on mTORC1 activity might therefore be expected to upregulate Treg generation—a phenomenon that in fact has been demonstrated in a mouse asthma model (Michalek et al. 2011).

Nonetheless, skepticism regarding a clinical role for AMPK activators in the control of autoimmunity is warranted, in light of the fact that there appear to be few if any reports that metformin therapy alleviates autoimmune disorders in diabetics. However, the possibility that it exerts a subtle effect in this regard cannot be ruled out at present; investigating its impact on disorders driven primarily by TH17 cells might be fruitful.

Practical strategies for implementing AMPK activation

The hypothesis that AMPK activators may, at least in some measure, confer health benefits comparable to those seen with sustained calorie restriction, prompts an inquiry into the range of health effects that might flow from chronic AMPK activation. The data cited above suggest that such activation may indeed reduce risk for atherosclerosis, heart attack, and stroke; reduce risk for ventricular hypertrophy while aiding control of congestive failure; ameliorate the severity of metabolic syndrome, reduce diabetes risk, and improve diabetic control; help to prevent weight gain; decrease risk for a number of types of cancer and serve as a worthwhile adjunct to cancer chemotherapy; reduce risk for dementia and possibly other neurodegenerative disorders; help to preserve the proper structure and function of bone and cartilage; and possibly help to prevent or control certain autoimmune disorders. The scope of these potential benefits is so vast that it does indeed lend credence to the notion that AMPK activation has "anti-aging" activity that may promote a notable augmentation of healthspan. Hence, it is of some importance to define practical measures which can upregulate AMPK activation in humans.

Metformin is the world's most widely used diabetic drug, typically administered in a dose of 500–850 mg twice daily. About a third of patients who use it experience some GI upset, which tends to become less significant over time; risk of GI side effects appears to be minimized if patients are started on a low dose that gradually is escalated. Otherwise, metformin is safe and well tolerated; the risk for lactic acidosis that prompted the banning of the related biguanide drug phenformin appears to be almost nonexistent with prescribed doses of metformin (Salpeter et al. 2010). For nondiabetics who seek to employ AMPK activation as a long-term health promotion strategy, metformin has the disadvantage that it is available only by prescription.

Berberine, on the other hand, is a natural compound, found in a number of medicinal herbs, that is currently available in its pure form as a nutraceutical in the USA. It is widely used in China for diabetes management. Typical dose regimens are 500 mg two to three times daily, or 300 mg three times daily; these are reported to achieve an improvement in glycemic control roughly comparable to that seen with metformin—accompanied by a greater reduction in LDL cholesterol (Zhang et al. 2008a; Gu et al. 2010). A clinical study in which diabetics received 500 mg twice daily noted that a small minority of patients experienced significant constipation; otherwise, berberine was well tolerated. In a study employing 500 mg three times daily, GI side effects were more common and sometimes necessitated dose reduction (albeit many of the affected patients were also

taking metformin and/or acarbose). As in the case of metformin, GI symptoms tend to diminish over time. Berberine is not efficiently absorbed, and innovations in delivery—microemulsification or cyclodextrin inclusion complexation—might enable lower doses to provide worthwhile benefit (Gui et al. 2008; Battu et al. 2010). Berberine may have considerable potential as a "life extension" nutraceutical.

Resveratrol, a polyphenol found in red wine and many other foods, has recently been widely promoted in the USA as an aging-retardant nutraceutical, owing to reports that it activates Sirt1 in cell cultures and improves survival in obese mice. Unfortunately, in humans, it is conjugated rapidly and completely following absorption; despite reasonably efficient absorption, orally administered resveratrol does not appear to achieve sustained serum levels of free resveratrol sufficient to inhibit mitochondrial ATP synthase and thereby activate AMPK (Walle et al. 2004; Boocock et al. 2007). Consistent with this, several recent controlled clinical studies have failed to observe an impact of oral resveratrol on insulin sensitivity, blood pressure, body composition, or other clinical parameters; in addition, AMPK activity in skeletal muscle or adipocytes was not influenced (Yoshino et al. 2012; Poulsen et al. 2013). However, oral resveratrol might have the potential to transiently activate AMPK in the intestinal mucosa. Resveratrol, as well as the AMPK activators metformin, berberine, AICAR, and acetic acid (see below), have been shown to boost intestinal production of the incretin hormone glucagon-like peptide-1 (GLP-1), which in turn stimulates hepatic AMPK activity; (Ben-Shlomo et al. 2011; Dao et al. 2011; Mulherin et al. 2011; Yu et al. 2010; Freeland and Wolever 2010) moreover, GLP-1 may exert protective effects on other tissues as well (Huisamen et al. 2008; Hattori et al. 2010; Villanueva-Penacarrillo et al. 2011). Perhaps this rationalizes the modestly favorable results of resveratrol supplementation (150 mg daily) reported in a recent clinical study (Timmers et al. 2011).

As noted, salicylate can bind to AMPK and activate it allosterically in a way that is complementary to the impact of AMP (Hawley et al. 2012). However, this effect is modest—the submillimolar concentrations of free salicylate that can be achieved with feasible clinical regimens of this drug (bearing in mind that 80–90 % of plasma salicylate is protein bound) appear capable of increasing enzyme activity by about 10–15 %. This may explain why the diabetic glycemic control achieved with salsalate therapy tends to be less impressive than that seen with metformin or berberine—and much of the glycemic reduction it does achieve may be mediated by downregulation of IkappaB kinase activation (Goldfine et al. 2010; Faghihimani et al. 2013; Yin et al. 1998). Nonetheless, employing salsalate in conjunction with either metformin or berberine may be an attractive option for managing diabetes, vascular disorders, or cancer; (McCarty 2010; Pierce et al. 2009; Lesniewski et al. 2011; McCarty and Block 2006) these agents may complement each other with respect to AMPK activation, NF-kappaB inhibition, and control of oxidative stress.

Remarkably, vinegar—dilute acetic acid—can also activate AMPK in some tissues. This likely reflects the fact that the initial step of acetate metabolism, in which acetate is phosphorylated, generates AMP in the process. Vinegar-mediated activation of AMPK has indeed been demonstrated in the liver of vinegar-fed rats, and in human endothelial cells in vitro (Kondo et al. 2009b; Sakakibara et al. 2006, 2010; Li et al. 2013). This effect will presumably be more transitory than that of AMPK-activating drugs, as acetate is rapidly oxidized following ingestion. Nonetheless, oral vinegar administration has exerted some intriguing effects, both in rodents and in clinical studies, that arguably are attributable to AMPK activation. In light of the favorable impact of metformin on weight control, it is interesting to note that vinegar administration suppresses weight gain in rats fed a high-fat diet and aids glycemic control in diabetic mice; (Kondo et al. 2009b; Sakakibara et al. 2006) moreover, in a placebo-controlled clinical trial, obese subjects ingesting 15–30 ml vinegar daily achieved modest but statistically significant weight loss compared to those receiving placebo vinegar (Kondo et al. 2009a). Moreover, in post-menopausal women, vinegar administration boosts flow-mediated vasodilation, an effect likely attributable to AMPK-mediated phosphorylation of eNOS (Sakakibara et al. 2010). Regular vinegar use may modestly improve glycemic control in human diabetics, and inclusion of vinegar in meals acutely lowers the postprandial glucose response, apparently by slowing the absorption of starch or polysaccharides (Johnston et al. 2009; Ostman et al. 2005; Johnston et al. 2010). These observations are intriguing in light of the common folkloric belief that apple cider vinegar can confer wide-ranging health protective benefits. And it is reasonable to speculate that some of the protective health benefits associated with moderate regular ingestion of alcohol may in fact be mediated by the acetic acid evolved by ethanol metabolism—and hence by AMPK

(McCarty 2001b). (Unfortunately, these are often counterbalanced by adverse effects of acetaldehyde and ethanol per se.) Moreover, other short-chain fatty acids generate AMP when metabolized and, hence, can activate AMPK (Peng et al. 2009). It recently has been credibly proposed that AMPK activation mediated by the short-chain fatty acids stemming from colonic metabolism of dietary fiber may be largely responsible for the favorable impact of high-fiber diets on control of metabolic syndrome; (Hu et al. 2010) conceivably, this phenomenon might also impact risk for colorectal cancer (Tang et al. 2011). Regular use of vinegar, fiber-rich diets, and moderate alcohol consumption may represent wholly nutritional strategies for evoking, to a modest degree, some of the health protection afforded by AMPK activation.

In assessing the research literature on clinical AMPK activators such as metformin and berberine, it is important to bear in mind the plasma concentrations of these agents which are clinically achievable. The plasma concentration of metformin that appears to be safe and effective is about 0.5–2.0 mg/l, or 4–16 μM (Scheen 1996). The therapeutic plasma concentration of berberine appears to be a bit under 1 μM (Zeng and Zeng 1999). Cell culture studies which employ concentrations in substantial excess of these values—as many if not most do—may or may not be clinically pertinent, as high concentrations could overdrive AMPK activation and perhaps influence additional targets. (Note however that in short-term studies, supraphysiological concentrations of metformin may be appropriate, as cellular uptake of metformin can be slow (Hawley et al. 2010).) And one must bear in mind that, even in clinical concentrations, metformin and berberine might have targets of action in addition to AMPK. For example, activation of atypical PKCs is suspected to contribute to metformin's impact on glucose uptake in skeletal muscle (Turban et al. 2012). Clinical doses of berberine modulate cytochrome p450 activities (Guo et al. 2012), and the fact that berberine suppresses LDL cholesterol more effectively than metformin has yet to be satisfactorily explained.

References

Abbasi F, Kamath V, Rizvi AA, Carantoni M, Chen YD, Reaven GM (1997) Results of a placebo-controlled study of the metabolic effects of the addition of metformin to sulfonylurea-treated patients. Evidence for a central role of adipose tissue. Diabetes Care 20(12):1863–1869

Abbasi F, Carantoni M, Chen YD, Reaven GM (1998) Further evidence for a central role of adipose tissue in the antihyperglycemic effect of metformin. Diabetes Care 21(8):1301–1305

Abidi P, Zhou Y, Jiang JD, Liu J (2005) Extracellular signal-regulated kinase-dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. Arterioscler Thromb Vasc Biol 25(10):2170–2176

Ahmad R, Sylvester J, Ahmad M, Zafarullah M (2011) Involvement of H-Ras and reactive oxygen species in proinflammatory cytokine-induced matrix metalloproteinase-13 expression in human articular chondrocytes. Arch Biochem Biophys 507(2):350–355

Alba G, El Bekay R, varez-Maqueda M et al (2004) Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils. FEBS Lett 573(1–3):219–225

Anisimov VN (2010) Metformin for aging and cancer prevention. Aging (Albany NY) 2(11):760–774

Anisimov VN, Berstein LM, Popovich IG et al (2011) If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY) 3(2):148–157

Arad M, Moskowitz IP, Patel VV et al (2003) Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff–Parkinson–White syndrome in glycogen storage cardiomyopathy. Circulation 107(22):2850–2856

Armour KE, Armour KJ, Gallagher ME et al (2001) Defective bone formation and anabolic response to exogenous estrogen in mice with targeted disruption of endothelial nitric oxide synthase. Endocrinology 142(2):760–766

Athar M, Elmets CA, Kopelovich L (2011) Pharmacological activation of p53 in cancer cells. Curr Pharm Des 17(6):631–639

Attane C, Foussal C, Le GS et al (2012) Apelin treatment increases complete fatty acid oxidation, mitochondrial oxidative capacity, and biogenesis in muscle of insulin-resistant mice. Diabetes 61(2):310–320

Austin SA, Santhanam AV, Katusic ZS (2010) Endothelial nitric oxide modulates expression and processing of amyloid precursor protein. Circ Res 107(12):1498–1502

Bai A, Yong M, Ma AG et al (2010a) Novel anti-inflammatory action of 5-aminoimidazole-4-carboxamide ribonucleoside with protective effect in dextran sulfate sodium-induced acute and chronic colitis. J Pharmacol Exp Ther 333(3):717–725

Bai A, Ma AG, Yong M et al (2010b) AMPK agonist downregulates innate and adaptive immune responses in TNBS-induced murine acute and relapsing colitis. Biochem Pharmacol 80(11):1708–1717

Banerjee R, Beal MF, Thomas B (2010) Autophagy in neurodegenerative disorders: pathogenic roles and therapeutic implications. Trends Neurosci 33(12):541–549

Bartke A (2005) Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging. Endocrinology 146(9):3718–3723

Battu SK, Repka MA, Maddineni S, Chittiboyina AG, Avery MA, Majumdar S (2010) Physicochemical characterization of berberine chloride: a perspective in the development of a solution dosage form for oral delivery. AAPS PharmSciTech 11(3):1466–1475

Baur JA, Pearson KJ, Price NL et al (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444(7117):337–342

Beauloye C, Bertrand L, Horman S, Hue L (2011) AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure. Cardiovasc Res 90(2):224–233

Benes J, Kazdova L, Drahota Z et al (2011) Effect of metformin therapy on cardiac function and survival in a volume-overload model of heart failure in rats. Clin Sci (Lond) 121(1):29–41

Ben-Shlomo S, Zvibel I, Shnell M et al (2011) Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. J Hepatol 54(6):1214–1223

Bergamini E, Cavallini G, Cecchi L et al (1998) A proposed mechanism of the antiaging action of diet restriction. Aging (Milano) 10(2):174–175

Bess E, Fisslthaler B, Fromel T, Fleming I (2011) Nitric oxide-induced activation of the AMP-activated protein kinase alpha2 subunit attenuates IkappaB kinase activity and inflammatory responses in endothelial cells. PLoS ONE 6(6):e20848

Blagosklonny MV (2010) Calorie restriction: decelerating mTOR-driven aging from cells to organisms (including humans). Cell Cycle 9(4):683–688

Bo S, Ciccone G, Rosato R et al (2012) Cancer mortality reduction and metformin. A retrospective cohort study in type 2 diabetic patients. Diabetes Obes Metab 14(1):23–29

Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR, Meier CR (2010) Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care 33(6):1304–1308

Boocock DJ, Faust GE, Patel KR et al (2007) Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. Cancer Epidemiol Biomarkers Prev 16(6):1246–1252

Bowker SL, Yasui Y, Veugelers P, Johnson JA (2010) Glucose-lowering agents and cancer mortality rates in type 2 diabetes: assessing effects of time-varying exposure. Diabetologia 53(8):1631–1637

Brown GC (2007) Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. Biochem Soc Trans 35(Pt 5):1119–1121

Brown KA, McInnes KJ, Hunger NI, Oakhill JS, Steinberg GR, Simpson ER (2009) Subcellular localization of cyclic AMP-responsive element binding protein-regulated transcription co-activator 2 provides a link between obesity and breast cancer in postmenopausal women. Cancer Res 69(13):5392–5399

Brown KA, Hunger NI, Docanto M, Simpson ER (2010) Metformin inhibits aromatase expression in human breast adipose stromal cells via stimulation of AMP-activated protein kinase. Breast Cancer Res Treat 123(2):591–596

Bulcao C, Ribeiro-Filho FF, Sanudo A, Roberta Ferreira SG (2007) Effects of simvastatin and metformin on inflammation and insulin resistance in individuals with mild metabolic syndrome. Am J Cardiovasc Drugs 7(3):219–224

Canto C, Auwerx J (2011) Calorie Restriction: is AMPK a key sensor and effector? Physiology (Bethesda) 26(4):214–224

Canto C, Gerhart-Hines Z, Feige JN et al (2009) AMPK regulates energy expenditure by modulating NAD+metabolism and SIRT1 activity. Nature 458(7241):1056–1060

Canto C, Jiang LQ, Deshmukh AS et al (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 11(3):213–219

Carling D, Mayer FV, Sanders MJ, Gamblin SJ (2011) AMP-activated protein kinase: nature's energy sensor. Nat Chem Biol 7(8):512–518

Cartee GD, Wojtaszewski JF (2007) Role of Akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. Appl Physiol Nutr Metab 32(3):557–566

Ceolotto G, Gallo A, Papparella I et al (2007) Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. Arterioscler Thromb Vasc Biol 27(12):2627–2633

Chari NS, Pinaire NL, Thorpe L, Medeiros LJ, Routbort MJ, McDonnell TJ (2009) The p53 tumor suppressor network in cancer and the therapeutic modulation of cell death. Apoptosis 14(4):336–347

Chen ZP, Mitchelhill KI, Michell BJ et al (1999) AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett 443(3):285–289

Chen Z, Peng IC, Sun W et al (2009a) AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. Circ Res 104(4):496–505

Chen Y, Zhou K, Wang R et al (2009b) Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. Proc Natl Acad Sci U S A 106(10):3907–3912

Ching YP, Davies SP, Hardie DG (1996) Analysis of the specificity of the AMP-activated protein kinase by site-directed mutagenesis of bacterially expressed 3-hydroxy 3-methylglutaryl-CoA reductase, using a single primer variant of the unique-site-elimination method. Eur J Biochem 237(3):800–808

Chong ZZ, Shang YC, Zhang L, Wang S, Maiese K (2010) Mammalian target of rapamycin: hitting the bull's-eye for neurological disorders. Oxidative Med Cell Longev 3(6): 374–391

Cicero AF, Rovati LC, Setnikar I (2007) Eulipidemic effects of berberine administered alone or in combination with other natural cholesterol-lowering agents. A single-blind clinical investigation. Arzneimittelforschung 57(1):26–30

Colombo SL, Moncada S (2009) AMPKalpha1 regulates the antioxidant status of vascular endothelial cells. Biochem J 421(2):163–169

Cooke JP (2004) The pivotal role of nitric oxide for vascular health. Can J Cardiol 20(Suppl B):7B–15B

Cool B, Zinker B, Chiou W et al (2006) Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab 3(6):403–416

Cortizo AM, Sedlinsky C, McCarthy AD, Blanco A, Schurman L (2006) Osteogenic actions of the anti-diabetic drug metformin on osteoblasts in culture. Eur J Pharmacol 536(1–2):38–46

Cuervo AM (2008) Calorie restriction and aging: the ultimate "cleansing diet". J Gerontol A Biol Sci Med Sci 63(6):547–549

Culjkovic B, Topisirovic I, Borden KL (2007) Controlling gene expression through RNA regulons: the role of the eukaryotic translation initiation factor eIF4E. Cell Cycle 6(1):65–69

Currie CJ, Poole CD, Gale EA (2009) The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52(9):1766–1777

Dagher Z, Ruderman N, Tornheim K, Ido Y (2001) Acute regulation of fatty acid oxidation and amp-activated protein kinase in human umbilical vein endothelial cells. Circ Res 88(12): 1276–1282

Dao TM, Waget A, Klopp P et al (2011) Resveratrol increases glucose induced GLP-1 secretion in mice: a mechanism which contributes to the glycemic control. PLoS ONE 6(6):e20700

De Leo V, La Marca A, Orvieto R, Morgante G (2000) Effect of metformin on insulin-like growth factor (IGF) I and IGF-binding protein I in polycystic ovary syndrome. J Clin Endocrinol Metab 85(4):1598–1600

De BA, Graff JR (2004) eIF-4E expression and its role in malignancies and metastases. Oncogene 23(18):3189–3199

Decensi A, Puntoni M, Goodwin P et al (2010) Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 3(11):1451–1461

Dellavalle A, Maroli G, Covarello D et al (2011) Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. Nat Commun 2:499

Deng G, Long Y, Yu YR, Li MR (2010) Adiponectin directly improves endothelial dysfunction in obese rats through the AMPK-eNOS Pathway. Int J Obes (Lond) 34(1):165–171

Desilets AR, Dhakal-Karki S, Dunican KC (2008) Role of metformin for weight management in patients without type 2 diabetes. Ann Pharmacother 42(6):817–826

Dhahbi JM, Mote PL, Fahy GM, Spindler SR (2005) Identification of potential caloric restriction mimetics by microarray profiling. Physiol Genomics 23(3):343–350

Di MS, Mazroui R, Dallaire P et al (2005) NF-kappa B-mediated MyoD decay during muscle wasting requires nitric oxide synthase mRNA stabilization, HuR protein, and nitric oxide release. Mol Cell Biol 25(15):6533–6545

Diogo CV, Machado NG, Barbosa IA, Serafim TL, Burgeiro A, Oliveira PJ (2011) Berberine as a promising safe anti-cancer agent—is there a role for mitochondria? Curr Drug Targets 12(6):850–859

Dolinsky VW, Morton JS, Oka T et al (2010) Calorie restriction prevents hypertension and cardiac hypertrophy in the spontaneously hypertensive rat. Hypertension 56(3):412–421

Dowling RJ, Goodwin PJ, Stambolic V (2011) Understanding the benefit of metformin use in cancer treatment. BMC Med 9:33

Drew BG, Fidge NH, Gallon-Beaumier G, Kemp BE, Kingwell BA (2004) High-density lipoprotein and apolipoprotein AI increase endothelial NO synthase activity by protein association and multisite phosphorylation. Proc Natl Acad Sci U S A 101(18):6999–7004

Egan D, Kim J, Shaw RJ, Guan KL (2011) The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. Autophagy 7(6):643–644

El-Assaad W, Buteau J, Peyot ML et al (2003) Saturated fatty acids synergize with elevated glucose to cause pancreatic beta-cell death. Endocrinology 144(9):4154–4163

Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD (2005) Metformin and reduced risk of cancer in diabetic patients. BMJ 330(7503):1304–1305

Faghihimani E, Aminorroaya A, Rezvanian H, Adibi P, Ismail-Beigi F, Amini M (2013) Salsalate improves glycemic control in patients with newly diagnosed type 2 diabetes. Acta Diabetol 50(4):537–543

Faubert B, Boily G, Izreig S et al (2013) AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. Cell Metab 17(1):113–124

Fidan E, Onder EH, Yilmaz M et al (2011) The effects of rosiglitazone and metformin on inflammation and endothelial dysfunction in patients with type 2 diabetes mellitus. Acta Diabetol 48(4):297–302

Foretz M, Ancellin N, Andreelli F et al (2005) Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. Diabetes 54(5):1331–1339

Freeland KR, Wolever TM (2010) Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-alpha. Br J Nutr 103(3):460–466

Fu YN, Xiao H, Ma XW, Jiang SY, Xu M, Zhang YY (2011) Metformin attenuates pressure overload-induced cardiac hypertrophy via AMPK activation. Acta Pharmacol Sin 32(7):879–887

Fu X, Zhao JX, Liang J, Zhu MJ, Viollet B, Du M (2013) AMP-activated protein kinase mediates myogenin expression and myogenesis via histone deacetylase 5. Am J Physiol Cell Physiol 305(8):C887–C895

Fulco M, Cen Y, Zhao P et al (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell 14(5):661–673

Gao Y, Li Y, Xue J, Jia Y, Hu J (2010) Effect of the anti-diabetic drug metformin on bone mass in ovariectomized rats. Eur J Pharmacol 635(1–3):231–236

Gao N, Zhao TY, Li XJ (2011) The protective effect of berberine on beta-cell lipoapoptosis. J Endocrinol Invest 34(2):124–130

Garton AJ, Yeaman SJ (1990) Identification and role of the basal phosphorylation site on hormone-sensitive lipase. Eur J Biochem 191(1):245–250

Geisler J, Haynes B, Ekse D, Dowsett M, Lonning PE (2007) Total body aromatization in postmenopausal breast cancer patients is strongly correlated to plasma leptin levels. J Steroid Biochem Mol Biol 104(1–2):27–34

Giannarelli R, Aragona M, Coppelli A, Del PS (2003) Reducing insulin resistance with metformin: the evidence today. Diabetes Metab 29(4 Pt 2):6S28–6S35

Gingras AC, Raught B, Gygi SP et al (2001) Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes Dev 15(21):2852–2864

Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE (2010) The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152(6):346–357

Gollob MH, Green MS, Tang AS et al (2001) Identification of a gene responsible for familial Wolff–Parkinson–White syndrome. N Engl J Med 344(24):1823–1831

Graff JR, Konicek BW, Carter JH, Marcusson EG (2008) Targeting the eukaryotic translation initiation factor 4E for cancer therapy. Cancer Res 68(3):631–634

Grange L, Nguyen MV, Lardy B et al (2006) NAD(P)H oxidase activity of Nox4 in chondrocytes is both inducible and involved in collagenase expression. Antioxid Redox Signal 8(9–10):1485–1496

Greco SJ, Sarkar S, Johnston JM, Tezapsidis N (2009) Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. Biochem Biophys Res Commun 380(1):98–104

Greer EL, Oskoui PR, Banko MR et al (2007) The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. J Biol Chem 282(41):30107–30119

Gu Y, Zhang Y, Shi X et al (2010) Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. Talanta 81(3):766–772

Guan S, Wang B, Li W, Guan J, Fang X (2010) Effects of berberine on expression of LOX-1 and SR-BI in human macrophage-derived foam cells induced by ox-LDL. Am J Chin Med 38(6):1161–1169

Gui SY, Wu L, Peng DY, Liu QY, Yin BP, Shen JZ (2008) Preparation and evaluation of a microemulsion for oral delivery of berberine. Pharmazie 63(7):516–519

Guo Y, Chen Y, Tan ZR, Klaassen CD, Zhou HH (2012) Repeated administration of berberine inhibits cytochromes P450 in humans. Eur J Clin Pharmacol 68(2):213–217

Habegger KM, Hoffman NJ, Ridenour CM, Brozinick JT, Elmendorf JS (2012) AMPK enhances insulin-stimulated GLUT4 regulation via lowering membrane cholesterol. Endocrinology 153(5):2130–2141

Hara T, Nakamura K, Matsui M et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441(7095):885–889

Harborne LR, Sattar N, Norman JE, Fleming R (2005) Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. J Clin Endocrinol Metab 90(8):4593–4598

Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8(10):774–785

Hardie DG (2011) Energy sensing by the AMP-activated protein kinase and its effects on muscle metabolism. Proc Nutr Soc 70(1):92–99

Hariharan N, Maejima Y, Nakae J, Paik J, DePinho RA, Sadoshima J (2010) Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. Circ Res 107(12):1470–1482

Harrison DE, Strong R, Sharp ZD et al (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460(7253):392–395

Hattori Y, Jojima T, Tomizawa A et al (2010) A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells. Diabetologia 53(10):2256–2263

Hawley SA, Ross FA, Chevtzoff C et al (2010) Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab 11(6):554–565

Hawley SA, Fullerton MD, Ross FA et al (2012) The ancient drug salicylate directly activates AMP-activated protein kinase. Science 336(6083):918–922

Haxhinasto S, Mathis D, Benoist C (2008) The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J Exp Med 205(3):565–574

He L, Sabet A, Djedjos S et al (2009) Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell 137(4):635–646

Hearle N, Schumacher V, Menko FH et al (2006) Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 12(10):3209–3215

Hellerstein MK (1999) De novo lipogenesis in humans: metabolic and regulatory aspects. Eur J Clin Nutr 53(Suppl 1): S53–S65

Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K (2009) Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer Res 69(19):7507–7511

Holland WL, Miller RA, Wang ZV et al (2011) Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med 17(1):55–63

Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 359(15):1577–1589

Hong Y, Hui SC, Chan TY, Hou JY (2002) Effect of berberine on regression of pressure-overload induced cardiac hypertrophy in rats. Am J Chin Med 30(4):589–599

Hong Y, Hui SS, Chan BT, Hou J (2003) Effect of berberine on catecholamine levels in rats with experimental cardiac hypertrophy. Life Sci 72(22):2499–2507

Hsu CC, Wahlqvist ML, Lee MS, Tsai HN (2011) Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. J Alzheimers Dis 24(3):485–493

Hu JP, Nishishita K, Sakai E et al (2008) Berberine inhibits RANKL-induced osteoclast formation and survival through suppressing the NF-kappaB and Akt pathways. Eur J Pharmacol 580(1–2):70–79

Hu GX, Chen GR, Xu H, Ge RS, Lin J (2010) Activation of the AMP activated protein kinase by short-chain fatty acids is the main mechanism underlying the beneficial effect of a high fiber diet on the metabolic syndrome. Med Hypotheses 74(1):123–126

Hu PF, Chen WP, Tang JL, Bao JP, Wu LD (2011) Protective effects of berberine in an experimental rat osteoarthritis model. Phytother Res 25(6):878–885

Huang X, Wullschleger S, Shpiro N et al (2008) Important role of the LKB1–AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. Biochem J 412(2):211–221

Huisamen B, Genade S, Lochner A (2008) Signalling pathways activated by glucagon-like peptide-1 (7–36) amide in the rat heart and their role in protection against ischaemia. Cardiovasc J Afr 19(2):77–83

Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, De ZF (2002) Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. J Clin Endocrinol Metab 87(12): 5702–5705

Iliopoulos D, Hirsch HA, Struhl K (2011) Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res 71(9):3196–3201

Ishii N, Matsumura T, Kinoshita H et al (2009) Activation of AMP-activated protein kinase suppresses oxidized low-density lipoprotein-induced macrophage proliferation. J Biol Chem 284(50):34561–34569

Iwabu M, Yamauchi T, Okada-Iwabu M et al (2010) Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature 464(7293):1313–1319

Jager S, Handschin C, St-Pierre J, Spiegelman BM (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104(29):12017–12022

Jakubowicz DJ, Seppala M, Jakubowicz S et al (2001) Insulin reduction with metformin increases luteal phase serum glycodelin and insulin-like growth factor-binding protein 1 concentrations and enhances uterine vascularity and blood

flow in the polycystic ovary syndrome. J Clin Endocrinol Metab 86(3):1126–1133
Jalving M, Gietema JA, Lefrandt JD et al (2010) Metformin: taking away the candy for cancer? Eur J Cancer 46(13): 2369–2380
Jang WG, Kim EJ, Bae IH et al (2011) Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. Bone 48(4):885–893
Jimenez-Sanchez M, Thompson F, Zavodsky E, Rubinsztein DC (2012) Autophagy and polyglutamine diseases. Prog Neurobiol 97(2):67–82
Jiralerspong S, Palla SL, Giordano SH et al (2009) Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 27(20): 3297–3302
Johnson JB, Laub DR, John S (2006) The effect on health of alternate day calorie restriction: eating less and more than needed on alternate days prolongs life. Med Hypotheses 67(2):209–211
Johnson JB, Summer W, Cutler RG et al (2007) Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radic Biol Med 42(5): 665–674
Johnston CS, White AM, Kent SM (2009) Preliminary evidence that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes mellitus. Diabetes Res Clin Pract 84(2):e15–e17
Johnston CS, Steplewska I, Long CA, Harris LN, Ryals RH (2010) Examination of the antiglycemic properties of vinegar in healthy adults. Ann Nutr Metab 56(1):74–79
Jones RG, Plas DR, Kubek S et al (2005) AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 18(3):283–293
Julius U (2003) Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Exp Clin Endocrinol Diabetes 111(5):246–250
Kanazawa I, Yamaguchi T, Yano S, Yamauchi M, Sugimoto T (2008) Metformin enhances the differentiation and mineralization of osteoblastic MC3T3-E1 cells via AMP kinase activation as well as eNOS and BMP-2 expression. Biochem Biophys Res Commun 375(3):414–419
Kang KY, Kim YK, Yi H et al (2013) Metformin downregulates Th17 cells differentiation and attenuates murine autoimmune arthritis. Int Immunopharmacol 16(1):85–92
Kim YD, Park KG, Lee YS et al (2008) Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes 57(2):306–314
Kimura T, Tomura H, Sato K et al (2010) Mechanism and role of high density lipoprotein-induced activation of AMP-activated protein kinase in endothelial cells. J Biol Chem 285(7):4387–4397
Kirpichnikov D, McFarlane SI, Sowers JR (2002) Metformin: an update. Ann Intern Med 137(1):25–33
Kisfalvi K, Rey O, Young SH, Sinnett-Smith J, Rozengurt E (2007) Insulin potentiates Ca2+ signaling and phosphatidylinositol 4,5-bisphosphate hydrolysis induced by Gq protein-coupled receptor agonists through an mTOR-dependent pathway. Endocrinology 148(7): 3246–3257
Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E (2009) Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. Cancer Res 69(16):6539–6545
Knowler WC, Barrett-Connor E, Fowler SE et al (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346(6):393–403
Kondo T, Kishi M, Fushimi T, Ugajin S, Kaga T (2009a) Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. Biosci Biotechnol Biochem 73(8):1837–1843
Kondo T, Kishi M, Fushimi T, Kaga T (2009b) Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. J Agric Food Chem 57(13):5982–5986
Kong W, Wei J, Abidi P et al (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 10(12):1344–1351
Kong WJ, Wei J, Zuo ZY et al (2008) Combination of simvastatin with berberine improves the lipid-lowering efficacy. Metabolism 57(8):1029–1037
Koo SH, Flechner L, Qi L et al (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437(7062):1109–1111
Kukidome D, Nishikawa T, Sonoda K et al (2006) 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 55(1):120–127
Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ (2010) Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. Diabetes Care 33(2):322–326
Lee WJ, Song KH, Koh EH et al (2005) Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. Biochem Biophys Res Commun 332(3):885–891
Lee YS, Kim WS, Kim KH et al (2006) Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 55(8):2256–2264
Lee TS, Pan CC, Peng CC et al (2010a) Anti-atherogenic effect of berberine on LXRalpha-ABCA1-dependent cholesterol efflux in macrophages. J Cell Biochem 111(1):104–110
Lee JM, Seo WY, Song KH et al (2010b) AMPK-dependent repression of hepatic gluconeogenesis via disruption of CREB.CRTC2 complex by orphan nuclear receptor small heterodimer partner. J Biol Chem 285(42):32182–32191
Lee YS, Kim YS, Lee SY et al (2010c) AMP kinase acts as a negative regulator of RANKL in the differentiation of osteoclasts. Bone 47(5):926–937
Lee IA, Hyun YJ, Kim DH (2010d) Berberine ameliorates TNBS-induced colitis by inhibiting lipid peroxidation, enterobacterial growth and NF-kappaB activation. Eur J Pharmacol 648(1–3):162–170
Lee CG, Boyko EJ, Barrett-Connor E et al (2011) Insulin sensitizers may attenuate lean mass loss in older men with diabetes. Diabetes Care 34(11):2381–2386
Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH (2012) The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. Int J Cancer 131(3):751–759

Leonhardt M, Langhans W (2004) Fatty acid oxidation and control of food intake. Physiol Behav 83(4):645–651

Lesniewski LA, Durrant JR, Connell ML, Folian BJ, Donato AJ, Seals DR (2011) Salicylate treatment improves age-associated vascular endothelial dysfunction: potential role of nuclear factor kappaB and forkhead Box O phosphorylation. J Gerontol A Biol Sci Med Sci 66(4):409–418

Li H, Miyahara T, Tezuka Y et al (1999) The effect of kampo formulae on bone resorption in vitro and in vivo. II. Detailed study of berberine. Biol Pharm Bull 22(4):391–396

Li F, Wang HD, Lu DX et al (2006) Neutral sulfate berberine modulates cytokine secretion and increases survival in endotoxemic mice. Acta Pharmacol Sin 27(9):1199–1205

Li HL, Yin R, Chen D et al (2007) Long-term activation of adenosine monophosphate-activated protein kinase attenuates pressure-overload-induced cardiac hypertrophy. J Cell Biochem 100(5):1086–1099

Li K, Yao W, Zheng X, Liao K (2009) Berberine promotes the development of atherosclerosis and foam cell formation by inducing scavenger receptor A expression in macrophage. Cell Res 19(8):1006–1017

Li D, Wang D, Wang Y, Ling W, Feng X, Xia M (2010a) Adenosine monophosphate-activated protein kinase induces cholesterol efflux from macrophage-derived foam cells and alleviates atherosclerosis in apolipoprotein E-deficient mice. J Biol Chem 285(43):33499–33509

Li J, Benashski SE, Venna VR, McCullough LD (2010b) Effects of metformin in experimental stroke. Stroke 41(11): 2645–2652

Li Y, Xu S, Mihaylova MM et al (2011) AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab 13(4):376–388

Li X, Chen H, Guan Y et al (2013) Acetic acid activates the AMP-activated protein kinase signaling pathway to regulate lipid metabolism in bovine hepatocytes. PLoS ONE 8(7):e67880

Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM (2009) New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 32(9):1620–1625

Linker K, Pautz A, Fechir M, Hubrich T, Greeve J, Kleinert H (2005) Involvement of KSRP in the post-transcriptional regulation of human iNOS expression-complex interplay of KSRP with TTP and HuR. Nucleic Acids Res 33(15): 4813–4827

Liu LZ, Cheung SC, Lan LL et al (2010) Berberine modulates insulin signaling transduction in insulin-resistant cells. Mol Cell Endocrinol 317(1–2):148–153

Lo YY, Conquer JA, Grinstein S, Cruz TF (1998) Interleukin-1 beta induction of c-fos and collagenase expression in articular chondrocytes: involvement of reactive oxygen species. J Cell Biochem 69(1):19–29

Lu J, McKinsey TA, Zhang CL, Olson EN (2000) Regulation of skeletal myogenesis by association of the MEF2 transcription factor with class II histone deacetylases. Mol Cell 6(2): 233–244

Lu DY, Tang CH, Chen YH, Wei IH (2010) Berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia. J Cell Biochem 110(3):697–705

Luque-Ramirez M, Escobar-Morreale HF (2010) Treatment of polycystic ovary syndrome (PCOS) with metformin ameliorates insulin resistance in parallel with the decrease of serum interleukin-6 concentrations. Horm Metab Res 42(11): 815–820

Ma X, Jiang Y, Wu A et al (2010) Berberine attenuates experimental autoimmune encephalomyelitis in C57 BL/6 mice. PLoS ONE 5(10):e13489

Mai QG, Zhang ZM, Xu S et al (2011) Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. J Cell Biochem 112(10): 2902–2909

Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW, Sonenberg N (2004) eIF4E—from translation to transformation. Oncogene 23(18):3172–3179

Marambaud P, Zhao H, Davies P (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem 280(45):37377–37382

Masoudi FA, Inzucchi SE, Wang Y, Havranek EP, Foody JM, Krumholz HM (2005) Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. Circulation 111(5):583–590

Massion PB, Balligand JL (2007) Relevance of nitric oxide for myocardial remodeling. Curr Heart Fail Rep 4(1):18–25

McCarty MF (2001a) Hepatothermic therapy of obesity: rationale and an inventory of resources. Med Hypotheses 57(3): 324–336

McCarty MF (2001b) Does regular ethanol consumption promote insulin sensitivity and leanness by stimulating AMP-activated protein kinase? Med Hypotheses 57(3):405–407

McCarty MF (2004) Chronic activation of AMP-activated kinase as a strategy for slowing aging. Med Hypotheses 63(2):334–339

McCarty MF (2005) High mitochondrial redox potential may promote induction and activation of UCP2 in hepatocytes during hepatothermic therapy. Med Hypotheses 64(6): 1216–1219

McCarty MF (2010) Salsalate may have broad utility in the prevention and treatment of vascular disorders and the metabolic syndrome. Med Hypotheses 75(3):276–281

McCarty MF (2011) mTORC1 activity as a determinant of cancer risk—rationalizing the cancer-preventive effects of adiponectin, metformin, rapamycin, and low-protein vegan diets. Med Hypotheses 77(4):642–648

McCarty MF (2013) Could carbohydrate-concentrated diets mimic calorie restriction in slowing the aging process? Available at: http://catalyticlongevity.org/prepub_archive/Slabber-essay.pdf

McCarty MF, Block KI (2006) Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. Integr Cancer Ther 5(3):252–268

McCarty MF, Barroso-Aranda J, Contreras F (2009) AMP-activated kinase may suppress NADPH oxidase activation in vascular tissues. Med Hypotheses 72(4):468–470

McFate T, Mohyeldin A, Lu H et al (2008) Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. J Biol Chem 283(33):22700–22708

McGarry JD, Leatherman GF, Foster DW (1978) Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty

acid oxidation by malonyl-CoA. J Biol Chem 253(12): 4128–4136
McGee SL, van Denderen BJ, Howlett KF et al (2008) AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. Diabetes 57(4):860–867
Mehenni H, Gehrig C, Nezu J et al (1998) Loss of LKB1 kinase activity in Peutz–Jeghers syndrome, and evidence for allelic and locus heterogeneity. Am J Hum Genet 63(6):1641–1650
Meneghini LF, Orozco-Beltran D, Khunti K et al (2011) Weight beneficial treatments for type 2 diabetes. J Clin Endocrinol Metab 96(11):3337–3353
Michalek RD, Gerriets VA, Jacobs SR et al (2011) Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol 186(6):3299–3303
Micic D, Cvijovic G, Trajkovic V, Duntas LH, Polovina S (2011) Metformin: its emerging role in oncology. Hormones (Athens) 10(1):5–15
Mihaylova MM, Vasquez DS, Ravnskjaer K et al (2011) Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 145(4): 607–621
Min SW, Cho SH, Zhou Y et al (2010) Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 67(6): 953–966
Monsuez JJ, Gesquiere-Dando A, Rivera S (2011) Cardiovascular prevention of cognitive decline. Cardiol Res Pract 2011: 250970
Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL (2011) Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. Endocrinology 152(11):4610–4619
Muoio DM, Seefeld K, Witters LA, Coleman RA (1999) AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. Biochem J 338(Pt 3):783–791
Musi N, Goodyear LJ (2003) AMP-activated protein kinase and muscle glucose uptake. Acta Physiol Scand 178(4):337–345
Musi N, Hirshman MF, Nygren J et al (2002) Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 51(7):2074–2081
Nath N, Giri S, Prasad R, Salem ML, Singh AK, Singh I (2005) 5-aminoimidazole-4-carboxamide ribonucleoside: a novel immunomodulator with therapeutic efficacy in experimental autoimmune encephalomyelitis. J Immunol 175(1):566–574
Nath N, Khan M, Paintlia MK, Singh I, Hoda MN, Giri S (2009a) Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. J Immunol 182(12):8005–8014
Nath N, Khan M, Rattan R et al (2009b) Loss of AMPK exacerbates experimental autoimmune encephalomyelitis disease severity. Biochem Biophys Res Commun 386(1):16–20
Nieuwenhuis-Ruifrok AE, Kuchenbecker WK, Hoek A, Middleton P, Norman RJ (2009) Insulin sensitizing drugs for weight loss in women of reproductive age who are overweight or obese: systematic review and meta-analysis. Hum Reprod Update 15(1):57–68
Oliveira SM, Ehtisham J, Redwood CS, Ostman-Smith I, Blair EM, Watkins H (2003) Mutation analysis of AMP-activated protein kinase subunits in inherited cardiomyopathies: implications for kinase function and disease pathogenesis. J Mol Cell Cardiol 35(10):1251–1255
Olmos Y, Valle I, Borniquel S et al (2009) Mutual dependence of Foxo3a and PGC-1alpha in the induction of oxidative stress genes. J Biol Chem 284(21):14476–14484
Ostman E, Granfeldt Y, Persson L, Bjorck I (2005) Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. Eur J Clin Nutr 59(9):983–988
Park H, Kaushik VK, Constant S et al (2002) Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 277(36):32571–32577
Pearson KJ, Baur JA, Lewis KN et al (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8(2):157–168
Pelletier JP, DiBattista JA, Roughley P, McCollum R, Martel-Pelletier J (1993) Cytokines and inflammation in cartilage degradation. Rheum Dis Clin North Am 19(3):545–568
Pelletier JP, Jovanovic D, Fernandes JC et al (1998) Reduced progression of experimental osteoarthritis in vivo by selective inhibition of inducible nitric oxide synthase. Arthritis Rheum 41(7):1275–1286
Pelletier JP, Lascau-Coman V, Jovanovic D et al (1999) Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. J Rheumatol 26(9):2002–2014
Peng L, Li ZR, Green RS, Holzman IR, Lin J (2009) Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 139(9):1619–1625
Phillips LK, Prins JB (2008) The link between abdominal obesity and the metabolic syndrome. Curr Hypertens Rep 10(2):156–164
Phillips SA, Ciaraldi TP, Kong AP et al (2003) Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. Diabetes 52(3):667–674
Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR (2009) Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation 119(9):1284–1292
Piwkowska A, Rogacka D, Jankowski M, Dominiczak MH, Stepinski JK, Angielski S (2010) Metformin induces suppression of NAD(P)H oxidase activity in podocytes. Biochem Biophys Res Commun 393(2):268–273
Poulsen MM, Vestergaard PF, Clasen BF et al (2013) High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 62(4):1186–1195
Praharaj SK, Jana AK, Goyal N, Sinha VK (2011) Metformin for olanzapine-induced weight gain: a systematic review and meta-analysis. Br J Clin Pharmacol 71(3):377–382
Puyal J, Ginet V, Grishchuk Y, Truttmann AC, Clarke PG (2011) Neuronal autophagy as a mediator of life and death: contrasting roles in chronic neurodegenerative and acute neural disorders. Neuroscientist 18(3):224–236
Qin X, Guo BT, Wan B et al (2010) Regulation of Th1 and Th17 cell differentiation and amelioration of experimental

autoimmune encephalomyelitis by natural product compound berberine. J Immunol 185(3):1855–1863
Rasouli N, Molavi B, Elbein SC, Kern PA (2007) Ectopic fat accumulation and metabolic syndrome. Diabetes Obes Metab 9(1):1–10
Rejnmark L (2008) Bone effects of glitazones and other antidiabetic drugs. Curr Drug Saf 3(3):194–198
Rodriguez-Moctezuma JR, Robles-Lopez G, Lopez-Carmona JM, Gutierrez-Rosas MJ (2005) Effects of metformin on the body composition in subjects with risk factors for type 2 diabetes. Diabetes Obes Metab 7(2):189–192
Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW (2010) Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 377(9759):31–41
Roussel R, Travert F, Pasquet B et al (2010) Metformin use and mortality among patients with diabetes and atherothrombosis. Arch Intern Med 170(21):1892–1899
Rozengurt E, Sinnett-Smith J, Kisfalvi K (2010) Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. Clin Cancer Res 16(9):2505–2511
Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T (2006) Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. Biochem Biophys Res Commun 344(2):597–604
Sakakibara S, Murakami R, Takahashi M et al (2010) Vinegar intake enhances flow-mediated vasodilatation via upregulation of endothelial nitric oxide synthase activity. Biosci Biotechnol Biochem 74(5):1055–1061
Salminen A, Hyttinen JM, Kaarniranta K (2011a) AMP-activated protein kinase inhibits NF-kappaB signaling and inflammation: impact on healthspan and lifespan. J Mol Med (Berl) 89(7):667–676
Salminen A, Kaarniranta K, Haapasalo A, Soininen H, Hiltunen M (2011b) AMP-activated protein kinase: a potential player in Alzheimer's disease. J Neurochem 118(4):460–474
Salpeter SR, Greyber E, Pasternak GA, Salpeter EE (2010) Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 4, CD002967
Samuels A, Perry MJ, Gibson RL, Colley S, Tobias JH (2001) Role of endothelial nitric oxide synthase in estrogen-induced osteogenesis. Bone 29(1):24–29
Sanchez AM, Candau RB, Csibi A, Pagano AF, Raibon A, Bernardi H (2012) The role of AMP-activated protein kinase in the coordination of skeletal muscle turnover and energy homeostasis. Am J Physiol Cell Physiol 303(5):C475–C485
Sanli T, Rashid A, Liu C et al (2010) Ionizing radiation activates AMP-activated kinase (AMPK): a target for radiosensitization of human cancer cells. Int J Radiat Oncol Biol Phys 78(1):221–229
Scarpello JH (2003) Improving survival with metformin: the evidence base today. Diabetes Metab 29(4 Pt 2):6S36–6S43
Scharrer E (1999) Control of food intake by fatty acid oxidation and ketogenesis. Nutrition 15(9):704–714
Scheen AJ (1996) Clinical pharmacokinetics of metformin. Clin Pharmacokinet 30(5):359–371
Schramm TK, Gislason GH, Vaag A et al (2011) Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. Eur Heart J 32(15):1900–1908
Selman C, Tullet JM, Wieser D et al (2009) Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326(5949):140–144
Semenza GL (2007) HIF-1 mediates the Warburg effect in clear cell renal carcinoma. J Bioenerg Biomembr 39(3):231–234
Sengupta S, Jang BC, Wu MT, Paik JH, Furneaux H, Hla T (2003) The RNA-binding protein HuR regulates the expression of cyclooxygenase-2. J Biol Chem 278(27):25227–25233
Sharma PK, Bhansali A, Sialy R, Malhotra S, Pandhi P (2006) Effects of pioglitazone and metformin on plasma adiponectin in newly detected type 2 diabetes mellitus. Clin Endocrinol (Oxf) 65(6):722–728
Shaw RJ (2009) LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. Acta Physiol (Oxf) 196(1):65–80
Simpson ER, Misso M, Hewitt KN et al (2005) Estrogen—the good, the bad, and the unexpected. Endocr Rev 26(3):322–330
Smith MR, Jaramillo M, Liu YL et al (1990) Translation initiation factors induce DNA synthesis and transform NIH 3 T3 cells. New Biol 2(7):648–654
Smith AR, Visioli F, Frei B, Hagen TM (2008) Lipoic acid significantly restores, in rats, the age-related decline in vasomotion. Br J Pharmacol 153(8):1615–1622
Smith DL Jr, Elam CF Jr, Mattison JA et al (2010) Metformin supplementation and life span in Fischer-344 rats. J Gerontol A Biol Sci Med Sci 65(5):468–474
Song G, Ouyang G, Bao S (2005) The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 9(1):59–71
St-Onge MP, Jones PJ (2002) Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. J Nutr 132(3):329–332
Sumarac-Dumanovic M, Jeremic D, Pantovic A et al (2013) Therapeutic improvement of glucoregulation in newly diagnosed type 2 diabetes patients is associated with a reduction of IL-17 levels. Immunobiology 218(8):1113–1118
Sun Y, Xun K, Wang Y, Chen X (2009) A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anticancer Drugs 20(9):757–769
Tan BX, Yao WX, Ge J et al (2011) Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. Cancer 117(22):5103–5111
Tang J, Feng Y, Tsao S, Wang N, Curtain R, Wang Y (2009) Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and biomedical investigations. J Ethnopharmacol 126(1):5–17
Tang Y, Chen Y, Jiang H, Nie D (2011) The role of short-chain fatty acids in orchestrating two types of programmed cell death in colon cancer. Autophagy 7(2):235–237
Tarkun I, Dikmen E, Cetinarslan B, Canturk Z (2010) Impact of treatment with metformin on adipokines in patients with polycystic ovary syndrome. Eur Cytokine Netw 21(4):272–277
Terkeltaub R, Yang B, Lotz M, Liu-Bryan R (2011) Chondrocyte AMP-activated protein kinase activity suppresses matrix degradation responses to proinflammatory cytokines interleukin-1beta and tumor necrosis factor alpha. Arthritis Rheum 63(7):1928–1937

Thomas GV, Tran C, Mellinghoff IK et al (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 12(1):122–127

Thornton C, Bright NJ, Sastre M, Muckett PJ, Carling D (2011) AMP-activated protein kinase (AMPK) is a tau kinase, activated in response to amyloid beta-peptide exposure. Biochem J 434(3):503–512

Timmers S, Konings E, Bilet L et al (2011) Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 14:612–622

Turban S, Stretton C, Drouin O et al (2012) Defining the contribution of AMP-activated protein kinase (AMPK) and protein kinase C (PKC) in regulation of glucose uptake by metformin in skeletal muscle cells. J Biol Chem 287(24):20088–20099

Turner N, Li JY, Gosby A et al (2008) Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. Diabetes 57(5):1414–1418

Tzoulaki I, Molokhia M, Curcin V et al (2009) Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. BMJ 339:b4731

UK Prospective Diabetes Study (UKPDS) Group (1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352(9131):854–865

Um JH, Park SJ, Kang H et al (2010) AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. Diabetes 59(3):554–563

Unger RH, Clark GO, Scherer PE, Orci L (2010) Lipid homeostasis, lipotoxicity and the metabolic syndrome. Biochim Biophys Acta 1801(3):209–214

Varady KA, Hellerstein MK (2007) Alternate-day fasting and chronic disease prevention: a review of human and animal trials. Am J Clin Nutr 86(1):7–13

Vazquez-Martin A, Oliveras-Ferraros C, Cufi S, Del BS, Martin-Castillo B, Menendez JA (2010) Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial–mesenchymal transition (EMT) status. Cell Cycle 9(18):3807–3814

Vazquez-Martin A, Oliveras-Ferraros C, Del BS, Martin-Castillo B, Menendez JA (2011) The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. Breast Cancer Res Treat 126(2):355–364

Vestergaard P, Rejnmark L, Mosekilde L (2005) Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. Diabetologia 48(7):1292–1299

Viana R, Aguado C, Esteban I et al (2008) Role of AMP-activated protein kinase in autophagy and proteasome function. Biochem Biophys Res Commun 369(3):964–968

Villanueva-Penacarrillo ML, Martin-Duce A, Ramos-Alvarez I et al (2011) Characteristic of GLP-1 effects on glucose metabolism in human skeletal muscle from obese patients. Regul Pept 168(1–3):39–44

Vingtdeux V, Giliberto L, Zhao H et al (2010) AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism. J Biol Chem 285(12): 9100–9113

Vingtdeux V, Chandakkar P, Zhao H, d'Abramo C, Davies P, Marambaud P (2011) Novel synthetic small-molecule activators of AMPK as enhancers of autophagy and amyloid-beta peptide degradation. FASEB J 25(1):219–231

Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004) High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 32(12):1377–1382

Wang W, Fan J, Yang X et al (2002) AMP-activated kinase regulates cytoplasmic HuR. Mol Cell Biol 22(10):3425–3436

Wang W, Yang X, Kawai T et al (2004) AMP-activated protein kinase-regulated phosphorylation and acetylation of importin alpha1: involvement in the nuclear import of RNA-binding protein HuR. J Biol Chem 279(46):48376–48388

Wang S, Xu J, Song P, Viollet B, Zou MH (2009) In vivo activation of AMP-activated protein kinase attenuates diabetes-enhanced degradation of GTP cyclohydrolase I. Diabetes 58(8):1893–1901

Wang S, Zhang M, Liang B et al (2010) AMPKalpha2 deletion causes aberrant expression and activation of NAD(P)H oxidase and consequent endothelial dysfunction in vivo: role of 26S proteasomes. Circ Res 106(6):1117–1128

Watt MJ, Holmes AG, Pinnamaneni SK et al (2006) Regulation of HSL serine phosphorylation in skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 290(3):E500–E508

Wei W, Zhao H, Wang A et al (2012) A clinical study on the short-term effect of berberine in comparison to metformin on the metabolic characteristics of women with polycystic ovary syndrome. Eur J Endocrinol 166(1):99–105

Wijkander J, Landstrom TR, Manganiello V, Belfrage P, Degerman E (1998) Insulin-induced phosphorylation and activation of phosphodiesterase 3B in rat adipocytes: possible role for protein kinase B but not mitogen-activated protein kinase or p70 S6 kinase. Endocrinology 139(1):219–227

Won JS, Im YB, Kim J, Singh AK, Singh I (2010) Involvement of AMP-activated-protein-kinase (AMPK) in neuronal amyloidogenesis. Biochem Biophys Res Commun 399(4): 487–491

Wright JL, Stanford JL (2009) Metformin use and prostate cancer in Caucasian men: results from a population-based case–control study. Cancer Causes Control 20(9):1617–1622

Wu Y, Song P, Xu J, Zhang M, Zou MH (2007) Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. J Biol Chem 282(13):9777–9788

Xia X, Yan J, Shen Y et al (2011) Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS ONE 6(2):e16556

Xiao B, Sanders MJ, Underwood E et al (2011) Structure of mammalian AMPK and its regulation by ADP. Nature 472(7342):230–233

Xie Z, Zhang J, Wu J, Viollet B, Zou MH (2008) Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. Diabetes 57(12):3222–3230

Xilouri M, Stefanis L (2010) Autophagy in the central nervous system: implications for neurodegenerative disorders. CNS Neurol Disord Drug Targets 9(6):701–719

Yap F, Craddock L, Yang J (2011) Mechanism of AMPK suppression of LXR-dependent Srebp-1c transcription. Int J Biol Sci 7(5):645–650

Yeung F, Hoberg JE, Ramsey CS et al (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23(12):2369–2380

Yin MJ, Yamamoto Y, Gaynor RB (1998) The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 396(6706):77–80

Yoshino J, Conte C, Fontana L et al (2012) Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 16(5): 658–664

Young A, Wu W, Sun W et al (2009) Flow activation of AMP-activated protein kinase in vascular endothelium leads to Kruppel-like factor 2 expression. Arterioscler Thromb Vasc Biol 29(11):1902–1908

Yu Y, Liu L, Wang X et al (2010) Modulation of glucagon-like peptide-1 release by berberine: in vivo and in vitro studies. Biochem Pharmacol 79(7):1000–1006

Zeng X, Zeng X (1999) Relationship between the clinical effects of berberine on severe congestive heart failure and its concentration in plasma studied by HPLC. Biomed Chromatogr 13(7):442–444

Zeng XH, Zeng XJ, Li YY (2003) Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 92(2):173–176

Zhang Y, Lee TS, Kolb EM et al (2006) AMP-activated protein kinase is involved in endothelial NO synthase activation in response to shear stress. Arterioscler Thromb Vasc Biol 26(6):1281–1287

Zhang Y, Li X, Zou D et al (2008a) Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin Endocrinol Metab 93(7):2559–2565

Zhang P, Hu X, Xu X et al (2008b) AMP activated protein kinase-alpha2 deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction in mice. Hypertension 52(5):918–924

Zhang CX, Pan SN, Meng RS et al (2011a) Metformin attenuates ventricular hypertrophy by activating the AMP-activated protein kinase-endothelial nitric oxide synthase pathway in rats. Clin Exp Pharmacol Physiol 38(1):55–62

Zhang ZJ, Zheng ZJ, Kan H et al (2011b) Reduced risk of colorectal cancer with metformin therapy in patients with type 2 diabetes: a meta-analysis. Diabetes Care 34(10): 2323–2328

Zhou H, Mineshita S (2000) The effect of berberine chloride on experimental colitis in rats in vivo and in vitro. J Pharmacol Exp Ther 294(3):822–829

Zhou G, Myers R, Li Y et al (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108(8):1167–1174

Zhou J, Zhou S, Tang J et al (2009) Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur J Pharmacol 606(1–3):262–268