# 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 have been suggested to have potential as calorie restriction mimetics for extending healthspan. In fact, an overview of 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. It is therefore reasonable to speculate that long term use of AMPK activators may indeed have a notably favorable impact on healthspan, and that such agents could reasonably be employed as adjuvants to lifestyle strategies – such as modified alternate-day fasting or carbohydrate-concentrated diets – intended to replicate the benefits of daily calorie restriction. 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.

### 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.[1-3] AMPK is a heterotrimer, consisting of α, β, and γ subunits. 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 possesses 2 sites each of which can bind either AMP, ADP, or ATP. At one of these sites, AMP binding allosterically boosts the kinase activity of the activated enzyme. At the other site, binding of either AMP or ADP suppresses the ability of phosphatases to remove the phosphate from Thr172 and hence deactivate the enzyme.[1] 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.[4]

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).[3] 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.[5] The natural metabolite AICAR is converted intracellularly to an analog of AMP which mimics AMP's activating impact on AMPK. And pharmaceutical companies are striving to develop drugs – of which A769662 is a prototype – which likewise can activate AMPK by binding to it directly.[6] 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.[4, 7] Analogously, lipoic acid has the potential to support AMPK activity by countering oxidant-mediated activation of neutral sphingomyelinase, whose product is ceramide.[8, 9]

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.[10] 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.[10-12] However, some key effects of AMPK – such as activation of the endothelial nitric oxide synthase (eNOS)[13, 14] – do not readily fit into this paradigm.

**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.[15] Evidence is currently conflicting as to whether the fuel deficit stress induced by calorie restriction regimens in rodents is sufficient to activate AMPK in rodents.[15] Such activation may however occur indirectly via up-regulation of adiponectin production in adipose tissue.[16] 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+.[17, 18] In light of the media hysteria generated recently by reports that the wine phytochemical resveratrol may exert a pro-longevity effect by activating Sirt1, it should be noted that this activation now appears to be indirect, mediated via resveratrol's impact on AMPK.[19, 20] (And, ironically, pharmacokinetic studies show that absorbed resveratrol is conjugated so rapidly in humans that it has little clinical potential as an AMPK activator.[21, 22])

AMPK mimics the impact of the growth factor down-regulation associated with calorie restriction by inhibiting activity of the mammalian target of rapamycin complex 1 (mTORC1).[23] This complex, via phosphorylation of its targets p70 ribosomal S6 kinase 1 (S6K1) and 4EBP1, up-regulates protein synthesis, and plays a key role in cell proliferation; its activity is suppressed by calorie restriction.[24, 25] 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.[12, 26, 27]

By inhibiting mTORC1 activity, and also via direct phosphorylation, AMPK stimulates ULK1, a kinase which is a key trigger for the process of macroautophagy.[28] This "cell cleansing" process is also abetted by Sirt1 and FOXO activity.[29] 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).[11, 30] The efficiency of macroautophagy declines with increasing age in mammals, and there is considerable speculation that the up-regulation of macroautophagy evoked by calorie restriction in many tissues contributes crucially to the life prolongation and "aging-retardation" achieved by such restriction.[31, 32] Indeed, concurrent up-regulation 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.[33]

At a systemic level, AMPK activation can modestly lower serum glucose and insulin levels by down-regulating hepatic gluconeogenesis (as discussed below) and hence slowing hepatic glucose output; this reduction in insulin can be expected to decrease IGF-I bioactivity by up-regulating hepatic production of IGFBP-1.[34-36] 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.

Down-regulation 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.[37] 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.[38-42] 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.[43, 44] Nonetheless, an improvement in markers for healthspan has been observed in resveratrol-treated mice even when lifespan has not been influenced.[44] 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.[45-48]

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[49-52] – 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 ten years of follow-up from MI, stroke, or all-causes was significantly lower.[53] 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.[54-59]

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.[13, 14] NO, in the modest physiological concentrations produced by eNOS, is well known to exert ant-atherosclerotic, anti-thrombotic, vasodilatory, and anti-hypertrophic effects crucial for preserving healthful structure and function of the vascular system.[60, 61] 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.[5, 62, 63] 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 is requires AMPK-mediated induction of the transcription factor kruppel-like factor 2.[64, 65]

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 down-regulate NF-kappaB activation and oxidative stress in various types of cells; the precise mechanisms responsible require further clarification.[66] 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.[67] A recent study with mouse and human endothelial cell cultures has demonstrated that AMPKα2 can modestly reduce proteasome activity; this likewise results in a down-regulation of NF-kappaB activation.[68] (A similar effect of AMPK on proteasomes has been reported in fibroblasts.[69]) This down-regulation of NF-kappa, in turn, results in decreased expression of various components of the NAPDH oxidase complex.[68] 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.[70-73] 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.[74] Whether these findings can be generalized to other types of cells remains to be determined; in any case, it is clear that AMPK can down-regulate 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.[75]

With respect to the role of foam cells in atherosclerosis, several studies – with one contrary exception[76] – report that agents which activate AMPK tend to boost cholesterol efflux and prevent lipid accumulation in foam cells exposed to oxidized LDL.[77-79] A related report indicates that AICAR-mediated AMPK activation suppresses the macrophage proliferation induced by exposure to oxidized LDL.[80]

Although hepatic activation of AMPK, mediated via metformin, appears to have a modest impact at best on serum levels of LDL – a curious fact, given that AMPK has the potential, like statins, to inhibit HMG-CoA reductase[81] - the AMPK activator berberine is notably effective for lowering serum LDL cholesterol.[82-85] However, 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.[86] 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.[57, 58] 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.[87-94] The protection afforded by AMPK in this regard reflects not only an effect on cardiomyocyte hypertrophy, but also an anti-fibrotic effect.[88, 91] Although the down-regulatory 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;[90] indeed, it is well established that effective NO activity tends to prevent cardiac hypertrophy.[61]

In a randomized clinical trial enrolling 156 patients with congestive heart failure, patients received berberine or placebo in addition to standard management.[95] 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 two year follow-up, mortality in the berberine group was about half as high as in the placebo group (7 vs. 13, $p<.02$). 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.

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**

The root cause of metabolic syndrome is inflammation in visceral adipocytes.[96] 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 up-regulation 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 "pollution" leads to the accumulation of triglycerides and other types of "ectopic fat" in tissues such as skeletal muscle, vascular endothelium, hepatocytes, and beta cells.[97, 98] 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, non-alcoholic 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.[99]

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 the serum IL-6 levels decline during metformin or berberine therapy in diabetics, are suggestive of an anti-inflammatory impact;[85, 100-102] however, metformin usually does not influence adiponectin levels.[103-105] A small amount of evidence suggests that metformin may improve the insulin sensitivity of adipocytes in diabetics.[106, 107] Whether or not AMPK helps "get to the root" of visceral adipocyte dysfunction, it clearly can mimic the anti-lipolytic 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.[108] 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.[109, 110] This likely explains why diurnal levels of serum free fatty acids tend to be suppressed by therapy with metformin or berberine.[106, 107, 111] Evidently, since excess free fatty acid exposure is a key mediator of the complications of metabolic syndrome, this down-regulatory 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.[112, 113] 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.[114] 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.[112, 115] 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[116] – it may lessen the exposure of beta cells to the ectopic fat metabolites that promote beta cell failure.[117-119]

The most important factor in the improved diabetic glycemic control imparted by metformin appears to be a down-regulation of hepatic gluconeogenesis that lessens hepatic glucose output and hence helps to moderate fasting glucose levels.[120] This reflects the fact that AMPK suppresses transcription of the genes coding for the key gluconeogenic enyzymes 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.[121, 122] Metformin's inhibition of CREB binding protein precisely mimics the impact of insulin in this regard.[121] AMPK also boosts the synthesis of the orphan nuclear receptor "small heterodimer partner", which likewise interferes with the transcription of these key genes.[123, 124] Berberine likewise suppresses gluconeogenesis in diabetic rats;[125] its clinical utility for glycemic control appears to be quite comparable to that of metformin.[85, 111]

**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.[126] 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.[126, 127] 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 anti-psychotic agents.[128-130] Hence, there is no reason to suspect that weight control benefits of AMPK activation will be confined to diabetics.[131]

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.[132-134] 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.[135-137] 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.[138, 139]

AMPK also suppresses hepatic de novo lipogenesis, via inhibition of the transcription and post-translational processing of the key transcription factor for lipogenic enzymes, SREBP-1c.[49, 140, 141] 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.[142]

A recent Japanese study has found that regular vinegar ingestion can promote modest weight loss in obese subjects.[143] This is pertinent in light of the fact – as discussed below – that vinegar (acetic acid) has the potential to activate hepatic AMPK.[144] Vinegar also induced UCP-2 in mouse liver – an effect which was blocked by inhibition of AMPK.[144]

**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.[145-153] 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 insulin sensitivity (associated with low serum levels of insulin and free IGF-I) – have also been linked to lower cancer risk in epidemiological analyses.[154] 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.[155] 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, ODC – and others that block apoptosis – survivin, Bcl-2, Bcl-xL, Mcl-2, dad1.[154, 156-159] Hence, mTORC1, acting via eIF4E, up-regulates 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.[160] The weak mRNAs whose translation is expedited by mRNAs also code for a number of proteins that render transformed cells more aggressive in their behavior, and more chemoresistant; not surprisingly, constitutive activation of mTORC1, and/or overexpression of eIF4E, is observed in a high proportion of advanced malignancies.[158]

Additionally, there is recent evidence that mTORC1 very rapidly up-regulates the activity of Gq-coupled receptors that can promote proliferation in some cell types by somehow activating the Raf-MEK-Erk1/2 pathway.[161] 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.[162, 163] 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.

The contribution of the mTORC1 signaling to cancer aggressiveness suggests 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.[164-166] 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;[167-169] this effect may contribute to a reduction in total cancer mortality in diabetics using metformin.[170] 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.[171-174] 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.[175-177] 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 down-regulate mTORC1 activity, complementing the more direct impact of AMPK in this regard.[36] 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.[178]

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.[179] 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.[180, 181] 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 down-regulating LKB1.[180, 182]

**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.[183-186] 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.[183, 187] On the other hand, overwhelmingly intense activation of autophagy can contribute to neuronal death when not properly balanced by biosynthesis.[188] These considerations have led some researchers to suggest that AMPK-mediated up-regulation of autophagy may have greater net utility in the early stages of neurodegenerative disorders, than in their advanced stages.[189] In other words, AMPK activation is best viewed as a preventive rather than therapeutic strategy for addressing neurodegeneration.

With respect to Alzheimers risk, AMPK activators have been shown to decrease extracellular amyloid-beta accumulation, owing to increased autophagic degradation of this protein.[190, 191] How AMPK

influences amyloid-beta production per se has been the subject of conflicting reports,[192-194] but in any case up-regulation 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.[190]

With respect to tau phosphorylation, a key factor in the formation of the neurofibrillatory tangles characteristic of Alzheimers, AMPK appears to have equivocal effects. Sirt1 activity opposes such phosphorylation, so AMPK has the potential to block tau phosphorylation via Sirt1 activation.[189, 195, 196] On the other hand, AMPK also has the potential to phosphorylate tau directly.[197] It is not yet clear which of these mechanisms is predominant during the elevation of Alzheimers.

The ability of AMPK to boost eNOS activity in the cerebral microvasculature may also aid Alzheimers 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.[198] 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 Alzheimers as they age.[199] 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.[200] 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 up-regulates 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.[201-206]

(Parenthetically, it should be noted that the ability of AMPK to down-regulate 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.[207] 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.[208])

Although risk 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.[209] 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.[210, 211] 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.[212-214] 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).[215] Suppression of RANKL expression and osteoclasts development has also been seen with berberine and other AMPK activators.[216, 217] 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.[215, 218, 219] 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.[220, 221]

AMPK may also aid 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 down-regulation of the tissue inhibitor of metalloproteinases.[222] Oxidative stress, NF-kappaB activation, and iNOS induction are key mediators of these effects.[223-227] 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.[228] 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 anti-catabolic 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.[229] 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.[230-233] 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.[231] 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.[231] 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.[234]

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.[235-238] Much further research addressing the immunomodulatory potential of AMPK activation is warranted.

**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.

Metformin is the world's most widely used diabetic drug, typically administered in a dose of 500-850 mg twice daily. A minority of patients who use it experience some GI upset, which tends to become less significant over time. Otherwise, it appears to be safe and well tolerated; the risk for lactic acidosis that prompted the banning of the related biguanide drug phenformin, appears to be almost non-existent with prescribed doses of metformin.[239] For non-diabetics who seek to employ AMPK activation as a longterm 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 U.S. It is widely used in China for diabetes management. Typical dose regimens are 500 mg 2-3 times daily, or 300 mg 3 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.[85, 111] A small minority of patients experience significant constipation, but otherwise it is well tolerated. Berberine is not efficiently absorbed, and innovations in delivery – microemulsification or cyclodextrin inclusion complexation – might enable lower doses to provide worthwhile benefit.[240, 241] Berberine may have tremendous potential as a "life extension" nutraceutical.

Resveratrol, a polyphenol found in red wine and many other foods, has recently been widely promoted in the U.S. as an aging-retardant nutraceutical, owing to reports that it activates Sirt1 in cell cultures and improves survival in obese mice. Unfortunately, it is conjugated rapidly and completely following absorption; despite reasonably efficient absorption, orally administered resveratrol do not appear to achieve sustained serum levels of free resveratrol sufficient to inhibit mitochondrial ATP synthase and thereby activate AMPK.[21, 22] However, oral resveratrol might have the potential to transiently activate AMPK in the intestinal mucosa. Resveratrol, as well as the AMPK activators metformin, berberine, and 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;[242-246] moreover, GLP-1 may exert protective effects on other tissues as well.[247-249] Perhaps this rationalizes the modestly favorable results of resveratrol supplementation (150 mg daily) reported in a recent clinical study.[250]

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 and of vinegar-fed rats, and in human endothelial cells in vitro.[144, 251, 252] 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;[144, 251] 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.[143] Moreover, in post-menopausal women, vinegar administration boosts flow-mediated vasodilation, an effect likely attributable to AMPK-mediated phosphorylation of eNOS.[252] 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.[253-255] 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![256] (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.[257] 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;[258] conceivably, this phenomenon might also impact risk for colorectal cancer.[259] 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.

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