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*Review Article*

# AMPK: A POTENT TARGET FOR TREATING OBESITY

**Taseen Gul, Henah Mehraj Balkhi and Ehtishamul Haq***

Department of Biotechnology, Ground Floor, Science Block, University of Kashmir - 190006, Jammu & Kashmir, India.

**ABSTRACT**

Obesity, a direct consequence of imbalance between energy intake and expenditure by the body has become a substantial health and economic burden worldwide. It leads to a variety of metabolic disorders and demands immediate introspection due to its increasing prevalence. The obesity epidemic is progressing worldwide at an alarming scale, and the development of new therapeutic strategies is of vital importance. One of the key regulators of the whole body energy homeostasis which has become the prime focus of attention as a therapeutic agent for treating obesity is AMP-activated protein kinase (AMPK). The present report discusses the various natural and synthetic compounds that exert antiobesity effects through AMPK mediated mechanisms.

**Keywords:** Obesity, AMPK, AICAR.

## INTRODUCTION

Obesity is a state characterized by increased body weight, specifically adipose tissue of adequate magnitude that produces adverse health consequences such as dyslipidemia, insulin resistance, hypertension, type 2 diabetes (T2D), cardiovascular disease and certain cancers[1]. According to projections for 2015 around 700 million people will be obese worldwide and because of its increasing prevalence, World Health Organisation has labelled obesity as the new epidemic of the 21st century.[2] The currently used anti obesity drugs, Orlistat (lipase inhibitor) and Sibutramine (appetite suppressant) suffer from a number of drawbacks including cardiovascular and gastrointestinal symptoms, elevated heart rate, blood pressure, abdominal pain, dyspepsia, diarrhea, flatulence, bloating etc,[3,4] thereby demanding an urgent need to plan novel modalities for the treatment of obesity. AMP-activated protein kinase (AMPK), a master regulator of energy homeostasis is a potential target for therapeutic agents that may meet this challenge.

The AMPK is a serine/threonine protein kinase comprised of a catalytic alpha subunit and two regulatory subunits.[5] It is evolutionary conserved and plays an utmost role in maintaining energy metabolism, thereby occupying a distinctive and central place in studies of obesity and other metabolic disorders. The kinases, CAMKKβ ($Ca^{2+}$/calmodulin-dependent protein kinase kinase beta) through $Ca^{2+}$ dependent pathway, LKB1 (Liver kinase B1) through AMP dependent pathway and TAK1 (TGF-β-activated kinase-1) activate AMPK by phosphorylation on α subunit at Threonine 172. In addition, hormones also adjust AMPK activity, adding another tier of control by balancing the cellular energy demand and supply with respect to the whole body energy requirements. The AMPK has a distinctive role in energy metabolism as it senses cellular energy status through phosphorylation and allosteric activation and thereby restores cellular energy balance through direct phosphorylation of downstream targets, eventually switching on and off ATP generating and ATP consuming pathways respectively.[6,7]

## ROLE OF AMPK IN METABOLISM

***In Liver***: AMPK in liver maintains the energy homeostasis by inhibiting the expression of gluconeogenic genes thereby decreasing the process of lipogenesis and glucose production in the liver. Studies have revealed that AMPK suppresses the transcription factors, CRTC2 and

FOXO1. These transcription factors bind to the CRE of gluconeogenic genes, PEPCK and G6Pase and induce their transcription. AMPK downregulate these transcriptional factors and thus inhibits gluconeogenesis.[8] AMPK regulates lipid metabolism via phosphorylation of several enzymes Acetyl-CoA carboxylase 1and 2 (ACC1, ACC2) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase leading to inhibition of fatty acid, cholesterol synthesis and increased fatty acid oxidation.[9] The mitochondrial glycerol-3-phosphate acyl transferase (mtGPAT) inactivated by AMPK regulates the channelling of acyl-CoA towards β-oxidation and away from lipid biosynthesis.[10] AMPK activation down regulates transcriptional factors SREBP-1c and ChREBP suppressing the expression of lipogenic genes, Fatty acid synthase and ACC.[11] The effect of AMPK activation on hepatic glucose production, in cultured hepatocytes, as well as in animal models, has inferred that AMPK activation suppresses endogenous hepatic glucose production and loweres the plasma glucose levels.[12] Thus, AMPK activation, in the liver, results in stimulation of fatty acid oxidation, inhibition of gluconeogenesis and fatty acid, cholesterol and triglyceride synthesis.

***In Muscle*:** The major site for glucose uptake is the skeletal muscle,[13] and both the insulin dependent and insulin independent pathways stimulate the process.[14] During exercise, AMPK seems to be the primary mediator for the glucose uptake stimulated by insulin independent pathways. Chronic AMPK activation leads to an increase in muscle Glut4, hexokinase and inactivates glycogen synthase in skeletal muscle.[15,16] Moreover, the activation of AMPK increase the expression of mitochondrial genes in skeletal muscle via induction of PGC-1α, suggesting that AMPK-mediated mitochondrial improvement may overcome the insulin resistance as well as metabolic inflexibility associated with obesity and Type II diabetes.[17,18]

***In adipose Tissue:*** In adipose tissue AMPK regulates lipogenesis and lipolysis, decreases the fatty acid uptake and triglyceride synthesis and increases the fatty acid oxidation. AMPK activation decreases adiposity through a feedback mechanism, which leads to inhibition of lipolysis, an energy-consuming process for the adipocytes. Many adipocyte derived hormones such as leptin, adiponectin, reduce fat mass via activation of AMPK in adipocytes. Activation of AMPK in human adipose tissue also leads to an increased expression of adiponectin, a potent insulin sensitizer, in skeletal muscles.[19]

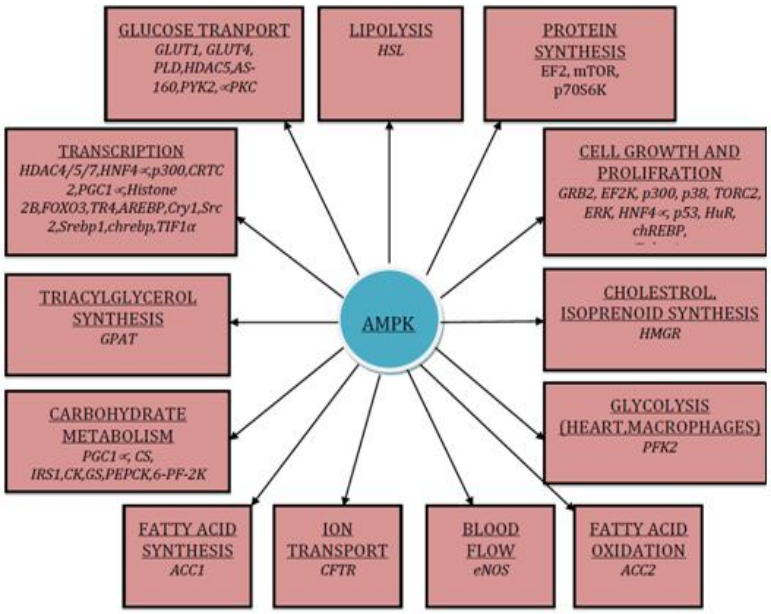


**Fig. 1: Physiological processes and genes regulated by AMPK**

AMPK mediates a number of physiological functions like carbohydrate and lipid metabolism, growth and differentiation, ion transport and blood flow, appetite regulation etc by regulating the genes (shown in italics).Abbreviations: TR4: testicular receptor4. p70S6K: p70 ribosomal S6 kinase. HuR: human receptor R. ACC: acetyl-Co-A carboxylase. AS-160: Akt substrate of 160 kDa. CFTR: cystic fibrosis transmembrane conductance regulator. ChREBP: carbohydrate responsive element binding protein. CK: creatine kinase. CS: citrate synthase. eNOS: endothelial nitric oxide synthase. ERK: extracellular signal-regulated kinases. GPAT: glycerol-3-phosphate acyltransferase. GRB2: growth factor receptor-bound protein 2. HDAC25: histone deacetylase 25. HSL: hormone sensitive lipase. HMGR: 3-hydroxy-3-methyl-glutaryl-CoA reductase. HNF4-α: hepatocyte nuclear factor 4α. IRS-1: insulin receptor substrate 1.p38: p38 mitogen-activated protein kinase. GS: glycogen synthase. PEPCK: phosphoenolpyruvate carboxy kinase.6-PF-2-Kinase: 6-phosphofructo-2-kinase. PGC-1α: peroxisome proliferator-activated receptor-γ-coactivator-1α.PYK2: proline-rich tyrosine kinase 2. SD: succinate dehydrogenase. SREBP-1: sterol regulatory element binding protein.GLUT: Glucose transporter. TORC2: target of rapamycin complex 2. HDAC5: histone deacetylase 5. FOXO3: forkhead box O3.HNF4α: Hepatocyte Nuclear Factor 4α. TIF: transcriptional intermediary factor. Src 1: steroid receptor coactivator 1. CRTS: cAMP-regulated transcriptional co-activator. AREBP: ARE-binding proteins. Cry 1: cryptochrome gene 1. mTOR: mammalian target of rapamycin.EF2: elongation factor. EF-2-K: elongation factor 2 kinase.

Thus, AMPK activation elicits a number of critical metabolic events (Figure-1), via modulating the expression of genes involved in energy metabolism and, therefore, has a potential to treat metabolic disorders like obesity.

## NATURAL ANTI-OBESITY COMPOUNDS ACTING BY MODULATING AMPK ACTIVITY

A wide range of natural compounds have shown promising results for targeting obesity through AMPK mediated mechanisms, the success to adopt such drugs in clinical practice will depend upon their rigorous long term toxicological screening. Some of these products and their mechanism of actions, are listed:

**α-lipoic acid (α-LA):** α-lipoic acid is a short chain fatty acid catabolizing decarboxylation of **α** keto acids.[20-24] It acts as a cofactor of mitochondrial enzymes, a potent antioxidant and used for the treatment of diabetic neuropathy. It regulates fuel metabolism, glucose transport and energy expenditure.[25,26] α-lipoic acid has shown to stimulate the whole body energy expenditure by significantly reducing plasma glucose levels, insulin and free fatty acids in blood of diet induced obese mice models. Incubation of C2C12 myotubes with α-lipoic acid has shown increased phosphorylation at Threonine 17 and, therefore, the activity of AMPKα2 subunit. Furthermore, the inhibition of CaMKK with STO-609 (a selective inhibitor for CaMKK) and silencing CaMKK expression abolished α-lipoic acid-stimulated AMPK activation, indicating that CaMKK mediates the α-lipoic acid induced AMPK activation in myotubes. However, in the hypothalamus, lipoic acid decreased AMPK activity leading to extreme weight loss in rodents by enhancing energy expenditure and reducing food intake.[27-29] α-LA decreases α2-AMPK phosphorylation, thereby activating ACC, a key enzyme in fatty acid biosynthesis. Thus, it follows that α-lipoic acid exerts anti obesity effects via regulation of AMPK activity.

**Berberine (BBR):** BBR is natural alkaloid isolated from *Rhizoma coptidis,* which has numerous biological, antimicrobial, metabolic and pharmacological properties.[30-33] BBR improves insulin action and glucose metabolism, enhances insulin sensitivity and reduces body weight and hyperlipidemia. BBR dependent metabolic changes, accompanied by change in hepatic and muscular gene expression pathways that enhance the fatty acid oxidation and reduce the lipogenesis. BBR remarkably elevates the expression of essential fatty acid oxidation genes such as Carnitine palmitoyl transferase1-α (CPT-1α), medium chain acyl-CoA dehydrogenase (mCAD), acyl-CoA oxidase (ACO), peroxisome proliferator-activated receptor-γ (PPARγ), and of uncoupling protein 2 (UCP2). Berberine also inhibits the mitochondrial function that increases the AMP/ATP ratio, thereby, explaining the activation of the AMPK pathway by Berberine and its

beneficial effects in the treatment of obesity and diabetes [34]. Studies on HepG2 cells and hamsters fed on high fat diet have inferred that BBR stimulate AMPK activity, fatty acid oxidation and relieves hyperlipidemia. Moreover, a number of conclusive *in vivo* and *ex vivo* experiments have shown that BBR induced effect in obese models involve the direct and indirect activation of AMPK in peripheral tissues for its effect on various metabolic processes including increasing fatty acid oxidation. BBR enhances phosphorylation levels of AMPK and ACC and stimulates AMPK activity in FAO cells (a rat hepatocyte cell line).[34-39] BBR increases fatty acid oxidation via AMPK activation, which in turn blocks differentiation of adipose tissue presumably via p38 MAPK–mediated phosphorylation of PPARγ.[30] Thus, BBR stimulates fatty acid oxidation and resets metabolic programs by directly modulating hepatic and muscular AMPK activity.

**Aloe:** Aloe species are known for their immunostimulant, anti-inflammatory, burn healing and antitumor activities. Aloe species significantly lower scavenger receptors like cluster of differentiation 36 and scavenger receptor A.[40-43] These receptors play a vital role in scavenging oxidized low density lipoproteins and also deliver long chain fatty acids. Once the expression of these scavenger receptors decreases, consequently adipocyte formation decreases. Aloe formulas have also shown to reduce expression of pro-inflammatory cytokines such as Interleukin-6 (IL-6), Interleukin-1β (IL-1 β), Nuclear Factor-Kappa B p65 (NF-κB p65), involved in the inflammatory response that ultimately cause the obesity induced metabolic disorders.[44-46] Processed Aloe Vera gel (PAG), an aloe formula, has shown a significant effect on hyperlipidemia and hypoglycaemia in *in vivo* models of obesity. Aloe formulas also increase mitochondrial biogenesis both in the white adipose tissue (WAT) as well as in muscles. They reduce obesity induced inflammatory response in muscles and suppress pro-inflammatory cytokines in WAT by activation of AMPK. This implies that, AMPK has a significant role in energy expenditure and metabolic disorders and, therefore, has a better potential to be used as a drug target for treating not only obesity but also obesity related metabolic disorders.[47,48]

**Reservatol:** Reservatol (3, 5, 4-trihydroxystilbene) is a naturally occurring polyphenol found in red wines and grape juice.[49,50] Reservatol has an extensive biological activity, and it has anti-cancer, ant-inflammatory and cardioprotective properties.[51,52] However, the convincing evidences from rodents fed on high fat diet and treated with Reservatol have shown its potential benefits in treating obesity. Reservatol diminished body fat content in mice fed a high fat diet and reduced the visceral fat and liver mass index in rats fed a high fat diet. Moreover, experiments on mice fed a high calorie diet demonstrated that Reservatol changed the expression of several genes and increased their survival and motor function. The proposed mechanism for Reservatol action indicates that NAD+ dependent protein deacetylase, Sirtuin 1 is necessary for its action. However, reports in high fat diet fed rats and obese Zucker rats have shown that Sirt1, is not the only factor, whereas some benefits of Reservatol also occur through activation of AMPK.[53-57]

**Cryptotanshinone:** Cryptotanshinone is a diterpene compound derived from *Salvia Militorrhiza Bungee*. Extracts from this herb harbour various biological activities and, thus used extensively for the treatment of variety of severe pathologies. Among these extracts, cryptotanshinone isolated from the dried roots treats several cardiovascular, haematological and metabolic diseases. It is one of the most active ingredients of the herb evaluated for treating various metabolic disorders including obesity.[58-60] It targets obesity via central loci of energy metabolism i.e. AMPK. Cryptotanshinone facilitates uptake of glucose, induces translocation of GLUT4 to the plasma membrane, however, long-term treatment with cryptotanshinone induced mRNA expression of GLUT1. Cells exposed to cryptotanshinone show increase in expression profiles of AMPK, p-AMPKα and ACC, an intracellular substrate of AMPK. Cryptotanshinone further enhances insulin induced glucose uptake with an increase in Akt activation which seems to be activated by AMPK upstream, with a parallel increase in expression of m-tor (mammalian target of rapamycin). This signalling cascade regulates significant physiological and cell regulatory processes. Cryptotanshinone may also have pathways in common with leptin or adiponectin for an activation of

AMPK. *In vitro* and *in vivo* studies of long term effects of cryptotanshinone on fatty acid metabolism genes results in suppression of ACC1, ACC2 expression and increase in carnitine palmitoyl transferase I m-RNA. Expression profiles of these genes involved in fatty acid metabolism are consistent with expression profiles on treatment with AMPK activators.[61-68] These findings thoroughly recommend that cryptotanshinone exerts potent antiobesity effects via AMPK, signifying potential of targeting AMPK for development of antiobesity drugs.

**Momordicosides:** Momordica Charantia L (bitter melon), a widely cultivated medicinal herb in many countries. The four cucurbitane glycosides (momordicosides Q, R, S&T), which are the principal chemical constituents of *M.Charantia,* have shown to exert hypoglycaemic effects in *in vivo* models.[69-74] These compounds have been extremely efficacious in stimulating GLUT4 translocation to membrane by increasing the phosphorylation of AMPK in both 3T3L1 adipocytes and L6 myocytes. These triterpenoids increase the phosphorylation of AMPK to a level similar to that of AICAR. Fatty acid oxidation and glucose disposal get enhanced by administering momordicoside S into HFD mice.[75-79] Results indicated that these triterpenoids, which might prove better therapeutics for treating obesity, also seem to work through activation of AMPK pathway.

**Phytoestrogens**: Phytoestrogens are biologically active plant substances and have shown protective effects against cancer, cardiovascular diseases, osteoporosis and chronic renal diseases.[4,80-85] Phytoestrogen such as Quercetin has shown to exhibit a wide range of functions including effects on adipogenesis in 3T3L1 cells by activating AMPK activity [86]. Studies have also reported the association between the capacity to reduce adiposity by phytoestrogen genistein and activation of AMPK.[87] Epigallocatechin-3-gallate, a major catechin of green tea, has shown anti obesity effects by suppressing adipose tissue formation in mice. Epigallocatechin-3-gallate has shown to suppress hepatic gluconeogenesis by AMPK activation, and the activation appears to be mediated by CaMKK.[88]

**SYNTHETIC ANTI-OBESITY COMPOUNDS ACTING BY MODULATING AMPK ACTIVITY**

**Metformin**: Metformin, an antihyperglycemic agent and widely used drug for treatment of type 2 diabetes.[89,90] The drug leads to weight loss in people suffering from Type 2 Diabetes. It limits the amount of glucose and increases muscle utilization of glucose in the liver and muscle respectively.[91-94] Metformin activates AMPK in hepatocytes, suppressing expression of lipogenic enzymes and inducing fatty acid oxidation. The activation of AMPK mediates a reduction in mRNA and protein expression of SREBP-1 (Sterol regulatory element binding transcription factor 1) which in turn downregulate fatty acid synthase (FAS), Spot-14 (S-14) in liver thus modulating circulating lipids contributing to lower hepatic lipid synthesis and fatty liver. The activation of AMPK by metformin by LKB1 in the liver to lower blood glucose levels indicate that AMPK activation mediates the beneficial effects of metformin.[95]

**Thiazolidinediones (TZD's):** TZDs are heterocyclic, PPARγ specific ligands, involved in regulation of energy homeostasis. They are clinically effective in improving glycaemia and ameliorating several risk factors for cardiovascular diseases. *In vitro* studies on rat EDL muscles treated with troglitazone showed a significant increase in phosphorylation of AMPK and ACC, along with a transient increase in the AMP/ATP ratio. TZD, such as rosiglitazone and pioglitazone, down regulate lipolysis as well as fatty acids in adipocytes and thus improve obesity related complications. Pioglitazone, used for the treatment of Type 2 diabetes also decreases triglycerides and C-reactive protein levels. It also lowers blood pressure and increases levels of HDL. Rosiglitazone also improves lipid abnormalities and sensitivity related to insulin. However, the release of adiponectin by adipocytes is probably the foremost effect of TZDs, leading to activation of AMPK in the liver to reduce glucose production.[96-98]

**AICAR:** 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) is a known activator of AMPK that induces allosteric changes in AMPK conformation and thereby leading to kinase activation. In 3T3L1 cells, AICAR inhibits adipocyte differentiation by down regulating key transcriptional factors

such as Sterol regulatory element-binding protein 1(SREBP1), CCAAT-enhancer-binding proteins(C/EBPα) and Peroxisome proliferator-activated receptor gamma (PPARγ) which strictly regulates adipocyte differentiation. AICAR proved remarkably effective in maintaining body weight and epididymal fat content, improving insulin sensitivity and glucose tolerance in diet induced obese mice models. However in rats, the chronic administration of AICAR resulted in significant changes, in skeletal muscle that included an increase in GLUT4 and glycogen stores, and increased activity of hexokinase and mitochondrial oxidative enzymes.[99-104]

**O2', O3', O5'-tri-acetyl-N6-(3-hydroxyl aniline) adenosine:** This drug named as WSO70117 is a synthetic compound and has shown lipid regulatory properties. Studies revealed that WS070117 improved lipid abnormalities associated with high fat diet (HFD)-induced lipid metabolism disorder. WS070117 treatment reduced body mass gain and significantly suppressed the increase of liver fat accumulation evoked by the HFD. WS070117 suppresses lipid accumulation possibly through activation of hepatic AMPK, which inactivates 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), thereby blocking the conversion of HMG-CoA to mevalonate and acetyl CoA carboxylase (ACC), a prominent rate-controlling enzyme involved in the biosynthesis of fatty acids. WS070117 treatment markedly reduced accumulation of neutral lipid content in OLA treated HepG2 cells suggesting that treatment with WSO7O117 block the progress of hepatocyte steatosis. In the liver tissue of HFD fed hamsters, WS070117 showed stimulatory effect on AMPK phosphorylation. Thus, the results suggest that WS070117 may prove a new pharmacological agent to treat or control lipid metabolism disorder and its anti-steatosis and anti-hyperlipidemia effects are attributed to the activation of AMPK.[105-113]

## DISCUSSION

The potential to reduce hypertriglyceridemia and elevated storage of triglycerides by inhibiting triglyceride and fatty acid synthesis, and stimulating fatty acid oxidation and also the ability to lower blood glucose by activation of AMPK suggests that modulators of AMPK kinase activity might prove effective remedy for treating obesity and related metabolic disorders. Reports have also shown that certain strains of mouse that are resistant to diet induced obesity (mice over expressing uncoupling protein-1 in white adipocytes, stearoyl-CoA desaturase-1 knockouts and mice over expressing uncoupling protein-3 in skeletal muscle) exhibit increased basal level of AMPK activity.[114-116] These findings have led to an intense interest in designing AMPK activators as potential therapies for type II diabetes and obesity. This review briefly introduces various natural and synthetic compounds for their potential antiobesity activities. The observational studies have shown that these compounds may treat obesity by reducing insulin resistance, adiposity, or by inhibiting lipogenesis or they may even target lipolysis in adipocytes or liver. A brief insight into the mechanism of action of these compounds indicates anti obesity effects to be mediated through AMPK. They either target AMPK directly or its upstream modulators, which in turn, change the expression of downstream targets of AMPK. Although these drugs have excellent therapeutic potential for treating obesity, but extensive *in vivo* studies need to be carried out to understand biological and molecular role of AMPK, and long term side-effects if any.

## CONCLUSION

AMPK plays a key role in regulating wide range of activities in lipid and glucose metabolism. The encouraging data from natural as well as synthetic anti-obesity compounds in preclinical studies acting by modulating AMPK activity suggest that targeting AMPK appears as a promising strategy for the treatment of obesity and related metabolic disorders. Although these compounds have some limitations with respect to dosage and specificity, alternative strategies for modulating AMPK should be developed for the treatment of obesity. Keeping in view the epidemic of obesity, the role of AMPK pharmacotherapy seems to be the most obvious armamentarium to combat the disease and attempts to develop novel therapies via AMPK mediated mechanisms are worthy of pursuit.

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