ATIC as a link between antirheumatic drugs and regulation of energy metabolism in skeletal muscle

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Abbreviations:
ADaM – allosteric drug and metabolite
AICAR – 5-aminoimidazole-4-carboxamide-1-β-D--ribofuranoside or 5-amino-4--imidazolecarboxamide riboside
AMPK – AMP-activated protein kinase
ATIC – aminoimidazole-4-carboxamide ribonucleotide formyltransferase/inosine monophosphate cyclohydrolase
IMP – inosine monophosphate
ZMP – N[1]-(β-D-5′-phosphoribofuranosyl)-5--aminoimidazole-4-carboxamide or 5-aminoimidazole-4-carboxamide-1-β--D-ribofuranosyl-5′-monophosphate

Abstract

Chronic inflammatory rheumatic diseases, such as rheumatoid arthritis, psoriatic arthritis, and systemic lupus erythematosus, increase the risk of developing insulin resistance, metabolic syndrome, and/or type 2 diabetes. While inflammation is thought to be a major mechanism underlying metabolic dysregulation in rheumatic diseases, antirheumatic drugs that exert direct metabolic effects in addition to suppressing inflammation, might be particularly useful to prevent metabolic complications. Here we review antirheumatic drugs, such as methotrexate, that inhibit ATIC, the final enzyme in the de novo purine biosynthesis, responsible for conversion of ZMP to IMP. Inhibition of ATIC results in accumulation of ZMP, thus promoting activation of AMP-activated protein kinase (AMPK), a major regulator of cellular energy metabolism and one of the most promising targets for the treatment of insulin resistance and type 2 diabetes. We focus especially on ATIC inhibition and AMPK activation in skeletal muscle as this is the largest and one of the most metabolically active tissues with a major role in glucose homeostasis. As an important site of insulin resistance, skeletal muscle is also one of the main target tissues for pharmacological therapy of type 2 diabetes. Finally, we review the metabolic effects of ATIC-inhibiting antirheumatic drugs and discuss whether these drugs might improve systemic glucose homeostasis by inhibiting ATIC and activating AMPK in skeletal muscle.

INTRODUCTION

Type 2 diabetes and cardiovascular diseases are highly prevalent and present a major public health challenge *(1)*. Chronic inflammatory rheumatic diseases, such as rheumatoid arthritis, psoriatic arthritis, and systemic lupus erythematosus, increase the risk of insulin resistance *(2)*, type 2 diabetes *(3)*, the metabolic syndrome *(4, 5)*, and/or cardiovascular complications *(6)*. Antirheumatic drugs suppress inflammation, but they are not all equally effective at reducing the risk of developing diabetes *(7, 8)* or cardiovascular events *(9)*. Some potent immunosuppressive and anti-inflammatory drugs, such as glucocorticoids or calcineurin inhibitors, may even increase the risk of metabolic dysregulation *(8, 10, 11)*. Given the high prevalence of metabolic as well as rheumatic diseases, drugs that help to maintain metabolic homeostasis and reduce the risk of metabolic complications would be particularly beneficial.

Skeletal muscle accounts for ~40% of body weight and 20–30% of basal oxygen consumption and is the largest metabolic tissue under the physiological conditions *(12)*. In type 2 diabetes, insulin resistance impairs insulin-stimulated glucose uptake and glycogen storage in skeletal muscle, thereby contributing to development of hyperglycaemia *(13–15)*.

Pharmacological agents that decrease insulin resistance and/or stimulate glucose uptake independently of insulin would therefore be useful for treatment of type 2 diabetes. In this respect, activation of AMP-activated protein kinase (AMPK) is one of the most promising strategies to improve metabolic dysregulation in skeletal muscle *(16–18)*. Activation of AMPK enhances insulin action and stimulates insulin-independent glucose uptake in skeletal muscle, thus improving metabolic homeostasis and opposing development of type 2 diabetes *(19, 20, 21, 22, 23, 24)*. However, most experimental AMPK activators that have been discovered or developed so far do not efficiently target AMPK isoforms which are expressed in skeletal muscle *(25–27)* or have poor pharmacokinetic properties *(28)*, highlighting the need for new approaches towards AMPK activation in skeletal muscle.

Interestingly, several antirheumatic drugs, including salicylate and methotrexate, have been shown to promote activation of AMPK *(29–31)*. On the other hand, both drugs were shown to be inhibitors of 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/inosine monophosphate (IMP) cyclohydrolase (ATIC) *(32–35)*, which is recognized as a promising target in development of new antidiabetic compounds *(30, 36)*. Anticancer drug pemetrexed, a compound related to methotrexate, also inhibits ATIC and activates AMPK *(37)*, but it is not used for treatment of rheumatic diseases and will not be discussed here. Here, we will review the evidence whether antirheumatic drugs might promote metabolic homeostasis in skeletal muscle by inhibiting ATIC.

AMPK as a pharmacological target in skeletal muscle

AMPK is a major cellular energy sensor and regulator of cellular metabolism *(38–41)*. AMPK is a heterotrimeric serine-threonine kinase comprising the catalytic α (isoforms α1 and α2) and the regulatory β (isoforms β1 and β2) and γ (isoforms γ1–3) subunits *(41, 42)*. AMPK senses cellular energy status primarily by monitoring changes in the AMP:ATP ratio *(41, 43)*, although changes in the ADP:ATP ratio also contribute *(44)*. Both AMP and ADP bind to the γ subunit and activate AMPK by promoting phosphorylation of AMPKα Thr172. In addition, AMP, but not ADP, also causes allosteric activation of AMPK *(40, 44–46)*. AMPK can also be activated independently of changes in adenine nucleotides *(47)* by an increase in cytoplasmic Ca^{2+} *(48–50)* or by a decrease in intracellular glucose concentration *(51, 52)*.

Numerous pharmacological activators of AMPK have emerged in the last three decades (reviewed in detail in *(18)*). Based on their mechanism of action, they can be divided into three major groups (Table 1). The first group comprises direct activators that bind to or close to the AMP-binding site. The prototypical representative of this group is AICAR (5-aminoimidazole-4-carboxamide-1-β-
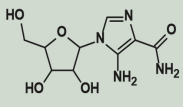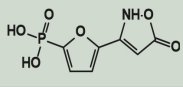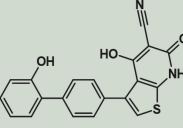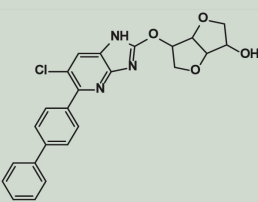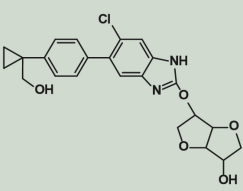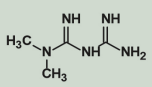
D-ribofuranoside or 5-amino-4-imidazolecarboxamide riboside), an adenosine analogue and the oldest and the most widely used experimental AMPK activator. AICAR is actually a prodrug that is intracellularly phosphorylated to ZMP (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl-5'-monophosphate), which directly binds to AMPK and activates it *(53)* (see below: *Intracellular metabolism of AICAR and ZMP*). ZMP binds to the AMP-binding sites on the γ subunit *(54)* and this is required for its ability to activate AMPK *(47)*. C2 is another pharmacological compound that binds to the γ subunit and activates AMPK (Table 1) *(55)*; however, it does not bind to the nucleotide-binding sites but next to them *(56)*.

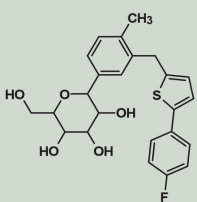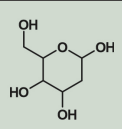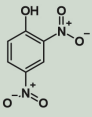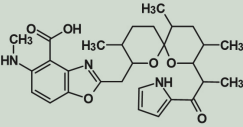To avoid ambiguity, it has to be stressed that different nomenclatures are in use for AICAR (a nucleoside) and ZMP (a nucleotide). Indeed, confusingly, different research fields have adopted different nomenclatures. Thus, AICAR is sometimes used to denote the nucleotide form ZMP *(57–63)*. However, in the vast majority of research literature on AMPK, the term AICAR (aka Acadesine) *(64)* refers to the non-phosphorylated precursor (nucleoside) of ZMP *(16, 18, 53)*. ZMP *(65, 66)* has also been referred to as AICAR-monophosphate *(37, 67)*, AICAribotide *(68, 69)*, Acadesine 5'-monophosphate *(64)*, as well as a Z-nucleotide *(66, 70)*. Correspondingly, AICAR can be referred to as Z-nucleoside, Z-riboside, and AICAriboside *(28, 59, 61, 63, 66, 69)*. The letter Z denotes 5-amino-4-imidazolecarboxamide (AICA, Z-base) based on the nomenclature for 5-amino-4-imidazolecarboxamide (Z) nucleotides established in 1980s *(65, 66, 70)*. Here we will follow the convention of the AMPK field and we will strictly use ZMP for the nucleotide and AICAR for the corresponding nucleoside.

The second group of AMPK activators comprises direct activators that bind outside the AMP-binding sites (Table 1), such as A-769662 *(25, 71)* and salicylate *(31)*. AICAR (as ZMP), A-769662, and salicylate activate AMPK allosterically and by stimulating phosphorylation and/or inhibiting dephosphorylation of Thr172 *(25, 31, 41, 47, 53, 72)*. However, while ZMP binds to the AMP-binding sites on the γ subunit *(54)*, A-769662 and salicylate bind to a specific pocket between the α and β subunits termed the allosteric drug and metabolite (ADaM) site *(73)*. Other compounds that activate AMPK by binding to this site include MK-8722 *(74)* and PF-739 *(75)* (Table 1).

The third group comprises indirect activators, which activate AMPK by inhibiting energy metabolism or by increasing intracellular Ca^{2+} concentrations (Table 1). Inhibitors of energy metabolism increase the AMP:ATP ratio, which results in AMP-stimulated AMPK activation via the γ subunit *(47)*. An increase in the AMP:ATP ratio underlies or at least contributes to AMPK activation by anti-diabetic drugs metformin and canagliflozin, which inhibit complex I of the respiratory chain *(76–80)*, as well

Table 1. Direct and indirect pharmacological AMPK activators. *ADaM site: allosteric drug and metabolite site.*

Group	Pharmacological activator	Chemical structure	Site of action (comments)
Direct activators that bind to or close to the AMP-binding site	AICAR		AMPK: AMP-binding site
	C2		AMPK: close to the AMP-binding site
Direct activators that bind outside the AMP-binding site	A-769662		AMPK: ADaM site
	Salicylate		AMPK: ADaM site
	MK-8722		AMPK: ADaM site
	PF-739		AMPK: ADaM site
Indirect activators	Metformin		Mitochondria (inhibits mitochondrial respiration and increases the AMP:ATP ratio)
	Canagliflozin		Mitochondria (inhibits mitochondrial respiration and increases the AMP:ATP ratio)
	2-deoxyglucose		Glycolysis (inhibits glycolysis and increases the AMP:ATP ratio)
	Dinitrophenol		Mitochondria (uncouples mitochondria and increases the AMP:ATP ratio)
	A23187		Plasma and organelle membranes (increases intracellular [Ca2+])

as experimental compounds, such as 2-deoxyglucose, which inhibits glycolysis, and dinitrophenol, which uncouples mitochondria *(47)*. Other indirect AMPK activators, such as Ca^{2+} ionophore A23187, increase intracellular Ca^{2+} concentrations, thus leading to AMPK activation via Ca^{2+}/calmodulin-dependent protein kinase kinase 2 *(47–50)*. Some compounds act by more than one mechanism. For instance, salicylate is a direct AMPK activator *(31)* as well as a mitochondrial uncoupler *(81)* and inhibitor of ATIC *(32)*.

Once activated, AMPK stimulates ATP-generating catabolic processes and inhibits ATP-consuming anabolic processes *(38, 41)*. In rat skeletal muscles, activation of AMPK with AICAR increases glucose uptake and fatty acid oxidation *(19, 82–84)*. However, it should be noted that effects of AICAR depend on the nutritional state as well as muscle (fibre) type, AICAR being less effective AMPK activator in oxidative than glycolytic muscles *(22, 83, 85)*. Activation of AMPK and increase in glucose uptake after treatment with AICAR have also been observed in skeletal muscles of insulin-resistant obese rats *(86)* and human subjects with type 2 diabetes *(87)*. Further, AICAR suppresses endogenous glucose production and decreases plasma triglycerides and fatty acids in insulin-resistant obese rats *(86)*. Similarly, administration of AICAR in subjects with type 2 diabetes reduces hepatic glucose output and suppresses lipolysis, thus reducing plasma glucose and free fatty acid concentrations *(88)*. However, beneficial effects on glucose homeostasis were not paralleled by improvements in lipid profile in all experimental models *(89)*. Finally, activation of AMPK probably underlies increased insulin sensitivity after muscle contraction or exercise *(90)*. Taken together, these and many other studies *(19–24)* suggest that pharmacological activators of AMPK could be used in the fight against insulin resistance and type 2 diabetes *(16, 17)*.

Most experimental AMPK activators have one or more shortcomings that prevent them from being used as clinical treatments for insulin resistance and type 2 diabetes *(91)*. For example, AICAR has off-target effects, including modulation of other AMP-sensitive enzymes, such as fructose-1,6-bisphosphatase *(92)* and glycogen phosphorylase *(93)*, and poor oral bioavailability *(28)*. Notably, human studies demonstrated that even intravenous infusion of AICAR results in plasma concentrations (~0.16–0.18 mM) that are below the threshold for activation of AMPK in skeletal muscle *(88, 94)*. A-769662 also shows poor oral bioavailability *(71)* as well as off-target effects, notably inhibition of Na^+/K^+-ATPase *(95)*. Finally, A-769662 and several other AMPK activators that bind to the ADaM site, preferentially activate the β1-containing AMPK complexes *(18, 25)*. This makes them less effective AMPK activators in tissues that express predominantly the β2-containing AMPK complexes *(75)*, which includes skeletal muscle *(26, 27)*.

Intracellular metabolism of AICAR and ZMP

As well as a pharmacological AMPK activator, AICAR is an endogenous purine precursor of ZMP *(61, 96–101)*. AICAR enters the cell via nucleoside transporters *(102–105)* and is converted to ZMP (AICAR-monophosphate) by adenosine kinase *(53, 66)*. As well as from AICAR, ZMP can be synthesized from AICA, an adenine analogue *(37, 70, 106)*. Following uptake into the cell, AICA is converted to ZMP in the reaction catalysed by adenine phosphoribosyltransferase (APRTase), thus mimicking conversion of adenine to the AMP in the salvage pathway of purine synthesis *(37, 70)*.

Once ZMP is formed, it likely has four possible fates. First, it can be phosphorylated to ZDP and/or ZTP *(61, 66, 107, 108)*. Second, ZMP can be dephosphorylated back to AICAR in a reaction catalysed by 5'-nucleotidase *(61, 107)*. AICA *(109)* and AICAR *(96, 97, 99)* are measurable in urine, indicating that dephosphorylation of ZMP is important under physiological conditions. Third, some evidence suggests that ZMP can be converted back to N-succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR or sZMP) by adenylosuccinate lyase *(66)*. Finally, ZMP can be converted to IMP by ATIC in the last two steps of the *de novo* purine synthesis pathway (Figure 1).

The physiological role of ATIC

ATIC, encoded by the *ATIC* gene (also known as the *PURH* gene), is a bifunctional enzyme responsible for the catalysis of the last two steps in the *de novo* purine biosynthesis, *i.e.* conversion of ZMP to IMP. ZMP is first formylated to formyl-AICAR (FAICAR) by AICAR formyltransferase (AICARFT) which uses N^{10}-formyl tetrahydrofolate (10-CHO-THF) as the formyl donor and then FAICAR is converted to IMP by IMP cyclohydrolase (IMPCH; also known as inosinicase) *(110, 111)*.

ATIC was first isolated by Flaks *et al.* in 1957 from chicken *(110)*. In 1991, Ni *et al.* cloned and sequenced chicken ATIC cDNA, which was the first eukaryotic ATIC cDNA to be cloned *(112)*. Human ATIC cDNA was cloned and sequenced a few years later *(113–115)*. Cloning of ATIC cDNA enabled the use of the site-directed mutagenesis and production of large quantities of easily purifiable recombinant ATIC protein in bacterial expression systems, which opened the door for mechanistic and structural studies. Chicken ATIC was also the first ATIC with a determined protein structure. The structure was determined by Greasley et al. in 2001 *(116)*. This and other ATIC structures that followed, including the structure of human ATIC *(117)*, advanced the understanding of the mechanism of action of ATIC and existing ATIC inhibitors and aided in the design of more potent and specific ATIC inhibitors *(36, 118–121)*.

Under physiological conditions, Z-nucleotides and nucleosides are present only in low intracellular concentrations *(61, 70)*. Indeed, even during treatment with low AICAR concentrations ZMP can remain below detection level *(30)*. However, pharmacological inhibition of ATIC promotes intracellular accumulation of ZMP *(29, 30, 37, 58, 122)*. Further, deficiency of ATIC in humans results in marked intracellular accumulation of ZMP and high urinary concentrations of AICAR (AICA-ribosiduria) *(61, 123)*, which highlights that ATIC is essential for normal ZMP and AICAR turnover. Indeed, deficiency of ATIC in humans leads to severe phenotype, characterized by blindness, mental retardation, epilepsy, and dysmorphia, underlining the physiological importance of ATIC *(61)*. Increased intracellular concentrations of ZTP were also observed in subjects with the Lesch-Nyhan syndrome, which is characterized by deficient salvage pathway of purine synthesis, and 5-phosphoribosyl-1-pyro-

phosphate synthetase (Figure 1) overactivity *(70)*, which both increase flux through the *de novo* pathway. Taken together, these studies show that increases in Z-nucleotide concentrations can be expected when ATIC function is suppressed or activity of the *de novo* pathway is markedly increased.

De novo purine synthesis and ATIC in skeletal muscle

ATIC is expressed in cultured skeletal muscle cells and skeletal muscle tissue *(30, 104, 115, 124)*. Further, the *de novo* purine synthesis pathway is active in cultured skeletal muscle cells *(125–128)* as well as skeletal muscle *(125, 129, 130)*, indicating ATIC is functionally important for muscle physiology. Treatment with exogenous AICAR in dogs results in marked increase in muscle IMP concentrations *(59)*, which again indicates that ATIC is functional
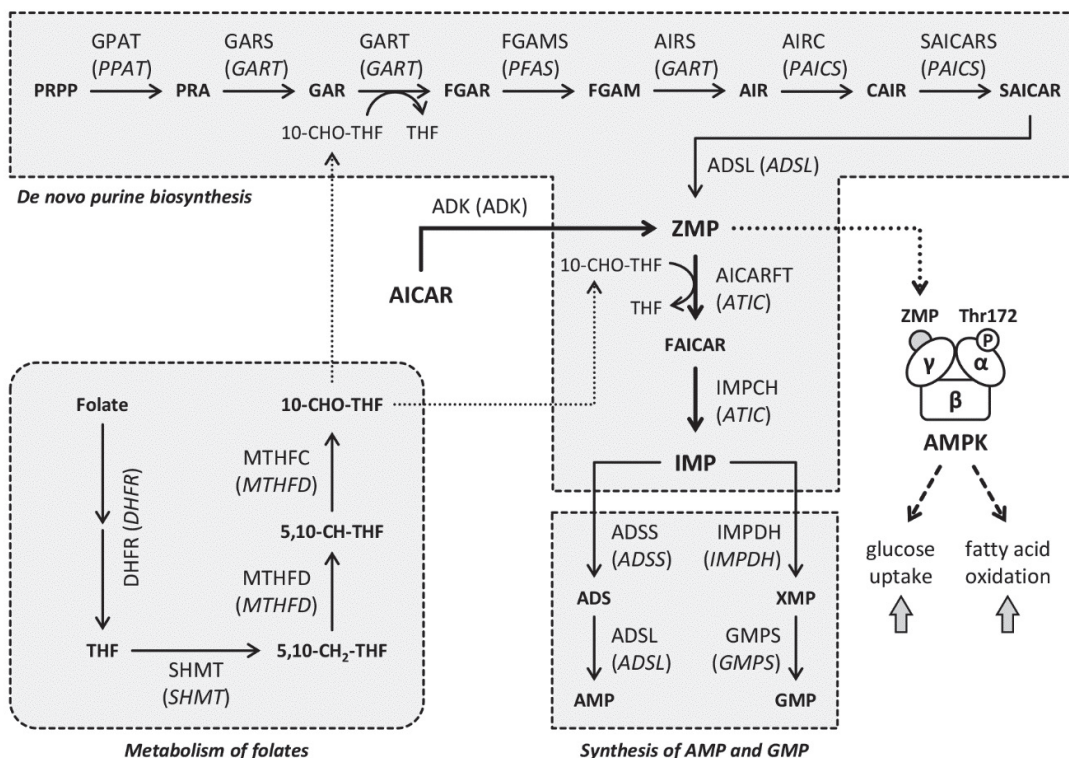

Figure 1. *De novo* **purine biosynthesis and AMPK activation.** *Full arrows indicate enzymatic reactions. Enzymatic activity and the gene encoding this enzymatic activity (in parentheses) are indicated for each enzymatic reaction.* **Intermediates:** *PRPP: 5-phosphoribosyl-1-pyrophosphate,* **PRA:** *phosphoribosylamine,* **GAR:** *glycinamide ribonucleotide,* **FGAR:** *formylglycinamide ribonucleotide,* **FGAM:** *formylglycinamidine ribonucleotide,* **AIR:** *5-aminoimidazole ribonucleotide,* **CAIR:** *carboxyaminoimidazole ribonucleotide,* **SAICAR:** *N-succinyl-5-aminoimidazole-4-carboxamide ribonucleotide,* **ZMP:** *5-aminoimidazole-4-carboxamide ribonucleotide,* **FAICAR:** *5-formaminoimidazole-4-carboxamide ribonucleotide,* **IMP:** *inosine monophosphate,* **ADS:** *adenylosuccinate,* **AMP:** *adenosine monophosphate,* **XMP:** *xanthosine monophosphate,* **GMP:** *guanosine monophosphate,* **THF:** *tetrahydrofolate,* **AICAR:** *5-amino-4-imidazolecarboxamide riboside.* **Enzymes: GPAT:** *glutamine phosphoribosylpyrophosphate amidotransferase,* **GARS:** *glycinamide ribonucleotide synthetase,* **GART:** *glycinamide ribonucleotide formyltransferase,* **FGAMS:** *formylglycinamidine ribonucleotide synthase;* **AIRS:** *aminoimidazole ribonucleotide synthetase,* **AIRC:** *aminoimidazole ribonucleotide carboxylase,* **SAICARS:** *succinylaminoimidazolecarboxamide ribonucleotide synthetase,* **ADSL:** *adenylosuccinate lyase,* **AICARFT:** *5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase,* **IMPCH:** *IMP cyclohydrolase,* **ADSS:** *adenylosuccinate synthetase,* **IMPDH:** *IMP dehydrogenase,* **GMPS:** *GMP synthetase,* **ADK:** *adenosine kinase,* **DHFR:** *dihydrofolate reductase,* **SHMT:** *serine hydroxymethyltransferase,* **MTHFD:** *methylenetetrahydrofolate dehydrogenase;* **MTHFC:** *methenyltetrahydrofolate cyclohydrolase;* **AMPK:** *AMP-activated protein kinase.*

in skeletal muscle. According to measurements in rat skeletal muscle, 0.3–1% of the total adenine nucleotide pool is turned over per hour *(131)*. However, the extent to which purines are synthesized via the *de novo* pathway depends also on activity of the salvage pathway, which suppresses *de novo* synthesis *(132)*. In skeletal muscle, *de novo* synthesis is thought to be particularly important after contractions, which result in a massive loss of adenine nucleotides from muscle into the bloodstream *(133)*.

ATIC as an entry point to modulate energy metabolism via AMPK in skeletal muscle?

ATIC is directly or indirectly suppressed by several antirheumatic drugs (Table 2): methotrexate, sulfasalazine, non-steroidal antirheumatic drugs (NSAID), and azathioprine. Interestingly, folinic acid (leucovorin), which is used to reduce methotrexate toxicity, also inhibits ATIC *(134)*. While all these drugs have several other targets, notably dihydrofolate reductase in the case of methotrexate and cyclooxigenases in the case of NSAID, suppression of ATIC is thought to be particularly important for antirheumatic actions of methotrexate and sulfasalazine *(57, 122, 135, 136)*. Methotrexate and sulfasalazine are especially interesting because they are widely used for chronic treatment of rheumatic diseases.

Antirheumatic treatment reduces the risk of diabetes in subjects with rheumatoid arthritis or psoriasis *(7)*. Suppression of inflammation likely represents one mechanism that underlies metabolic improvements with antirheumatic treatment *(144)*. However, direct metabolic effects of antirheumatic drugs could also contribute. Indeed, while all drugs used for treatment of inflammatory rheumatic diseases suppress inflammation and immune function, they are not all equally effective at reducing the risk of diabetes *(7, 8)*. The most effective seem to be inhibitors of tumour necrosis factor-α (TNF-α) and hydroxychloroquine *(7, 8, 145)*, neither of which acts via ATIC.

Dysregulated TNF-α signalling plays a major role in pathogenesis of rheumatic diseases and its suppression with biologicals, such as etanercept and infliximab, effectively suppresses their progression *(146, 147)*. Infusion of TNF-α opposes insulin-stimulated glucose disposal in humans *(148, 149)*. It is therefore not surprising that suppression of TNF-α in rheumatic patients protects against diabetes *(7, 8)*. Hydroxychloroquine is an antirheumatic and antimalarial drug that has recently been in focus of intense research efforts since it suppresses severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) *in vitro (150, 151)* and might be useful for treatment of coronavirus disease-19 (COVID-19), although its clinical effectiveness needs to be verified *(152–157)*. Use of hydroxychloroquine in rheumatic patients has been linked to improvements in metabolic status and protection against diabetes *(158)*. Mechanism of action of hydroxychloroquine involves inhibition of lyso-

somal activity, autophagy, and Toll-like receptor signalling, but how these effects lead to improvements in metabolic homeostasis has not been established *(159)*.

Although less potent as regards metabolic actions, methotrexate has also been rather consistently linked with at least mild improvements in glucose homeostasis and/or protection against diabetes (Table 3). Methotrexate (amethopterin) is a folate antagonist that was first used for treatment of cancer *(160)*. Anticancer effects of high doses of methotrexate, which may lead to peak plasma concentrations as high as 1000 µM (or more) *(161)*, are thought to be the result of inhibition of dihydrofolate reductase, which suppresses thymidylate and consequently DNA synthesis, although inhibition of ATIC and other enzymes also contributes *(34)*. In rheumatology, low-dose methotrexate is used *(63, 96, 146, 162)*, which produces peak plasma concentrations below 1 µM (~100–500 nM) *(163–165)*. During treatment with low-dose methotrexate, inhibition of ATIC is thought to be particularly important for antirheumatic effects of methotrexate *(57, 58, 166)*.

Sulfasalazine, a conjugate of 5-aminosalicylic acid and sulfapyridine, is another widely used antirheumatic drug *(146)*. However, compared with methotrexate, relatively few studies examined metabolic effects of sulfasalazine. Molecular mechanisms underlying its anti-inflammatory and immunosuppressive effects are complex *(167, 168)*, involving modulation of various cellular processes, including inhibition of ATIC *(32, 122)*. Interestingly, sulfasalazine has been suggested to reduce blood glucose concentrations in patients with type 2 diabetes *(169)*. Further, animal studies suggest sulfasalazine may protect against diabetic retinopathy and neuropathy *(167, 168)*.

An important question is whether antirheumatic drugs, such as methotrexate and sulfasalazine, can exert protective metabolic effects by inhibiting ATIC and promoting AMPK activation in skeletal muscle. There are at least four lines of evidence directly or indirectly supporting this notion. First, methotrexate was shown to activate AMPK or enhance AICAR-stimulated AMPK activation in cultured cancer and skeletal muscle cells *(29, 30, 104)*. Further, methotrexate enhances AICAR-stimulated AMPK activation and downstream metabolic effects in isolated mouse skeletal muscle *(30)*. Second, *in vivo* evidence supports the notion that methotrexate can activate AMPK in tissues. Indeed, methotrexate increased phosphorylation of AMPK not only in cultured human umbilical vascular endothelial cells, but also in aorta in mice *in vivo (194)*. Third, in the *db/db* mice methotrexate upregulated GLUT4 in skeletal muscle and reduced serum glucose and insulin concentrations *(174)*, which is consistent with muscle AMPK activation. Finally, Cpd14, a new experimental ATIC inhibitor, activates AMPK and improves glucose homeostasis in obese mice *(36)*.

As mentioned above, ATIC is not the only pharmacological target of methotrexate. Other targets are dihydro-

Table 2. Overview of antirheumatic drugs that inhibit ATIC. *K_i values may depend on the enzyme used in the assay (human or chicken) and on the assay conditions. Source of the enzyme is indicated in the table while assay conditions can be found in the references. References are listed chronologically. Abbreviations: DMARD – disease modifying antirheumatic drugs; NSAID – non-steroidal anti-inflammatory (antirheumatic) drugs; PBMC – peripheral blood mononuclear cell; 7-OH-MTX - 7-hydroxy-MTX (a major metabolite of MTX).*

Class	Drug	Inhibition of ATIC activity	Refs.
DMARD	Methotrexate (MTX), amethopterin	MTX increases urinary AICA excretion in patients with leukemia.	(137, 138)
		MTX-pentaglutamate is >2,000-fold more effective inhibitor of ATIC than MTX-monoglutamate.	(34, 35)
		MTX is a non-competitive inhibitor, while polyglutamylated MTX acts as a competitive inhibitor; polyglutamylated MTX inhibits chicken liver ATIC with K_i of 3.15 µM.	(33)
		MTX produces ZMP accumulation in cultured MCF-7 cells.	(139)
		MTX (in low concentrations) produces ZMP accumulation in malignant lymphoblasts.	(140)
		7-OH-MTX inhibits human ATIC (from MCF-7 breast cancer cells) with K_i of 0.03-180 µM (K_i depends on the folate cofactor and glutamylation of 7-OH-MTX).	(141)
		MTX inhibits chicken liver ATIC with K_i of 0.11 mM.	(32)
		Treatment with MTX increases ZMP concentration in murine splenocytes (*in vivo*).	(58)
		7-OH-MTX inhibits chicken liver ATIC with K_i of 133 µM.	(142)
		MTX increases urinary AICA excretion in patients with psoriasis.	(69)
		MTX increases urinary AICA excretion in patients with rheumatoid arthritis.	(63)
		MTX enhances ZMP accumulation in AICAR-treated MDA-MB-231 cells.	(29)
		MTX enhances ZMP accumulation in AICAR-treated skeletal muscle cells *in vitro*.	(30)
	Sulfasalazine (SSZ), sulphasalazine, alazosulfapyridine, salicylazosulfapyridine, salazopyrin, azulfidine, sulfazine, azopyrin	Sulfasalazine inhibits chicken liver ATIC with K_i of 22 µM.	(32)
		Sulfasalazine increases ZMP content in murine splenocytes (*in vivo*).	(122)
	Azathioprine, imuran	Azathioprine and its metabolite thioinosinic acid (TIMP) are competitive inhibitors of ATIC from chicken liver (K_i for azathioprine and TIMP are 120 and 39 µM, respectively) and mouse PBMCs (K_i for azathioprine and TIMP are 90 and 110 µM, respectively).	(143)
NSAID	Aspirin	K_i = 11 mM (chicken liver ATIC)	(32)
	Ibuprofen	Chicken liver ATIC is inhibited by 14–22% with 2 mM ibuprofen.	(143)
		K_i = 1.5 mM (chicken liver ATIC)	(32)
	Indomethacin	K_i = 0.35 mM (chicken liver ATIC)	(32)
	Mefenamic acid	K_i = 3.8 mM (chicken liver ATIC)	(32)
	Naproxen	Chicken liver ATIC inhibited by 32–46% with 2 mM naproxen.	(143)
		K_i = 0.99 mM, (chicken liver ATIC)	(32)
	Salicylic acid	K_i = 1.1 mM (chicken liver ATIC)	(32)
	Sulindac	K_i = 0.13 mM (chicken liver ATIC)	(32)
Folate	Leucovorin, folinic acid, 5-formyltetrahydrofolic acid	Leucovorin pentaglutamate inhibits human ATIC (from MCF7 cells) with K_i of 3 µM.	(134)

Table 3. **Metabolic effects of antirheumatic drugs that inhibit ATIC.** *References are listed chronologically. Abbreviations: RA - rheumatoid arthritis; PsA - psoriatic arthritis; T2D - type 2 diabetes.*

Drug	Metabolic effects	Refs.
Methotrexate (MTX)	MTX decreased glycogen content of the liver and glucose level and level of nonesterified fatty acids in the liver perfusate (experiment with isolated perfused rat liver).	(170)
	MTX activated glucose release from endogenous glycogen (glycogenolysis) (experiment with isolated perfused rat liver).	(171)
	MTX reduced risk of metabolic syndrome in RA patients older than 60 years.	(172)
	MTX did not significantly reduce HbA_{1c} concentration in diabetes patients with RA. (However, the study was not powered to detect a difference in MTX.)	(145)
	MTX treatment of RA or PsA was linked to reduced risk of developing diabetes.	(7)
	Obese mice treated with MTX displayed reduced serum levels of insulin and glucose, and an improvement of insulin sensitivity.	(173)
	MTX increased skeletal muscle GLUT4 mRNA expression and GLUT4 protein level and reduced serum glucose and insulin levels in diabetic (*db/db*) mice.	(174)
	Long-term MTX therapy was associated with a lower rate of dyslipidemia.	(175)
	MTX therapy and MTX-polyglutamates were associated with lower concentrations of HbA_{1c} in patients with RA.	(11)
	MTX reduced concentration of HbA_{1c} in patients with RA or PsA, but urinary AICAR or erythrocyte ZMP were not increased.	(96)
	MTX numerically (but non-significantly) reduced the risk of diabetes in RA patients.	(8)
	MTX in PsA patients did not appear to have hyperglycaemic effects (there were no significant changes between HbA_{1c} levels before and after MTX therapy).	(176)
Sulfasalazine (SSZ)	SZZ was linked to increased risk of hypoglycaemia and improved glycaemic control in T2D.	(169)
	SSZ prevented loss of retinal ganglion cells and degeneration of retinal capillaries in diabetic (streptozocin-treated) rats, indicating it protects against diabetic retinopathy.	(167)
	SSZ blocked development of tactile allodynia and ameliorated mechanical hyperalgesia in diabetic (streptozocin-treated) rats, indicating it protects against diabetic neuropathy.	(168)
Leucovorin	Leucovorin reduced glucose uptake and storage of glycogen in isolated rat diaphragm.	(177)
Naproxen	Naproxen reduced serum glucose levels and increased hepatic glycogen and serum insulin levels in normal and diabetic (streptozocin-treated) mice. Naproxen also reduced weight, serum glucose and resistin levels, while it elevated serum insulin, C-peptide, and adiponectin levels in obese mice.	(178)
Salicylate	Salicylate reduced glycosuria in a patient with diabetes mellitus.	(179)
	Salicylate reduced glycosuria and blood glucose concentrations in diabetic (alloxan-treated) rats.	(180)
	Salicylate reduced glycosuria and hyperglycaemia in rats treated with cortisone.	(181)
	Salicylate reduced liver glycogen content in mice.	(182)
	Salicylate caused hyperglycaemia in rats.	(183)
	Salicylate increased glucose uptake in perfused rat hearts.	(184)
	Salicylate increased plasma insulin levels and reduced plasma glucose levels in mild diabetic patients. Salicylate also improved glucose tolerance in these patients.	(185)
	Salicylate inhibited the development of diabetic retinopathy.	(167)
	Salicylate prevented fat-induced insulin resistance in rats: salicylate prevented lipid-induced decrease in whole body and skeletal muscle glucose uptake, skeletal muscle glycolysis and glycogen synthesis.	(186)
	Salicylate was shown to be a direct AMPK activator.	(31)
	Salsalate (a prodrug of salicylate) reduced HbA_{1c} in diabetic patients. Fasting glucose and triglyceride levels decreased with salsalate, but weight and low-density lipoprotein cholesterol levels increased.	(187)
	Salicylate activated AMPK, stimulated glucose uptake and decreased ATP, phosphocreatine, and glycogen contents in rat skeletal muscles.	(188)
	Salicylate uncoupled mitochondria and improved glucose homeostasis in mice independently of AMPK.	(81)
	Salicylate attenuated development of diabetic nephropathy in diabetic mice.	(189)

Aspirin	Aspirin abolished glycosuria and lowered the fasting blood sugar to normal or near normal in mild to moderately severe diabetic patients.	(190)
	Aspirin decreased serum glucose response and increased serum insulin response to oral glucose in normal and diabetic subjects. Aspirin also decreased fasting serum free fatty acids in normal and diabetic subjects and fasting serum triglycerides in diabetic patients.	(191)
	Aspirin prevented the increase in plasma glucose levels and attenuated the rise in insulin levels as well as insulin resistance in the glucose-fed rats.	(192)
	Aspirin inhibited the development of diabetic retinopathy.	(167)
	Aspirin attenuated insulin resistance in muscles of obese rats: aspirin reversed diet-induced decrease of insulin-induced Tyr phosphorylation of insulin receptor β and IRS-1 and Ser phosphorylation of Akt.	(193)

folate reductase, methylenetetrahydrofolate reductase, thymidylate synthase, as well as glycinamide ribonucleotide formyltransferase (GART) *(34, 57)*. Notably, GART is an enzyme upstream of ATIC in the *de novo* pathway of purine synthesis (Figure 1), whose inhibition would therefore tend to suppress *de novo* synthesis, reduce endogenous intracellular ZMP levels *(140)*, and AMPK activity. In its native (monoglutamate) form methotrexate is approximately equally effective inhibitor of GART and ATIC *(34)*. However, once inside the cell methotrexate is glutamylated and methotrexate-polyglutamates are much more effective ATIC inhibitors than GART inhibitors *(34)*. Due to formation of methotrexate-polyglutamates, exposure to low concentrations of methotrexate primarily inhibits ATIC, thus leading to intracellular accumulation of endogenous ZMP *(140)*. Consistent with this notion, low-dose methotrexate, which is used for treatment of rheumatic diseases, produces intracellular accumulation of ZMP in mice *(58)* and increases urinary excretion of ZMP metabolite AICA in subjects with rheumatoid or psoriatic arthritis *(63, 69)*. In contrast, exposure to high concentrations of methotrexate inhibits both enzymes, which blocks ZMP synthesis and accumulation despite ATIC inhibition *(140)*. Under these conditions, methotrexate alone does not activate AMPK, but it effectively enhances AMPK activation by exogenous AICAR *(29, 30, 104)*.

Finally, it needs to be emphasized that methotrexate, even when used in low dosages, might lead to toxicity, including suppression of the bone marrow, loss of hair, and liver fibrosis *(57, 195, 196)*. In addition, therapy with methotrexate may lead to a small increase in the risk of skin cancer *(196)*. Although adverse effects can be controlled to some extent by administration of folic or folinic acid (leucovorin) *(196–198)*, toxicity of methotrexate or related compounds would be a limiting factor in treatment of metabolic disorders. Nevertheless, patients who need methotrexate to treat their rheumatic disease might benefit from its metabolic effects.

CONCLUSIONS AND PERSPECTIVES

In summary, antirheumatic drugs that inhibit ATIC, such as methotrexate, might exert direct metabolic effects by promoting AMPK activation in skeletal muscle and other tissues. Activation of AMPK would tend to benefit patients with inflammatory rheumatic diseases by ameliorating metabolic dysregulation. Further, AMPK activation was linked to suppression of inflammation *(199, 200)*, indicating AMPK might be important for anti-inflammatory and immunosuppressive effects of these drugs. Several effective anti-inflammatory and immunosuppressive drugs promote metabolic dysregulation, especially when used in combination *(8, 10, 11)*. In contrast, antirheumatic drugs that also inhibit ATIC seem to be beneficial for controlling both inflammation and metabolic dysregulation. Development of new compounds with such characteristics might therefore be particularly relevant for patients with chronic inflammatory diseases and increased risk of metabolic dysregulation, including type 2 diabetes. Finally, since chronic low-grade inflammation plays a role in obesity and type 2 diabetes *(18, 201)*, compounds that simultaneously oppose both pathological processes might also be useful for treatment of metabolic diseases.

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