

AMPK Metabolic Activ8r™ Introduction to AMPK – the Energy Regulator

What is AMPK?

Adenosine monophosphate-activated protein kinase (AMPK) is a key fuel sensor of cellular energy status, regulating cellular and whole-body homeostasis. AMPK is activated by ratios of adenosine monophosphate (AMP)/adenosine triphosphate (ATP) and or adenosine diphosphate (ADP)/ATP(1).

When cellular energy, ATP, levels fall, AMPK induces catabolic pathways that generate ATP – the energy needed by cells to function, while inhibiting anabolic pathways and other pathways that consume ATP(1). This energy switch can influence multiple pathways including lipid and glucose metabolism, protein metabolism, autophagy, and mitochondrial biogenesis(2).

Biochemical Structure and Mechanism of AMPK

AMPK is built from three protein subunits, that together form a functional enzyme. These subunits consist of an alpha (α) catalytic subunit, a beta (β) and regulatory gamma (γ) subunit. These subunits encode different genes and regulate different metabolic pathways(2). AMPK can be found in several tissues in the body, including the liver, brain, and skeletal muscle. AMPK is known as the fuel of the cell, working to ensure ATP levels are maintained under energetic stress situations such as exercise, starvation, hypoxia, and rapid cell growth(1).

Figure 1: AMPK Metabolic Regulation Pathways

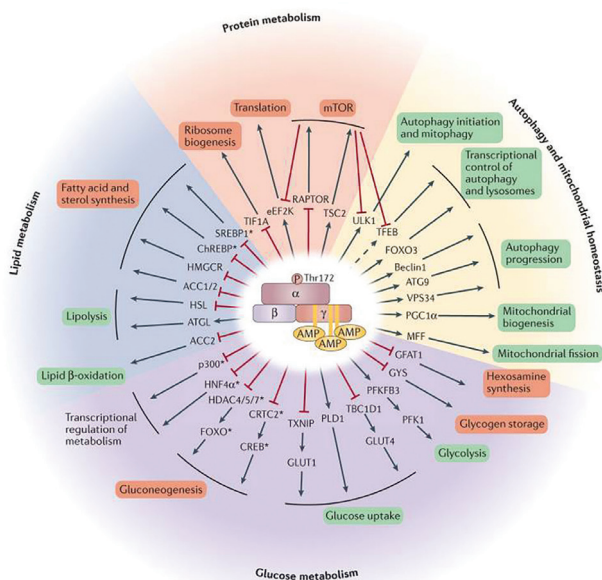
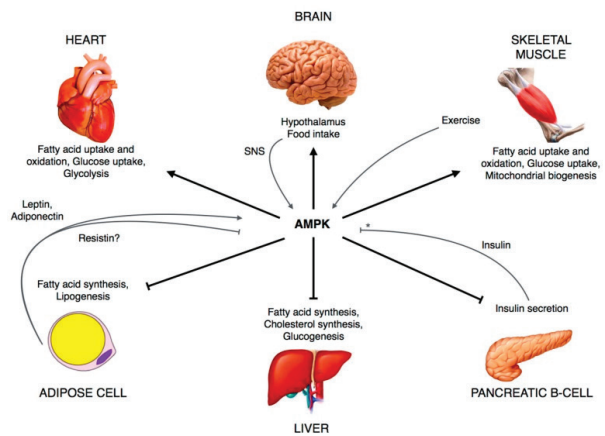


Figure 2: Feedback Loops of AMPK



The binding of AMP and ADP result in activation of AMPK. Once activated, AMPK phosphorylates key targets to rewire metabolism. Dependent on which gene is triggered, the following processes may result – hepatic fatty acid oxidation, ketogenesis, stimulation of skeletal muscle fatty acid oxidation and glucose uptake, inhibition of cholesterol synthesis, lipogenesis and triglyceride synthesis, inhibition of adipocyte lipogenesis, inhibition of adipocyte lipolysis, and modulation of insulin secretion by pancreatic β -cells(2).

Aging can reduce AMPK sensitivity to cellular stress, impairing downstream signaling and maintenance of cellular energy balance. Alterations in AMPK signaling decrease mitochondrial biogenesis, increase cellular stress and induce inflammation. Therefore, AMPK has been identified as a key target for slowing the aging process, increasing fat oxidation pathways, and glucose metabolism and stimulating insulin secretion and sensitivity.

BioGenixUSA has created a unique AMPK formulation, AMPK Metabolic Activ8r™ helps switch on pathways to increase insulin secretion and sensitivity, increase fat oxidation via BAT activation, reduce post-workout muscle inflammation and provide antioxidants for protection against the aging process. This review will breakdown the formulation ingredients including the mechanism and clinical research supporting their effectiveness.

Metabolic Cellular Regulation – AMPK Metabolic Activ8r™

- Supports Visceral Fat Metabolism
- Anti-Aging Support
- Weight Management
- Appetite Support
- Blood Glucose Support

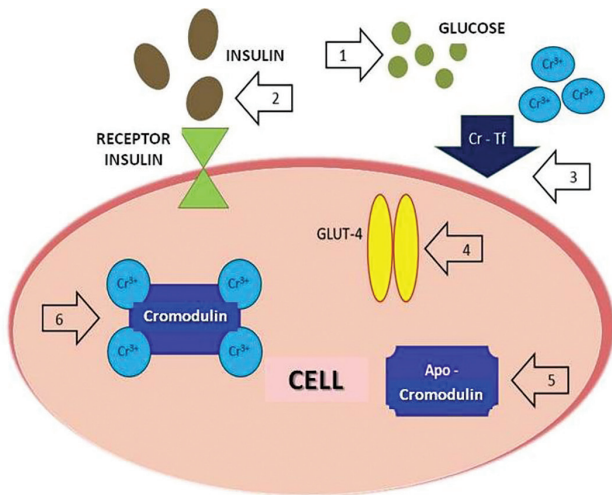
AMPK Metabolic Activ8r™ Ingredient Breakdown

Chromium – Glucose Transporter Regulation

Chromium is an essential mineral needed in the diet. It has commonly been used in the treatment of diabetes to reduce food intake, body weight, and increase glucose metabolism, particularly in those with type 2 diabetes(3).

Trivalent chromium, such as chromium picolinate found in AMPK Metabolic Activ8r™, has been shown to improve plasma membrane-based aspects of glucose transporter GLUT-4 translocation and glucose transport into adipocytes via lowering plasma membrane cholesterol, and mediating AMPK(4).

Figure 3: Chromium and GLUT-4 Translocation



Glucose homeostasis is regulated by the ability of insulin to stimulate the storage and metabolism of glucose, helping to return elevated plasma glucose levels toward normal. In muscle and adipose tissue, insulin accelerates removal of excess glucose by regulating the GLUT-4 transporter(4). GLUT-4 cycles continuously between the plasma membrane and one or more intracellular compartments, with most of the transporter residing within the cell interior. Removal of GLUT-4 from the plasma membrane, is associated membrane cholesterol.

Chromium picolinate increases membrane fluidity and can decrease cholesterol content of the plasma membrane. In one study, it was shown that GLUT-4 redistribution in chromium picolinate treated cells only occurred in cells cultured with high glucose concentrations that resembled the diabetic state, and not in non-diabetic conditions(4). Therefore, accumulation of GLUT-4 transporter at the plasma membrane maybe dependent on the chromium picolinate induced cholesterol loss.

Examination of the effect of chromium picolinate on proteins involved in cholesterol homeostasis showed that the activity of sterol regulatory element-binding protein (SREBP), a membrane-bound transcription factor ultimately responsible for controlling cellular cholesterol balance, was upregulated by chromium picolinate(4). In addition, ABCA1, a major player in mediating cholesterol efflux was decreased, consistent with SREBP transcriptional repression of the ABCA1 gene(4).

Chromium and AMPK Regulation

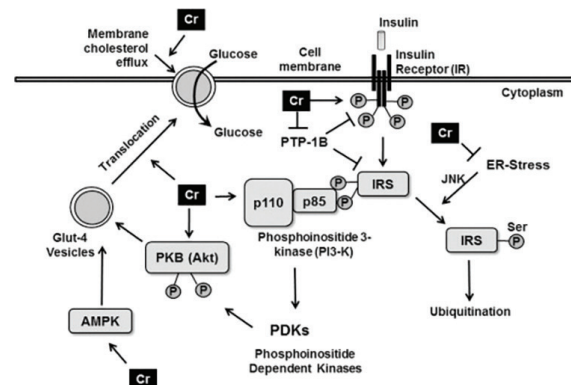
In addition to regulating GLUT-4 translocation, research has shown that chromium picolinate treated cells increase AMPK activity. AMPK phosphorylates and inhibits HMGR (3-hydroxy-3-methyl-glutaryl coenzyme A reductase), the rate-limiting enzyme in cholesterol synthesis. Therefore, chromium picolinate could potentially mediate its beneficial effects on GLUT-4 and glucose transport regulation via AMPK activation(5).

In a mechanistic study, AMPK's ability to mediate the effects of chromium picolinate on GLUT-4 and glucose transport regulation was tested. Hyperinsulinemia-induced insulin-resistant L6 myotubes displayed excess membrane cholesterol and diminished cortical F-actin essential for effective glucose transport regulation. These membrane and cytoskeletal abnormalities were associated with defects in insulin-stimulated GLUT-4 translocation and glucose transport.

Supplementing the culture medium with pharmacologically relevant doses of chromium picolinate protected against membrane cholesterol accumulation, F-actin loss, GLUT-4 dysregulation, and glucose transport dysfunction. Insulin signaling was neither impaired by hyperinsulinemic conditions nor enhanced by chromium picolinate, whereas chromium picolinate increased AMPK signaling.

Mechanistically, RNA-mediated depletion of AMPK abolished the protective effects of chromium picolinate against GLUT-4 and glucose transport dysregulation. Together these findings suggest that chromium picolinate via increasing AMPK activity, positively impacts skeletal muscle cell insulin(5).

Figure 4: Molecular Mechanisms of Chromium in Alleviating Insulin Resistance



In another study, the effects of chromium picolinate was examined in insulin-resistant adipocytes, for stimulating gene transcription and secretion of adiponectin and resistin(6). Adiponectin is a hormone that is released by fat tissue to help with insulin sensitivity and inflammation. Resistin is a cysteine-rich peptide secreted by adipocytes, immune cells, and epithelial cells. High levels of resistin have been associated with Metabolic Syndrome. This proinflammatory cytokine causes the resistance of peripheral tissues to insulin and is considered by many researchers as a possible link between obesity and type 2 diabetes.

Using immunoblotting, ELISA and real-time reverse transcription–polymerase chain reaction (RT-PCR), the effects of chromium picolinate was investigated for 24 h on AMP-activated protein kinase (AMPK) to determine whether this pathway contributed to the regulation of adiponectin and resistin expression and secretion(6).

Results showed that chromium picolinate significantly inhibited the secretion of resistin, but not adiponectin, by normal and insulin-resistant 3T3-L1 adipocytes in vitro⁵. Chromium picolinate markedly elevated levels of phosphorylated AMPK and acetyl CoA carboxylase in 3T3-L1 adipocytes. Importantly, inhibition of AMPK completely abolished the chromium picolinate-induced suppression of resistin secretion. In conclusion, the data suggest that chromium picolinate inhibits resistin secretion via activation of AMPK in normal and insulin-resistant 3T3-L1 adipocytes(5).

Berberine – Glucose Metabolism Regulation via AMPK

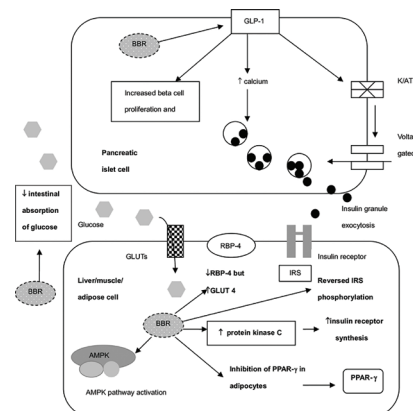
Berberine is an alkaloid extracted from *Berberis vulgaris* and has traditional uses in Chinese and Ayurvedic medicine for approximately the last 2500 years. It has shown to be effective for improving blood glucose disposal, improving insulin efficiency, lipid metabolism and body composition(7).

Its effectiveness is comparable to pharmaceuticals used for the same purposes including for the treatment of diabetes, obesity, and metabolic syndrome.

Berberine has been shown to regulate glucose and lipid metabolism, working on both adipocytes and myocytes, and within these cell types berberine induces a variety of metabolic effects consistent with AMPK activation.

Berberine enhances insulin secretion, stimulates glycolysis, suppresses adipogenesis by inhibiting PPAR γ and C/EBP α function, activating AMPK pathway and increasing glycokinase activity⁸. Berberine stimulates G protein, reduces intestinal glucose absorption by inhibiting α -glucosidase activity, and up-regulating the expression of GLUT-4 translocation, stimulating glucose up-take, and upregulating glucagon like peptide (GLP-1) genes(8).

Figure 5: Berberine’s Effects on Glucose Metabolism



In two animal models of insulin resistance and in insulin-responsive cell lines, the effects of berberine were investigated. Berberine reduced body weight and caused a significant improvement in glucose tolerance without altering food intake in *db/db* mice. Similarly, berberine reduced body weight and plasma triglycerides and improved insulin action in high-fat-fed Wistar rats(9).

Berberine downregulated the expression of genes involved in lipogenesis and upregulated those involved in energy expenditure in adipose tissue and muscle(9). Berberine treatment resulted in increased AMPK activity in 3T3-L1 adipocytes and L6 myotubes, increased GLUT-4 translocation in L6 cells in a phosphatidylinositol 3' kinase-independent manner, and reduced lipid accumulation in 3T3-L1 adipocytes(9). These findings suggest that berberine displays beneficial effects in the treatment of diabetes and obesity at least in part via stimulation of AMPK activity.

Berberine may also inhibit Protein-Tyrosine Phosphatase 1B (PTP1B), thus increasing insulin sensitivity¹⁰. In diabetic mice, berberine was shown to mimic insulin action by increasing glucose uptake ability by 3T3-L1 adipocytes and L6 myocytes in an insulin-independent manner, inhibiting phosphatase activity of PTP1B, and increasing phosphorylation of IR, IRS1 and Akt in 3T3-L1 adipocytes⁽¹⁰⁾. In diabetic mice, berberine lowers hyperglycemia and improves impaired glucose tolerance, but does not increase insulin release and synthesis⁽¹⁰⁾.

Clinical Research for Treatment of Type 2 Diabetes

Berberine is one plant alkaloid that has significant positive research to support its effectiveness for the treatment of Type 2 Diabetes Mellitus (T2DM), comparable to pharmaceutical drugs used for the same purpose. In a meta-analysis of twenty-seven randomized controlled clinical trials, including 2569 patients, the effectiveness of berberine was compared to various interventions⁽¹¹⁾.

In the treatment of T2DM, it was shown that berberine with lifestyle intervention tended to lower the level of fasting plasma glucose (FPG), postprandial glucose (PPG) and HbA1c than lifestyle intervention alone or placebo⁽¹¹⁾. The same as berberine combined with oral hypoglycaemics to the same hypoglycaemics; but there was no statistical significance between berberine and oral hypoglycaemics.

As for the treatment of hyperlipidemia, berberine with lifestyle intervention was better than lifestyle intervention, berberine with oral lipid lowering drugs was better than lipid lowering drugs alone in reducing the level of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), and raising the level of high density lipoprotein cholesterol (HDL-C)⁽¹¹⁾.

In a comparative study between berberine and oral lipid lowering drugs, there was no statistical significance in reducing the level of TC and LDL-C, but berberine showed better effects in lowering the level of triglycerides (TG) and raising the level of HDL-C. In the treatment of hypertension, berberine with lifestyle intervention tended to lower the level of blood pressure more than the lifestyle intervention alone or placebo did(11). Additionally, no serious adverse reaction was reported in the 27 experiments.

In another systematic review and meta-analysis, the efficacy and safety of berberine for the treatment of T2DM was assessed. Fourteen randomized trials of berberine were compared with lifestyle modification, placebo and or oral hypoglycaemic intervention involving 1068 participants(12).

Compared with lifestyle modification with or without placebo, the cointervention of berberine and lifestyle modification showed significantly hypoglycaemic and antidyslipidemic response(12). Compared with oral hypoglycaemics including metformin, glipizide, or rosiglitazone, berberine did not demonstrate a significantly better glycaemic control but showed a mild antidyslipidemic effect.

Compared with oral hypoglycaemic drugs, cointerventions with berberine and the same oral hypoglycaemics showed a better glycaemic control.

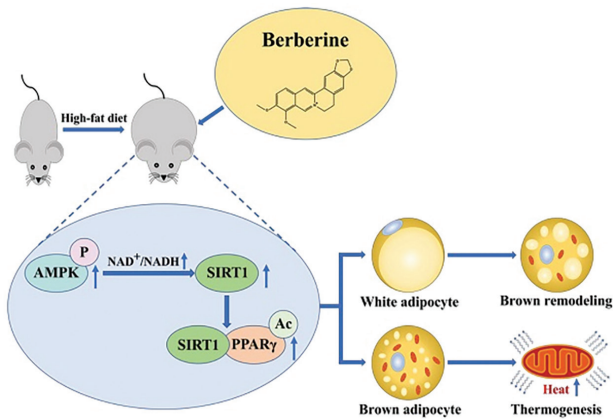
No serious adverse effects from berberine were reported(12). Berberine is considered efficacious for treating hyperglycaemia and dyslipidemia in T2DM(12).

BAT Activation and Body Weight Loss

Brown adipose tissue (BAT) dissipates metabolic energy and mediates non-shivering thermogenesis, allowing for a boost in energy expenditure. This is regulated by uncoupling protein 1 (UCP1). There are two types of thermogenic adipocytes, brown adipocytes that are abundant in BAT with high levels of UCP1, and beige adipocytes that are abundant in white adipose tissue (WAT) with low levels of UCP1 under basal conditions(13). Beige adipocytes have potential to express comparable levels of UCP1 to brown adipocytes in response to stimuli such as cold temperatures or β 3-adrenergic receptor agonists.

The transcriptional factor, PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM16) acts as a master regulator of brown/beige adipogenesis through its interactions with transcriptional factors such as PPAR γ , C/EBP β , PPAR γ co-activator 1 α (PGC-1 α), zinc finger protein 516, and C-terminal-binding protein. 1. Animal studies have indicated that the anti-obesity effect of berberine might relate to its pro-thermogenesis effect in mature adipocytes via AMPK-PGC-1 α pathway(13).

Figure 6: Berberine Modulates Deacetylation of PPAR γ to Promote Adipose Tissue Remodeling and Thermogenesis via AMPK/SIRT1



A study showed, chronic berberine treatment promoted BAT by stimulating the expression of brown adipogenic genes, enhanced BAT thermogenesis, and energy expenditure in diet-induced obese mice and chow-fed lean mice(13). Consistently, berberine facilitated brown adipocyte differentiation in both mouse and human primary brown preadipocytes. The study also found that berberine increased the transcription of PRDM16, by inducing the active DNA demethylation of PRDM16 promoter, which might be driven by the activation of AMPK and production of its downstream tricarboxylic acid cycle intermediate α -ketoglutarate(13). Moreover, chronic berberine administration had no impact on the BAT thermogenesis in adipose-specific AMPKa1 and AMPKa2 knockout mice(13).

In summary, the study showed that berberine intervention promoted recruitment and activation of BAT and AMPK-PRDM16 axis was indispensable for the pro-BAT and pro-energy expenditure properties of berberine(13). The findings suggest that berberine may be a promising drug for obesity and related metabolic disorders in humans partially through activating BAT.

R-Alpha Lipoic Acid – Antioxidant Appetite Control

Alpha lipoic acid (ALA) is an organosulfur compound produced by plants, animals, and humans. In the Krebs cycle, ALA plays important roles in various chemical reactions, acting as a cofactor for some enzymatic complexes involved in energy generation in the mitochondria of the cell(14).

Structurally speaking ALA has a single chiral center and asymmetric carbon, resulting in two optical isomers R and S-lipoic acid. Thus, ALA has two enantiomeric forms called S and R enantiomers, which are mirror images of each other. The R form is present naturally in many sources of plants, vegetables, fruits, and meat. ALA although produced by the body, it is not enough to fulfill energy requirements of the body, therefore in addition to eating a diet rich in ALA, supplementation of the R-form should be considered for maximum benefits. This is the form provided in AMPK Metabolic Activ8r.

ALA is a potent antioxidant, and supplementation has shown many benefits including reduction of inflammation associated with heart disease, diabetes, liver disease and neurological decline associated with aging. ALA has also been shown to reverse oxidative damage related to the effects of aging, assist in weight loss, appetite control and metabolism of fats in the body. It's also a potent regulator of insulin secretion and sensitivity. Metabolic action of ALA is mediated by activation of AMPK, via PGC-1 α , a regulator of mitochondrial biogenesis.

AMPK, Aging and Appetite Control

ALA has been shown to decrease and inhibit hypothalamic AMPK activity, the result is a mimicking of caloric surplus, causing appetite suppression. The hypothalamus controls the body's temperature and hunger. This effect may be secondary to increasing glucose uptake into the hypothalamus and are reversed upon AMPK activation in the hypothalamus(15).

Rats were given ALA at 0.25, 0.5, and 1% of the overall food intake for 2 weeks. Over a period of 14-weeks, the supplementation was able to reduce body weight relative to placebo control(15).

The reduction in food intake in rats was not secondary to a conditioned taste aversion, from ALA's sharp taste(15).

In another rat study, using 200mg/kg of ALA, oral intake showed that this suppression of hypothalamic AMPK paired with adipose expression of AMPK helped to normalize changes in fat mass associated in a model of menopause(16). After treatment with ALA, the elevation of leptin and adiponectin levels and the activation of hypothalamic AMPK and phosphorylated acetyl-CoA carboxylase (ACC), as induced by ovariectomy, were significantly suppressed. Meanwhile, decreased fat mass and increased phosphorylated AMPK and phosphorylated ACC expression in the white adipocyte tissue (WAT) were observed in ovariectomized rats treated with ALA(16).

Other studies have reproduced similar results. In a study in older mice fed 0.75% ALA, a reduction in food relative to control was quantified at ranges of 18%(17). ALA supplementation improved body composition, glucose tolerance, and energy expenditure in the aged mice. ALA also increased skeletal muscle mitochondrial biogenesis with increased phosphorylation of AMPK and mRNA expression of a key transcriptional regulator of energy metabolism, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 α) and GLUT-4.

Besides body fat mass, ALA decreased lean mass and attenuated phosphorylation of mammalian target of rapamycin (mTOR) signalling in the skeletal muscle(17). In cultured cells, ALA increased glucose uptake and palmitate β -oxidation, but decreased protein synthesis, which was associated with increased phosphorylation of AMPK and expression of PGC-1 α and GLUT-4, and attenuated phosphorylation of mTOR and p70S6 kinase(17).

The study showed that ALA improves skeletal muscle energy metabolism in the aged mouse possibly through enhancing AMPK-PGC-1 α -mediated mitochondrial biogenesis and function(17). One thing to note, ALA increases lean mass loss possibly by suppressing protein synthesis in the skeletal muscle by downregulating the mTOR signalling pathway(17). Thus, ALA may be a promising supplement for treatment of obesity and/or insulin resistance in older patients but may not be helpful for muscle wasting.

ALA, AMPK and Insulin Secretion and Sensitivity

The effects of ALA on pancreatic β -cell function, insulin secretion, AMPK-signalling pathway, mitochondrial function, and cell growth were assessed in isolated rat islets and β -cells treated with ALA or known AMPK-activating compounds(18). Acute or chronic treatment of islets and β -cells with ALA led to dose-dependent rises in phosphorylation of the AMPK α -subunit and ACC.

Chronic exposure to ALA, and other AMPK activating compounds, including metformin caused a reduction in insulin secretion(18).

Unlike metformin, ALA also acutely inhibited insulin secretion. Examination of the effect of ALA on mitochondrial function showed that acute treatment with this compound elevated reactive oxygen species (ROS) production(18). This study demonstrated that ALA directly affects β -cell function. The chronic effects of ALA include AMPK activation and reductions in

ALA and BAT

Uncoupling Protein-1 (UCP1) is a transporter present in the inner membrane of the mitochondria that plays a role in non-shivering thermogenesis (NST). UCP1 are present in the inner membrane of brown adipose tissue (BAT) mitochondria. UCP1 uncouples mitochondrial respiration from adenosine-5'-triphosphate (ATP) synthesis. When activated, it causes a leak that dissipates the electrochemical proton gradient that builds up across the inner mitochondrial membrane during BAT fatty acid oxidation(19). This electrochemical proton gradient drives the conversion of adenosine-5'-diphosphate (ADP) to ATP, by ATP synthase(19).

The presence of active UCP1 eliminates the negative feedback inhibition exerted by high ATP and/or low ADP levels, leading to very high rate of fatty acid oxidation that directly produces heat. Because of its large amount of active UCP1 proteins, BAT is thus the only organ that literally can “burn” fat(19).

One study showed that ALA could activate UCP1 in Zucker Diabetic Fatty (ZDF) rats. The study fed 6-week old ZDF rats and their lean counterparts, ZL rats, with a standard diet or chow diet supplemented with ALA at 1 g/kg feed for 6-weeks(20). At 12-weeks of age the ZDF rats feed the standard diet, exhibited an increase in systolic blood pressure, epididymal fat weight per body weight, hyperglycemia, hyperinsulinemia, insulin resistance, adipocyte hypertrophy and a rise in basal superoxide (SO) anion production in gastrocnemius muscle and a downregulation of epididymal UCP-1(20).

Treatment with ALA prevented the development of hypertension, the rise in whole body weight and SO production in gastrocnemius muscle, but failed to affect insulin resistance, hyperglycemia, and hyperinsulinemia in ZDF rats(20). ALA treatment resulted in a noticeable increase of pancreatic weight and a further adipocyte hypertrophy, along with a decrease in epididymal fat weight per body weight ratio associated with an upregulation of epididymal UCP-1 in ZDF rats(20).

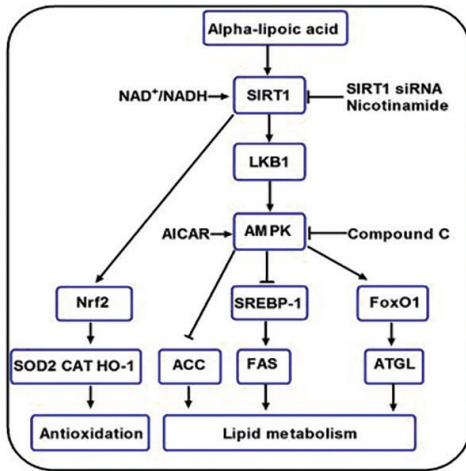
These findings suggest that ALA was efficacious in preventing the development of hypertension, which could be related to its antioxidant properties. The anti-visceral obesity effect of ALA appears to be mediated by its antioxidant properties and the induction of UCP-1 protein at the adipose tissue level in ZDF rats(20). It's suggested that ALA exerts its anti-visceral obesity effects through its antioxidant properties, leading to the activation of UCP-1 protein expression at the adipose tissue level in ZDF rats(20). The beneficial impact of ALA to decrease the body weight gain in ZDF rats is consistent with the reduction of NADPH oxidase hyperactivity in adipose tissue(21).

ALA, AMPK and Lipid Metabolism

ALA has been shown to down-regulate triglyceride content by inhibiting fatty acid esterification and de novo lipogenesis – the conversion of excess carbohydrates into fatty acids that are esterified and stored as triglycerides. These effects appear to be mediated by reduction in fatty acid synthase (FAS), and other key regulator enzyme levels. ALA increased AMPK and ACC phosphorylation, while the presence of an AMPK inhibitor reversed the inhibition observed on FAS protein levels.

ALA down-regulates key lipogenic enzymes, inhibiting lipogenesis and reducing triglyceride accumulation through the activation of AMPK signaling pathway in human subcutaneous adipocytes from overweight/obese subjects(22).

Figure 7: ALA and Lipid Metabolism



In a study in rats, dietary supplementation with ALA reduced body weight and adiposity in control and high-fat fed rats. ALA also reduced basal hyperinsulinemia as well as the homeostasis model assessment (HOMA) levels, an index of insulin resistance, in high-fat-fed rats, which was in part independent of their food intake lowering actions(23). Furthermore, AMPK phosphorylation was increased in white adipose tissue (WAT) from ALA-treated rats as compared with pair-fed animals(23).

Dietary supplementation with ALA also upregulated adiponectin gene expression in WAT, while a negative correlation between adiposity-corrected adiponectin levels and HOMA index was found(23). This data suggests that the ability of ALA supplementation to prevent insulin resistance in high-fat diet-fed rats might be related in part to the stimulation of AMPK and adiponectin in WAT(23).

Clinical Research – ALA & Weight Loss

Based on ALA’s metabolic effects, the use of ALA for weight loss was evaluated. In one study, 77 healthy overweight obese women were randomly assigned into four groups, placebo, 1300 mg eicosapentaenoic acid (EPA), 300 mg of ALA or 1300 mg EPA + 300 mg ALA per day for 10-weeks(24). All subjects also received a 30% energy restricted diet. The group receiving the ALA showed significantly higher body weight loss and a drop in leptin levels, without a change in metabolic rate(24).

In another study, the action of ALA on body weight, waist circumference and lipid metabolism in 170 overweight or obese patients was evaluated(25). ALA group received orally 1200 mg ALA per day for 8 weeks, then after a 4-week washout intervention this group continued to receive placebo for 8 weeks(25). The exact opposite sequence of ALA and placebo interventions was used for placebo group. According to mixed model statistical analysis, ALA administration showed a significant body weight and waist circumference reduction(25).

***Gymnema sylvestre* - Insulin Control, Secretion & Sensitivity**

Gymnema sylvestre is a plant used in traditional Ayurvedic medicine, for its therapeutic benefits when it comes to controlling blood sugar. The phytoconstituent responsible for its ability to suppress sweet cravings include the triterpene saponins, gymnemic acids, gymnemagenin and gurmarin(26). *Gymnema sylvestre* has shown positive effects on blood sugar homeostasis, controlling sugar cravings, and promoting regeneration of the pancreas(26).

Gymnema sylvestre's mode of action is through the stimulation in insulin secretion from the pancreas and by delaying glucose absorption in the blood. The chemical structure of gymnemic acids, is also like sugar, filling the receptors in the taste buds preventing its activation by the sugar molecules in food(26). In the intestine, gymnemic acids attach to the receptor present in the external layer of the intestine, thereby preventing the absorption of sugar molecules by the intestine, leading to a reduction in blood sugar levels(26).

Gurmarin acts in a similar manner by interfering with the ability of taste buds on the tongue to differentiate between sweet and bitter.

Hypoglycemic effect of gymnemic acids includes a cascade of events starting from modulation of incretin activity which triggers insulin secretion and release. Incretins are protein hormones, whose functions include the modulation of glucose metabolism by stimulating the release of insulin by the β cells and, at the same time, inhibiting the release of glucagon by pancreatic α cells.

It also increases regeneration of pancreatic islet cells to enhance enzyme mediated uptake of glucose. This process decreases glucose and fatty acid assimilation in the small intestine and interferes in the ability of receptors in mouth and intestine to sensation of sweetness.

The action of gymnemic acid is like that of incretin-mimetic mechanism of action. Gymnemic acid has been found to interact with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis pathway(26). Research indicates that the acyl moieties present in gymnemic acids play important role for the GA-induced smearing of GAPDH and G3PDH and play an integral role in the antihyperglycemic activity of GA derivatives(26).

AMPK and *Gymnema sylvestre*

In one study, the anti-hyperglycemic mechanism of gymnemic acids was evaluated in type 2 diabetes mellitus (T2DM) rats. The results indicated that gymnemic acids decreased fasting blood glucose concentrations by 26.7% and lowered insulin concentrations by 16.1% after oral administration at a dose of 80 mg/kg/day for 6-weeks(27).

The data showed that real-time polymerase chain reaction and western blot indicated that gymnemic acids upregulated the level of phosphatidylinositol-3-kinase (PI3K) and glycogen synthesis (GS) and promoted the phosphorylation of protein kinase B (Akt) while downregulated the expression of glycogen synthesis kinase-3 β (GSK-3 β) in T2DM rats(27). In addition, key proteins involved in AMPK-mediated gluconeogenesis were downregulated in GA-treated T2DM rats. In summary, the hypoglycemic mechanisms of gymnemic acids may be related to promoting insulin signal transduction and activating PI3K/Akt- and AMPK-mediated signaling pathways in T2DM rats(27).

Clinical Research Support – Glycemic Control

In a randomized, double-blind placebo-controlled clinical trial was conducted in 30 patients with impaired glucose tolerance (IGT). Half of the patients randomly received *Gymnema sylvestre* in doses of 300 mg twice per day, while the other half received placebo. Measurements were taken before and after intervention(28).

There was a significant reduction in 2-h oral glucose tolerance test (OGTT) (9.1 ± 1.2 vs. 7.8 ± 1.7 mmol/L, $P = .003$), A1C ($5.8 \pm 0.3\%$ vs. $5.4 \pm 0.4\%$, $P = .025$), body weight, body mass index, and low-density lipoprotein cholesterol levels in the *Gymnema sylvestre* group(28). At the end of the intervention, 46.7% of the patients obtained normal values in A1C. *Gymnema sylvestre* administration in patients with IGT decreased 2-h OGTT and A1C, increasing insulin sensitivity. There were also improvements in anthropometric measures and the lipid profile(28).

A systematic review and meta-analysis evaluated the effect of *Gymnema sylvestre* supplementation on glycemic control in T2DM(29). This meta-analysis consisted of 10 studies with a total of 419 participants that showed that *Gymnema sylvestre* supplementation significantly reduced fasting blood glucose (FBG) (SMD 1.57 mg/dl, 95% CI 2.22 to -0.93), postprandial blood glucose (PPBG) (SMD 1.04 mg/dl, 95% CI 1.53 to -0.54), and glycated haemoglobin (HbA1c) compared to baseline(29). Further, the study also found that *Gymnema sylvestre* significantly reduces triglycerides (SMD 1.81 mg/dl, 95% CI 2.95 to -0.66), and total cholesterol (SMD 4.10 mg/dl, 95% CI 7.21 to -0.99) compared to baseline(29).

The study shows that *Gymnema sylvestre* supplementation is effective in improving glycemic control and reducing lipid levels in T2DM patients and suggests that such supplementation might be used as an effective therapy for the management of T2DM and its associated complications to an extent(29).

L-Ergothioneine – Anti-aging Antioxidant

L-ergothioneine (ET) is a thiol form of the natural amino acid histidine that can be considered an anti-aging, super antioxidant compound. Thiol-based antioxidants are considered the most powerful antioxidants in nature. One of the most common thiol-based compounds is glutathione.

ET is also found naturally present in the body. Blood levels of ET start to decline with advancing age. Plasma ET levels continue to decline in elderly populations as evidence in Asian and Australian epidemiological studies, which showed that ET was inversely correlated with age(30). This decline in blood ET could be due to several factors such as increased turnover, diet changes or altered ET transporter function (ETT). This suggests that lower blood levels of ET are a risk factor for age-related disorders and promote health and longevity.

Although ET can be obtained from the diet, and is found in plants and foods including mushrooms, black beans, red meat, rye and oats, supplementation with ET could have a more profound effect on aging prevention.

Structure & Function of ET

Chemically speaking – ET is a tautomeric, which is a structural isomer that readily interconverts between forms. ET exists in the thione form, which makes it more resistance to oxidation, unlike the super antioxidant glutathione, which is rapidly oxidized. ET can scavenge ROS (reactive oxygen species), RNS (reactive nitrogen species) and even RCS (reactive chlorine species) protecting cells from damage(31).

In the body, ET is concentrated in cells and tissues frequently exposed to oxidative stress. It has also been shown that there are specific transporters for ET in the body, with abundant levels in tissues(32). ETT move ET throughout the body, via the blood, storing it in locations that are high in free radical activity. Cells lacking ETT are more susceptible to oxidative stress, resulting in increased mitochondrial DNA damage, protein oxidation, cytokine inflammatory response and lipid peroxidation(32). ET therefore acts as a strong antioxidant and anti-inflammatory compound. Additionally, it prevents lipid peroxidation and protects both mitochondria and DNA.

ET also acts as a senolytic, which destroys senescent cells in old tissues to rejuvenate, turning back the progression of age-related conditions. Senescent cells accumulate in many tissues with aging. ET works by clearing out damaged and dying cells that are just taking up space.

Super Antioxidant

ET has been shown to be a highly effective adaptive antioxidant. ET tends to accumulate at sites of tissue injury. This increase in ET accumulation seems to be related to an increased expression of the gene that encodes the ET transporter, and thus increase in transporter activity. Studies have suggested that ET plays an antioxidant role, but only when levels of free radical oxidation increase due to tissue damage.

In one study, ET supplementation resulted in a trend to decrease oxidative damage, but not in healthy tissue and only coming into play when oxidative damage becomes excessive due to tissue injury, toxin exposure or disease(32). In another study, ET supplementation resulted in ET levels in plasma and whole blood were significantly elevated and continue to elevate in the blood for up to 4-weeks after administration stopped. What's even more interesting is that ET retention in the body is high(33).

ET has also been found to have higher antioxidant capacity, particularly compared to other common antioxidants such as glutathione, CoQ10 and Vitamin C. A study showed that ET was the most active scavenger of free radicals as compared to the super antioxidant glutathione, and other antioxidants(34).

In another study, ET was found to eliminate all oxidants up to 3000% better than glutathione and decreases lipid peroxidation 200% better than glutathione³⁵. It also has a higher half-life, 5400% times longer than glutathione.

Compared with CoQ10, ergothioneine was more than twice as effective as CoQ10, when cells were exposed to a toxin and analyzed for their ability to limit lipid peroxidation. The study found ergothioneine was 270% better than CoQ10(36). An in vitro study showed that mushroom derived ergothioneine outperformed Vitamin C and glutathione in its scavenging ability against reactive species(37).

DNA Protection

ET has been shown to prevent free-radical induced DNA damage, by eliminating ROS, RNS and the two DNA-destroying acids hypochlorous and hypobromous –both of which are considered reactive chlorine species (RCS). Most antioxidants cannot protect against RCS.

RCS is a strong free radical that attacks mitochondrial DNA, producing mutagenic DNA lesions. These lesions can alter the DNA, resulting in wrong DNA encoding and messaging, producing the wrong proteins. ET can not only scavenge ROS but it can also protect and repair both nuclear and mitochondrial DNA.

In one in vitro study, it was shown that ET exposure of human derived cells, protects mitochondrial DNA from oxidative stress when ETT is not expressed(32). ET treatment effectively prevented free-radical induced DNA damage in a dose dependent manner. When ETT was removed, ET treated cells yielded much higher viability rates, a 40% increase, over those without treatment. ET can directly protect against DNA damage, intercepting damage induced by UV radiation before it could negatively affect cells. The ETT location therefore helps transport ET to defend against substances that could damage it, protecting cellular integrity.

Anti-Inflammatory Support

In addition to ET's antioxidant capacity, ET also has anti-inflammatory properties. ET has been shown to inhibit numerous pathways of inflammation including pro-inflammatory cytokines, interleukin-6 (IL-6) and IL-8, myeloperoxidase (MPO) and NfKappaB.

Multiple studies in vitro cell models have shown that ET can modulate levels of inflammatory cytokines and mitigates oxidative damage. In studies on mice, it was shown that ET provided protection against acute lung injury brought on by cytokines(38).

In another study on mice exposed to cigarette smoke for 6-months, with elevated numbers of inflammatory cells in the lungs and increased inflammatory cytokines, those with knocked out ETT had more severe reactions than those who didn't(39). Cells that express ETT accumulate higher levels of ET.

White Willow Bark – Anti-Inflammatory Control

White willow bark, also known as *Salix alba*, contains the active ingredient salicylate. Salicylates are hormones produced by plants in response to infection, which are critical in their defence against attack by pathogens⁴⁰. Salicylate and derivatives such as salicin are transported to neighbouring tissues, triggering defensive responses. Salicylates can therefore be regarded as the equivalent of cytokines operating in the plant version of the innate immune system.

White willow bark is an anti-inflammatory and analgesic. The active salicin is metabolized to salicylic acid, which is a non-selective inhibitor of cyclooxygenase (COX-1 and COX-2). This action blocks the production of prostaglandins, which cause pain and inflammation, raise blood pressure and body temperature⁽⁴⁰⁾.

In addition to white willow bark's action on COX, it can also target NF-κB, a transcription factor for the inflammatory process(40). Inhibiting NF-κB (IκB) retains NF-κB in the cytoplasm, preventing its translocation to the nucleus until IκB is phosphorylated by the kinase complex containing IκKβ, thus triggering IκB degradation.

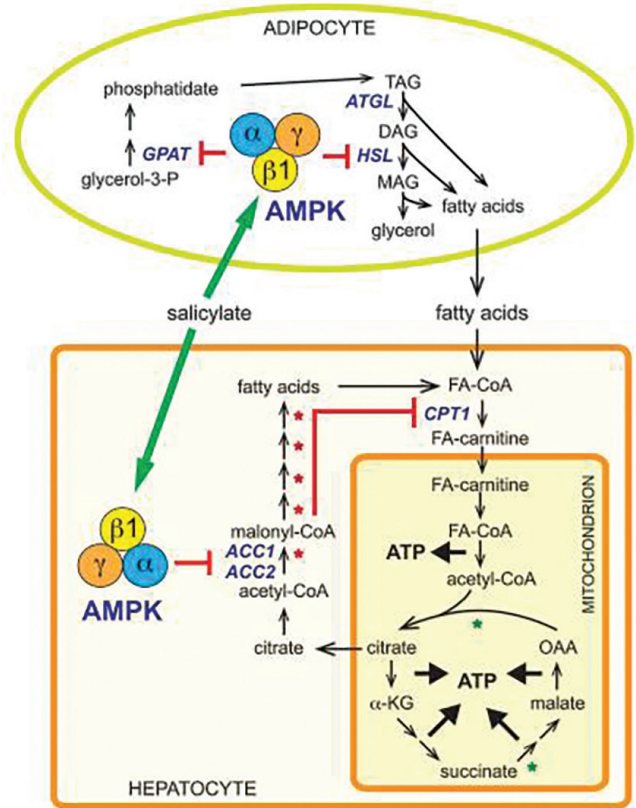
High concentrations of salicylates inhibit NF-κB function in B and T cell lines and inhibition of IκKβ was proposed as the mechanism. However, IκKβ inhibition by salicylate are competitive with ATP, so the effect is greatly reduced at normal physiological ATP concentrations.

Activation of AMPK by Salicylates

Salicylate has also been shown to be an activator of AMPK. Salicylate can uncouple mitochondrial respiration from ATP synthesis at higher concentrations. Treatment of mice with salicylate at doses that generated plasma concentrations of 1-2 mM activated AMPK and increased ACC phosphorylation in tissues expressing the β1 isoform, including liver and adipose tissue(41). This was associated with a reduction in circulating free fatty acids as well as in the respiratory exchange ratio, the latter signifying a metabolic shift from carbohydrate to fatty acid oxidation.

Importantly, these effects were eliminated in AMPK-β1 null mice, showing that AMPK activation is required for these effects of salicylate on lipid metabolism.

Figure 8: Effects of Salicylate on Lipid Metabolism Mediated by AMPK



Salicylate activates AMPK in both hepatocytes and adipocytes. In hepatocytes, AMPK activation causes phosphorylation of the ACC1 and ACC2 isoforms of ACC lowering malonyl-CoA(40). This inhibits fatty acid synthesis, while also promoting fatty acid oxidation by relieving inhibition of carnitine palmitoyl-CoA transferase-1(CPT1), thus helping to generate ATP(40).

AMPK promotes oxidative metabolism by increasing expression of oxidative enzymes, while downregulating lipogenic enzymes. In adipocytes, AMPK activation inhibits fatty acid synthesis by phosphorylation of ACC1 and triacylglycerol (TAG) synthesis by inactivation of glycerol phosphate acyl transferase (GPAT), while inhibiting TAG breakdown by phosphorylation of hormone sensitive lipase (HSL), the enzyme that converts DAG to monoacylglycerol (MAG)(40).

This reduces the release of fatty acids into the bloodstream – lipolysis. Salicylate reduces the accumulation of lipids in hepatocytes enhancing insulin sensitivity.

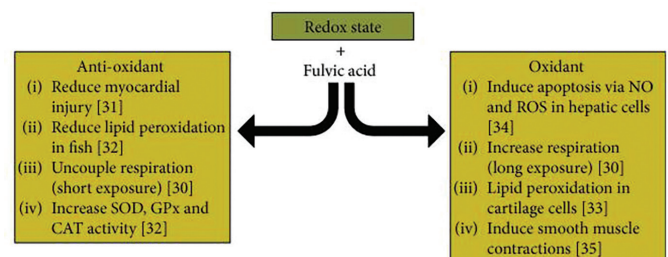
Fulvic Acid – Absorption & Chelation

Fulvic acid is a subclass of diverse compounds known as humic substances, which are by-products of organic degradation from microorganisms. Fulvic acid consist of small molecular weight, hydrophilic, carboxylic-containing molecules including a mixture of covalently linked phenolic, quinoid and benzene carboxylic acid compounds(42). Depending on where the fulvic acid is derived can change its composition of oxygen, nitrogen, aromatic ring and carbon content. Fulvic acid is soluble at all pHs, has a small molecular weight and a high oxygen content(42).

Anti-Inflammatory & Pro-Inflammatory

There is some evidence to suggest that fulvic acid can work as both an anti-inflammatory and a pro-inflammatory. In human monocyte models, fulvic acid was shown to reduce TNF- α expression after exposure to the endotoxin Lipopolysaccharide (LPS) (43). Fulvic acid was also shown to reduce COX2 and prostaglandin E2 (PGE2) secretion after homocysteine stimulation in primary human monocytes. Additionally, it can blunt interleukin release.

Figure 9: Redox Balance of Fulvic Acid



Fulvic acid may help with redox balance, allowing it to induce oxidation pathways – inducing apoptosis via NO and ROS, increase respiration, lipid peroxidation and inducing smooth muscle contractions; as well as antioxidant pathways by reducing lipid peroxidation, uncoupling respiration, and increasing SOD, glutathione and CAT activity(42).

Gut Health & Nutrient Absorption

Fulvic acid has been shown to influence the soil microbe composition and is able to conjugate or bind itself to various minerals increasing uptake in plants(42). Fulvic acid is therefore suggested to improve the gut flora, nutrient and mineral absorption and may help heal the gut.

Fulvic acid was shown to influence the bioavailability of heavy metals in animal models. Fulvic acid can increase the absorption of copper in porcine oviductal epithelial cells and reduce its toxicity via chelation(44). Fulvic acid has been shown to mediate drug delivery in rats as well. A low bioavailable drug, when conjugated to fulvic acid, increased absorption across the rat intestinal sac along with concentrations of the drug in blood plasma(45).

Another study showed the interaction between fulvic acid and transferrin (TF), a potential delivery agent for anti-cancer drugs(45). Results showed that fulvic acid binds to TF and forms a new complex. This study indicated a mechanism of the interaction between fulvic acid and TF, which may provide information for possible design of methods to deliver drug molecules via transferrin to target tissues and cells effectively(45).

AMPK Metabolic Activ8r™ – the Energy Regulator

AMPK is a key regulator of the metabolism, that is directly impacted by aging. As the body experiences metabolic slow down, due to stress and oxidative damage, AMPK sensitivity reduces, impairing downstream signaling and maintenance of the cellular energy balance. This results in the increase of inflammation, reduction of fat oxidation, resistance to insulin secretion, sensitivity, and poor glucose metabolism.

BioGenixUSA has created a unique AMPK formulation, AMPK Metabolic Activ8r™ to help switch on pathways to increase insulin secretion and sensitivity, increase fat oxidation via BAT activation, reduce post-workout muscle inflammation and provide antioxidants for protection against the aging process. This formulation delivers ingredients to support visceral fat metabolism, weight management, appetite support, blood glucose support, weight management and help regenerate the body, which helps reduce the aging process.

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