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ADENYLATE KINASE AND 5'-AMP-ACTIVATED PROTEIN KINASE CONTRIBUTION TO THE REGULATION OF SKELETAL MUSCLE ENERGETICS

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Abstract

It is well documented that adenine nucleotides (ATP, ADP and 5'-AMP) are the primary high-energy phosphate-carrying species in all living cells. There is a wealth of studies concerning the ADP role in the regulation of muscle energetic processes. The 5'-AMP contribution to the regulation of muscle metabolism was mostly discussed in the context of glycogen phosphorylase activity and glycogen breakdown as well as purine nucleotide cycle role in energy metabolism in contracting muscle. Numerous studies during last decade have indicated that 5'-AMP-producing adenylate kinase reaction and 5'-AMP per se play a fundamental role in the regulation of cellular metabolism under stress conditions in the living systems mostly due to 5'-AMP contribution to the activation of protein kinase named 5'-AMP-activated protein kinase (AMPK). At present AMPK structure, properties and regulatory mechanism are well recognized and confirm its key role as a metabolic signal activating catabolic processes in the face of increased energy demands (for example under fasting condition, during ischemia or contraction). In contracting skeletal muscle AMPK activity is elevated causing phosphorylation (i.e. inactivation) of a key enzyme of *de novo* free fatty acid (FFA) synthesis – acetylCoA-carboxylase. In consequence malonyl-CoA concentration - a potent inhibitor of FFA entry into mitochondria and its oxidation decreases promoting fat utilization but decreasing carbohydrate contribution to energy-providing processes. Additionally, in the muscle AMPK at least partially contributes to the regulation of exercise-induced glucose uptake and glycogen synthesis. There is a few studies indicating that persistent muscle AMPK activation in endurance-trained muscle induces mitochondrial biogenesis and increases the activity of mitochondrial enzymes, thus may be responsible for greater contribution of aerobic metabolism to overall energy production in trained muscle. Additionally, elevated AMPK activity affects the expression of genes encoding uncoupling protein-3 (UCP-3) and peroxisome-proliferator-activated γ coactivator -1 (PGC-1) – both contributing to fat metabolism in the muscle.

Key words: muscle, energetic demands, adenylate kinase, 5'-AMP-activated protein kinase

Introduction

The remarkable feature of striated muscle compared to other tissues is its ability to cope with rapid fluctuations in the metabolic rate. This ability is reflected by pronounced changes in ATP turnover in contracting vs. resting muscle. However, the changes in ATP content are much less indicating that skeletal muscles have evolved an accurate and sensitive metabolic control system (1).

It is well established that adenine nucleotides (ATP, ADP and 5'-AMP) are the primary high-energy phosphoryl-carrying species in all living cells. On the other hand, they are major regulator of ion channel

gating, gene expression and receptor – or protein kinase-mediated signal transduction and communication between mitochondria and nucleus with major role of their ratios and rates of exchange between different intracellular compartments (2,3,4,5,6,7,8).

However, despite a wealth of studies the mechanism govering adenine nucleotide metabolism under increased energetic demands of the muscle is far from being fully elucidated.

There is a plethora of studies concerning ADP role in the regulation of muscle oxidative phosphorylation (9,10). However, 5'-AMP contribution to the regulation of muscle metabolism was mostly discussed in the context of glycogen phosphorylase activity and glycogen breakdown as well as purine nucleotide cycle role in energy metabolism in contracting muscle (11,12,13,14). Numerous last decade studies have indicated that 5'-AMP-producing adenylate kinase reaction and 5'-AMP per se play a fundamental role in the regulation of cellular metabolism under stress conditions in the heart, liver and skeletal muscle (15,16).

Adenylate kinase and energy transfer in the muscle

Adenylate kinase (adenylate monophosphate deaminase - EC 2.7.4.3 - AK) by virtue of its ability to catalyze the reaction 2 ADP $\longrightarrow$ ATP + AMP has been considered a key enzyme in the synthesis and regulation of adenine nucleotides in the living cell. By regulating ADP and AMP levels, AK renders these nucleotides in metabolic signaling determining the cellular response to increasing energetic demands (17).

In noncontracting rat diaphragm muscle AK- catalyzed reaction provided approx. 7% of total ATP in contrast to 88% provided by creatine kinase-catalyzed reaction (18). However, a gradual CK inhibition (up to 98%) with 2,4-dinitrofluorobenzene resulted in elevated AK activity, 5'-AMP production and metabolic shift from CK system to AK system indicating their functional interrelation.

Increased adenylate kinase phosphotransfer rate (up to 12-fold) was proportional to contractile frequency and equivalent elevation in the rate of lactate producing glycolysis and depression in creatine kinase (CK) catalyzed reaction (19). Moreover, AK phosphotransfer was approx. 20-fold accelerated when mitochondrial oxidative phosphorylation was impaired due to oxygen deprivation or KCN action.

The above data clearly indicated that AK – catalyzed reaction in concert with CK-catalyzed reaction have capability to couple ATP utilization with its generation by glycolytic and/or oxidative processes depending on cell metabolic status.

Adenylate kinase structure and localization

Both human and animal skeletal muscle AK is a single polypeptide built of 194 amino acid residues and well known primary structure with an acetylmethionine at the N-terminus and a lysine at C-terminus and molecular weight approx. 22 kDa (20).

AK is a monomer consisted of approx. 60% of α-helix and stranded parallel beta-sheets (21). AK contains two distinct nucleaotide binding sites – Mg-ATP site which binds MgATP and MgADP and the AMP site, which is specific for AMP and uncomplexed ADP (22). It has been demonstrated that *in vitro* MgATP is bound to Lys21 orienteering the triphosphate chain of MgATP to a proper conformation required for catalysis. This interaction dictates the interactions between the substrate and the actives site residues of histidine (23). The enzyme is inhibited by potassium ferrate, an analog of orthophosphate and a potent oxidizing agent disrupting cysteine and

tyrosine residues (Cys-25, Tyr-95) essential for enzyme activity (24).

Comperative studies have indicated that AK activity is high in white muscle and somewhat lower in red muscle of mammals, including human (25).

Tanabe et al. (26) isolated cDNA for three adenylate kinase (AK) isoenyzmes in rat tissue – AK1, AK2 and AK3. Tissue-dependent activities of AK1 and AK2 paralleled the contents of mRNA. Moreover, tissue with high AK1 levels showed low AK2 levels and vice versa, suggesting that tissue-specific expression of the AK1 and AK2 genes are inversely regulated. AK3 was detected in most rat tissues suggesting that AK3 gene expression is constitutive.

It is worthy to note that tissues with high energy demand as skeletal muscle are particularly rich in isoenzyme AK1 – localized in the cytosol and clustered with myofibrills or bound to membranes (27-29). AK2 and AK3 are less abundant in skeletal muscle and are confined to the mitochondrial intermembrane space and mitochondrial matrix (30). Recently it has been found that there are two AK isoenzymes targeted to the mitochondrial matrix AK3 and AK4 (previously named AK3). New AK3 isoenzyme is highly expressed in the heart, skeletal muscle and liver and moderately in other tissue as pancreas and kidney (31).

AK distribution in different intracellular compartments implicates that enzyme contributes to the intracellular energy transfer and communication between ATP-consuming and ATP-producing sites (17,32, 33).

Adenylate kinase gene deletion and muscle energetics

The crucial role of AK in energy transfer within the cells is supported by findings concerning AK gene deletion and subsequent disruption in energy metabolism in the mice (34). In $AK^{-/-}$ animals decreased potential of myofibers to sustain adenine nucleotide ratio has been noted despite increased glycolytic, guanylate and creatine phosphate contribution to energy production. In addition, the maintainance of contractile activity in AK-gene depleted animals was associated with higher ATP turnover rate and larger amount of ATP consumed per contraction.

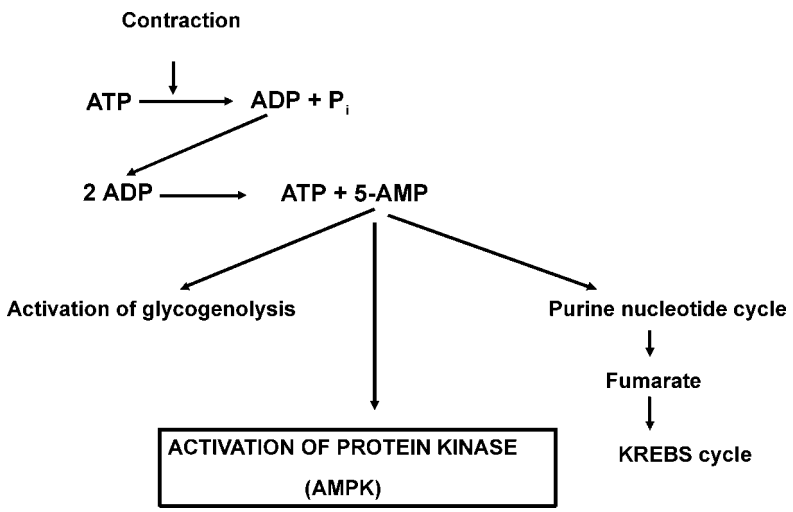

Fig. 1. *5'-AMP contribution to energy-providing processes in contracting muscle*

Furthermore, genetic ablation of AK1 gene brought about several metabolic adaptations in muscle metabolism as fiber-specific elevation in phosphotransfer capacities of 3-phosphoglycerate kinase and pyruvate dehydrogenase in fast twitch- but not in slow-twitch soleus muscle, concomitantly with increased mitochondrial adenine nucleotide translocator (ANT) and creatine kinase protein (35).

The above data indicated that AK1 deficiency caused substantial rearrangement in muscle energy- transfer system.

Adenylate kinase and heart energetics

It should be stressed that AK activity is of importance in the regulation of energy metabolism also in the cardiac muscle. In AK1 knockout animals energy transfer in the heart was markedly affected under ischemic conditions resulting in accelerated loss of contractile activity (36).

On reperfusion AK1 knockout hearts demonstrated lower ATP, GTP, ADP and GDP levels and decreased ATP/P_i and creatine phosphate/P_i ratio. However, AK1 knockout hearts still maintained approx. 40% of 5'-AMP turnover probably due to increased flux through other AK isoenzymes.

The above data enabled hypothesis to be made that adenylate kinase together with creatine kinase and glycolytic enzymes substantially contribute to energy metabolism in the muscle and other tissues functioning within phosphotransfer system securing energy homeostasis and cell economy under stress conditions (37).

A fundamental role of adenylate kinase-catalyzed reaction in energy homeostasis was even further supported by the studies which demonstrated that 5'-AMP is a potent activator of a specific protein kinase named 5'-AMP activated protein kinase – the enzyme recognized at present as a key regulator of cells' energetics at ATP deficiency.

5'-AMP-activated protein kinase as a key sensor of ATP depletion in the living cells

5'-AMP activated protein kinase (AMPK) structure and localization

AMPK belongs to a family of serine/treonine protein kinases that have been highly conserved throughout evolution since rat liver AMPK primary sequence has a remarkable degree of identity to protein

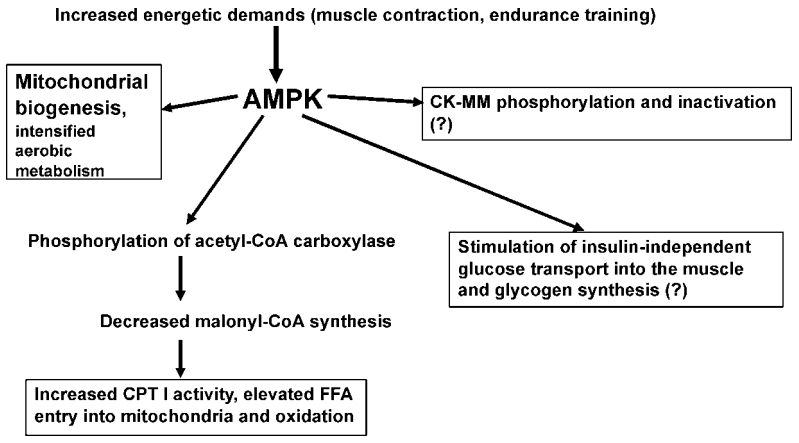

Fig. 2. *Exercise-induced contribution of AMPK to the regulation of muscle energetics*

kinases isolated from the yeast and plants (38-40). In the rat AMPK protein content is tissue-specific (41). In liver, kidney, brain, mammary glands, heart and lung AMPK mRNA levels ranged over 1-4 amol/μg total RNA. Adipose tissue contains less than 1 amol of AMPK protein per μg of total RNA. Relatively large amount of AMPK protein is expressed in the skeletal muscle (20 amol/μg total RNA) suggesting a substantial role of AMPK in skeletal muscle metabolism.

AMPK is a heterotrimeric complex composed of a catalytic α subunit with a molecular weight 62.3 kDa and two regulatory subunits ß and γ with a molecular weight 40 kDa and 38 kDa, respectively (42,43). It has been found that there are multiple genes encoding each subunit of which α and ß exist in two isoforms termed 1 and 2 and γ exist in three isoforms termed 1, 2 and 3 (44). AMPK complexes containing α2 catalytic subunit have a greater dependence of 5'-AMP than that containing α1 isoform. Interestingly, a significant proportion of α2 but not α1 is localized in the nucleus probably involved in the direct regulation of gene expression (45).

However, modification of ß subunit due to phosphrylation and miristoylation modulate enzyme activity and subunit cellular localization indicating pronounced complexity of AMPK regulation (46,47).

Regulation of AMPK activity

The enzyme is activated by a fall in the ATP/AMP ratio within the cell in response to metabolic stresses such as hypoxia, ischaemia, heat shock and pharmacological inhibition of glycolysis and oxidative phosphorylation as well as muscle contraction (3). In addition, the regulatory mechanism of AMPK activity involves allosteric activation by 5'-AMP possibly binding on the γ subunit (48). Additionally, AMPK can be activated by phosphorylation of Thr172 on the a catalytic subunits by an upstream protein kinase (AMPK kinase - AMPKK) and inhibited by dephosphorylation by protein phospatase such as PP-2C (49). It is worth noting that 5'-AMP binding to AMPK making it a better substrate for phosphorylation by AMPKK and a worse substrate for deactivation by PP-2C (50,51).

Furthermore, in vitro studies has indicated that phosphocreatine (CrP) inhibits AMPK in dose-dependent manner (52). In the presence of physiological CrP concentration of 20 mM the activity of AMPK was less than 50% of that found in the absence of CrP. Additionally, recent data have indicated that 48 h incubation of L6 rat skeletal muscle cells in the presence of 0.5 mM creatine brings about approximately 2-fold increase in the phosphorylation and activity of both α1 and α2 isoforms of AMPK (53).

The above data clearly indicate that AMPK activity within the cell is sensitive to the fluctuation in energy status since both elevated ATP breakdown and 5'-AMP accumulation and depleted phosphocreatine stores and increased creatine content bring about the elevation in enzyme activity.

In other words AMPK action shifts metabolic processes from anabolic to catabolic and protect the cell against energy deficit in the states of accelerated ATP utilization (48,49).

AMPK contribution to the regulation of liver and heart energetics

The contribution of AMPK to the regulation of energy-providing metabolic pathways was throughly studied in the liver. In isolated rat liver heat shock, arsenite treatment or physical exercise and subsequent ATP depletion activates adenylate kinase and induces the increase in 5'-AMP content – a potent allosteric activator of AMPK. In consequence 5-fold elevation in AMPK occurs. In addition, 5'-AMP activates AMPK by a phosphorylation of AMPK ki-

nase causing more than 20-fold enzyme activation. In consequence, the increase in 5'-AMP content causes approx. 100-fold overall activation of AMPK, which phosphorylates i.e. inhibits a key enzymes of fatty acid synthesis – acetylo-CoA carboxylase (ACC) and promotes ATP-providing FFA oxidation. By this mechanism, ATP is preserved protecting the liver against failure due to energy deficit (54).

There is a plethora of studies concerning AMPK role in the regulation of cardiac muscle metabolism which indicate that the elevation in AMPK activity due to increased AMP/ATP ratio is responsible for accelerated fatty acid oxidation observed during reperfusion of ischemic hearts (55-58). Recently it has been suggested that in humans dominant mutations in the γ2-regulatory subunit of AMPK are responsible for novel myocardial glycogen storage disease characterized by hyperthrophy, ventricular pre-excitation and conductance system defects (59).

AMPK and acetyl-CoA carboxylase

The above mentioned data have suggested that the relationship between AMPK activation in the face of decreased ATP/5'-AMP ratio in the liver and the heart promote fatty acid oxidation affecting a key enzyme of *de novo* fatty acid synthesis from carbohydrates – acetyl-CoA carboxylase (ACC).

Acetyl-CoA carboxylase catalyses carbohydrate-originated acetyl-CoA conversion into malonyl-CoA, a potent inhibitor of carnitine palmitoyltransferese I (CPT I) and acyl-CoA transport into mitochondria (60,61). In mammals ACC exists in two isoforms termed ACC-1 and ACC-2 (or ACC-α and ACC-ß). AMPK phosphorylates and inactivates both isoforms at the equivalent site.

Data from ACC knockout mice suggests that the malonyl-CoA – the product of ACC-catalyzed reaction produced by ACC-2 is exclusively involved in regulation of fatty acid oxidation, whereas that produced by ACC-1 is utilized in fatty acid synthesis. Activation of AMPK by cellular stress or exercise caused ACC-1 and ACC-2 phosphorylation thus decreasing both isoforms activity and malonyl-CoA synthesis and promotes FFA transport into mitochondria and oxidation (62,63).

Until now it is not clear if AMPK is also involved in phosphorylation and activation of malonyl-CoA degradating enzyme – malonyl-CoA decarboxylase (MCD) because data concerning the relationship between AMPK and MCD activities are contradictory.

Habinowski et al. (64) have not demonstrated the AMPK action on MCD activity in rat tissues. On the contrary, Ruderman et al. (65) have noted significant elevation in MCD activity in the muscle in response to AMPK activation and hypothesized that AMPK may regulate malonyl-CoA content by two strictly coordinated pathways - phosphorylation and inactivation of ACC-2 and depressing malonylo-CoA synthesis and phosphorylation and activation of MCD – promoting malonylo-CoA degradation.

However, despite all doubts concerning AMPK and MCD relationship at present it is well documented that AMPK- induced ACC phosphorylation and subsequent inactivation is effective as a signal promoting FFA transport into mitochondria and oxidation.

AMPK and regulation of energy-providing processes in striated muscle

As was mentioned earlier, in contrast to the liver and the heart, in skeletal muscle relatively large amount of AMPK protein is expressed, but under rest conditions enzyme activity is low in comparison with other tissues (e.g. liver) (42). However, at

present several reports describe a potential role for AMPK in the regulation of energy-providing pathway in the muscle under condition of increased energetic demands (e.g. exercise) and this function is perfectly consistent with the overall hypothesis for AMPK in energy conservation in the living systems (66)

AMPK and creatine kinase activity

In tissues characterized by high energetic demands (muscle, brain and heart) a close link between AMPK and creatine-phosphocreatine system has been noted (67). Both muscle and brain creatine kinase isoforms (CK-MM and CK-BB, respectively) undergo phosphorylation in the presence of AMPK. In case of CK-MM phosphorylation brought about approx. 60% inhibition of the enzyme activity and this effect was almost completely reversed by dephosphorylation of CK-MM by protein phosphatase 2C (PP-2C). Similarly, in differentiating muscle cells CK activity was markedly lower in the presence of AMPK activator – analog of adenosine – AICAR (5'amino-4-imidazolecarboximide ribonucleoside).

The above data taken together with mentioned earlier inhibition of AMPK by phosphocreatine and enzyme activation by creatine as well as stimulation of free fatty acid oxidation due to elevated AMPK activity, it seems feasible that AMPK plays a substantial role in anaerobic-aerobic transition in contracting muscle. ***In other words AMPK action protects the cell against energy deficit in the states of accelerated ATP*** and phosphocreatine ***utilization*** promoting mitochondrial ATP resynthesis.

AMPK activation and acetyl-CA carboxylase activity in the muscle

In vitro, in C2C12 myoblasts and in differentiated C2C12 myotubes, 5'-AMP analog – 5-amino-4-imidazolecarboxamide (AICA) – a potent AMPK activator, inhibited intracellular triacyloglycerol synthesis and increased free fatty acid oxidation (68). Additionally, AICA blocked insulin's antioxidative and lipogenic effects, increasing free fatty acid oxidation by 78% and decreasing triacylglycerol synthesis by 43%.

In electrically stimulated rat gastrocnemius muscle it has been found that the rapid decrease in acetyl-CoA carboxylase activity was caused by enzyme phosphorylation and was accompanied by reciprocal changes in the activity of $\alpha 2$ isoform of AMPK (69,70).

However, it should be pointed out that the effect of elevated AMPK activity on ACC and malonyl-CoA synthesis is strongly dependent on nutritional status. It has been demonstrated that AICAR administration significantly decreased ACC activity, malonylo-CoA levels and increased FFA oxidation in the muscle of fed rats, but was unable to stimulated high FFA oxidation and further decrease low malonylo-CoA seen in overnight fasted animals, despite marked inhibition of ACC activity in both fed and fasted animals (71).

Until know data concerning AMPK and ACC response to exercise in humans are fragmentary and equivocal.

Fuji et al. (72) has demonstrated that exercise-induced AMPK activation in skeletal muscle is isoform specific. In comparison to the resting state AMPK $\alpha 2$ but not $\alpha 1$ activity significantly increased at 20 and 60 min bicycle ergometer exercise at 70% $\dot{V}O_2$max and remained at a higher level with 30 min of recovery. On the other hand, no increase in AMPK $\alpha 2$ activity has been noted in same subjects exercising 20 min at 50% $\dot{V}O_2$max. Similarly, Wojtaszewski et al. (73) have shown that $\alpha 2$ isoform of AMPK in the *vastus lateralis* muscle markedly increased in response to 60 min cycling at 75% $\dot{V}O_2$max, but not following 90 min cycling at 50% $\dot{V}O_2$max. On the con-

trary it has been found that 30 s bicycle sprinting exercise brought about 2-3-fold increase in the *vastus lateralis* $\alpha 1$ and $\alpha 2$ AMPK activity, induced acetyl-CoA carboxylase phosphorylation at Ser 79 and decline in its activity (74). The above data indicated that exclusively exercise at a higher intensity leads to increases in AMPK $\alpha 2$ and this isoform may be involved in the metabolic responses to increased energetic demands in the working muscle.

On the contrary, Wojtaszewski et al. (75) have noted the elevation in tight muscle $\alpha 2$ AMPK activity during prolonged low-intensity exercise (3.5 h, 45% $\dot{V}O_2$max). In addition, in the above study the dissociation between AMPK activity and ACC phosphorylation in the muscle has been demonstrated, despite significant elevation in free fatty acid oxidation. The authors postulated that AMPK activation during prolonged exercise is not sufficient to maintain an increased ACC phosphorylation which peaked after 1 h of exercise and returned to basal level at the end of muscular work in contrast to AMPK which activity gradually increased during 3.5 h of exercise.

The elevation in both $\alpha 1$ (1.5-fold) and $\alpha 2$ (5-fold) isoforms activity has been also demonstrated in response to 20 min cycling at 40% and 59% $\dot{V}O_2$max but not in response to cycling at 79% $\dot{V}O_2$max (76). In addition, at lower intensities ACC phosphorylation and parallel increase in fat oxidation has been shown.

Wojtaszewski et al. (77) measured ACC-2 phosphorylation and substrate balance in skeletal muscle of male athletes at rest, during cycling (60 min, 70% $\dot{V}O_2$max.) and 60 min into recovery in relation to muscle glycogen content. In glycogen-depleted state at rest muscle $\alpha 1$ and $\alpha 2$ AMPK activity was respectively by 160% and 145% higher, ACC-2 phosphorylation at Ser 221 – by 137% higher than in glycogen-loaded state resulting in pronounced elevation in FFA oxidation. During exercise similar changes were observed, however no apparent differences in muscle high-phosphate content has been noted.

The above data indicated that human muscle AMPK activity and ACC-2 phosphorylation are sensitive to exercise intensity, but also and to the energetic status of the body and it may be a reason for contradictory results concerning AMPK response to muscular work of different energetic demands.

It is worth noting that elevated AMPK but depressed ACC-2 and increased free fatty acid oxidation may be of special importance in the face of increased energetic demands during muscular work shifting substrate utilization from limited carbohydrate stores to practically unlimited fat stores. Taking into account that glycogen is essential for overall energy-providing aerobic metabolism in contracting muscle, AMPK – activated free fatty acid oxidation possibly plays a pivotal role in substrate selection and sparing of muscle glycogen (78).

Endurance training effects on AMPK activity in the muscle

It is well recognized that endurance training brings about the shift from anaerobic to aerobic metabolism in the muscle (79). Recent data have demonstrated training-induced decrease in muscle malonyl-CoA content and increased free fatty acid entry into mitochondria and oxidation (for review see 80). However, details concerning the mechanism of training effects on muscle metabolism still remains elusive.

In the rat after endurance training significant depression in ACC activity leading to diminished malonyl-CoA synthesis and promoting free fatty acid entry into mitochondria and oxidation has been noted in red but not in white muscles. The analysis of AMPK isoforms in trained muscle revealed that after training there

was a 3-fold elevation in $\gamma 3$ isoform suggesting its special role in the adaptation of the muscle to endurance training (81,82). In addition, in trained rats attenuated AMPK response to a single exercise bout in red muscles has been found suggesting adaptational enzyme response to regular physical activity.

In endurance-trained men the level of $\alpha 1$ AMPK protein was by 85% higher than in sedentary individuals, whereas the levels of the remaining subunits ($\alpha 2$, ß1, ß2, $\gamma 1$, $\gamma 2$ and $\gamma 3$) were similar in trained subjects and sedentary controls (83). In addition, in endurance trained men AMPK activation in response to exercise was blunted (by 38%) compared with the sedentary group (by 253%). Similarly, less pronounced AMPK response to a single exercise bout in endurance-trained vs. untrained men (2.3 fold and 4.2 fold, respectively) has been found by Yu et al. (84).

In humans Langfort et al. (85) have noted that 4 weeks one leg training resulted in significant elevation in $\alpha 1$ AMPK protein content with no effect on $\alpha 2$ AMPK expression in trained vs. untrained leg. Similarly, Frøsig et al. (86) have revelaed that endurance training increases protein content of $\alpha 1$, ß2 and $\gamma 1$ subunit isoforms of AMPK (by 41, 34 and 26%, respectively) in the muscle of endurance trained leg. In contrast, the protein content of regulatory $\gamma 3$ isoform decreased by 62%, whereas no effect of training has been shown for $\alpha 2$, ß1 and $\gamma 2$ isoforms. Additionally AMPK activity associated with the $\alpha 1$ and $\alpha 2$ isoforms increased in trained muscle by 94 and 49% respectively.

The above data have indicated that both AMPK protein content and activity in human skeletal muscle are highly susceptibile to endurance training.

On the contrary, in well-trained athletes 3 weeks of high - intensity training (7 sessions of 8 x 5 min at 85% $\dot{V}O_2max$) did not affect AMPK activity and ACC phosphorylation in response to 90 min exercise, despite marked elevation in whole body fat utilization (87).

Assuming complicated nature of the regulation of human muscle energetics during contraction with pronounced contribution of nutritional status, muscle characteristics, physical fitness and training duration more studies are needed to established details concerning AMPK role in endurance training effects.

5'-AMP-activated protein kinase and carbohydrate metabolism in the muscle

AMPK and glucose transport

Most studies concerning the relationship between AMPK activity and glucose metabolism in the muscle focused on the regulation of cellular glucose uptake under basal conditions. There is a wealth of studies indicating that AICAR treatment and subsequent elevation in AMPK activity causes approx. 2-fold increase in glucose uptake by perfused rat hindquarter (88-90). Similar effect of AICAR on glucose transport has been noted in the isolated rat muscle in vitro concomitantly with close correlation between $\alpha 1$ and $\alpha 2$ AMPK activities and the rate of glucose transport (91).

The above data suggested that the elevation in AMPK activity plays a substantial role in the regulation of insulin-independent glucose uptake in the muscle. This mechanism is probably of special importance during muscle contraction when marked activation of AMPK activity occurs due to elevation in ATP breakdown and 5'-AMP accumulation in the cell.

It is well documented that in contracting muscle there is a pronounced elevation in glucose uptake in comparison with the resting conditions (92). Additionally, the effect of contraction is additive to insulin action suggesting different mecha-

nism of action of both stimuli (93). On the other hand, in electrically stimulated rat muscle AICAR treatment has no effect on 3-methyl-glucose transport suggesting similar mechanism of action of muscle contraction and AMPK stimulation (94).

Moreover, it has been noted that chronic AMPK activation brings about increased expression of glucose transporter GLUT- 4 and stimulates hexokinase II activity in rat muscles and in this way stimulate glucose uptake (95-97).

However, Derave et al. (98) have shown that the effect of AMPK activation on glucose transport is limited to fast-twitch muscle because in slow-twitch muscle the elevation in glucose uptake during contraction occurs without elevation in AMPK activity. Additionally, studies with mouse models with α1 and α2 AMPK genes deletion have indicated that α1 gene deletion does not affect glucose homeostasis (99). However, α2 deleted mouse presented high plasma glucose concentrations together with other anomalies concerning carbohydrate metabolism (low plasma insulin levels, insulin resistance and depressed muscle glycogen stores). Furthermore, α2 but not α1 AMPK gene deletion abolished AICAR-induced 3-deoxy-glucose uptake by the isolated muscles indicating that exclusively α2 subunit of AMPK is the main donor of AICAR-stimulated AMPK activity and is responsible for AICAR-induced glucose uptake. (100). On the contrary, neither α1 nor α2 gene deletion affected contraction-induced glucose uptake similar in wild and AMPK gene knockout mouse. In wild animals both AICAR treatment and contraction increased α2 and α1 AMPK activity. In a1 knockout mouse contraction induced the elevation in α2 AMPK activity, but the inverse was noted in α2 gene deletion.

The above data have demonstrated that α2 AMPK activation is necessary for the effects of AICAR on muscle glucose uptake, whereas during contraction the two a isoforms seem to substitute for each other in terms of activity, which may explain the normal glucose uptake despite the lack of either α1 or α2 AMPK in knockout animals.

AMPK and glycogen synthesis and breakdown in the muscle

Although there is a wealth of studies indicating that AMPK plays a role in increasing glucose uptake in contracting skeletal muscle, the role of AMPK in intramuscular glycogen metabolism is much less clear and not well understood.

AMPK and glycogen synthase activity

In vitro no effect of AICAR-induced AMPK stimulation of glycogen synthase activity in the rat muscle has been noted (101). On the other hand, *in vivo* AICAR treatment increases glycogen synthase activity more in white than in red rat muscles. Suprisingly, in both red and white muscles the elevation in glycogen phosphorylase activity has been found. Additionally, AICAR treatment results in increased glucose-6-phosphate content and glycogen stores in the muscle indicating that the effect of AMPK activation on glycogen synthase overwhelmed that on glycogen phosphorylase activity. Moreover, in the rat 5 days AICAR injection (1 mg/kg) resulted in 60-100% increase in GLUT-4 expression, 2.5-fold elevation in hexokinase II activity and 2-fold rise in glycogen content in the muscle (97).

Therefore, it could not be excluded that the elevation in AMPK activity affects glycogen stores mostly due to mass-action of increased glucose uptake. It should be pointed out that glucose-6-phosphate is a potent allosteric glycogen synthase (GS) activator. In this context AMPK-induced increase in hexokinase II activity and ele-

vated muscle glucose-6-phosphate possibly stimulate GS and affects glycogen synthesis.

The contribution of AMPK to the activation of GS has been confirmed in a mouse model in which muscle AMPK activity was specifically blocked ("lazy mouse") leading to depleted intramuscular glycogen stores and increased contraction-induced tiredness (102).

On the other hand, overexpression of the enzyme does not affect muscle glycogen concentrations (103). Furthermore, other study (104) has indicated that AMPK activation may contribute to the depressed glycogen synthase activity. In human myoblasts *in vitro* it has been noted that in the absence of glucose in the medium and low glycogen synthase activity AMPK is stimulated but the stimulation is abolished when glucose is added and glycogen synthase activity increases. Moreover, stimulation of AMPK by hydrogen peroxide or AICAR brings about the decrease in glycogen synthase activity. Additionally, using mouse models in which AMPK activity was specifically blocked (kinase dead) or knock out, it has been revealed that AMPK is a glycogen synthase kinase, phosphorylating and decreasing GS activity and glycogen stores (105).

It is worth noting that the inactivation of glycogen synthase in the presence of AICAR was eliminated by protein phosphatase treatment indicating that AICAR leads to phosphorylation (i.e. deactivation) of glycogen synthase in skeletal muscle (106).

Recently it has been demonstrated that AMPK ß subunit contains a functional glycogen binding domain and glycogen-bound AMPK is fully active (107). The above data indicated that there is an architectural link between AMPK and cellular glycogen and suggested a substantial role of AMPK in the regulation of glycogen metabolism.

However, it could not be excluded that AMPK contribution to the regulation of muscle glycogen stores is related to initial muscle glycogen concentration. It seems feasible since Wojtaszewski et al. (106) have found that both basal and AICAR-induced AMPK activity in the muscle is lower (by 50%) in animals fed high-carbohydrate diet and supercompensated muscle glycogen content in comparison with those with diet-and exercise-induced moderately lowered glycogen stores. In addition, both basal and AICAR-induced muscle 3-deoxy-glucose uptake and glycogen synthase activity were depressed in the muscle with high glycogen content. Because glycogen level did not alter adenine nucleotide concentrations, these data suggested that glycogen content in the muscle directly affect the activation state of AMPK.

The association between muscle glycogen stores and AMPK activity was also found in contracting muscle (108). In subjects with McArdle disease characterized by high intramuscular glycogen stores and depressed glycogenolysis, low-intensity muscular work resulted in elevated α2-AMPK but depressed GS activity in the muscle. In contrast, in control subjects no effect of muscle contraction on α2-AMPK activity has been noted, however GS activity was elevated in response to exercise. Additionally, neither GS kinase 3 – phosphorylating and deactivating GS nor protein phosphatase 1 – dephosphorylating and activating GS were affected by contraction. Thus, it has been postulated that muscle glycogen stores, AMPK and possibly protein kinase A (PKA) contribute to the regulation of GS activity and the resultant GS activity depends on the relative strength of various stimuli.

All the above data clearly depict complicated nature of the associations between AMPK activity and glycogen synthesis in the muscle. At least at rest and plentiful

glycogen stores AMPK activity is depressed, leading to decreased glucose transport and phosphorylation and glycogen synthesis is inhibited. In contrast, when muscle glycogen is low, AMPK is activated, glucose transport and phosphorylation is elevated contributing to increased glycogen synthesis. Therefore, it could be postulated that the relationship between glycogen synthesis and AMPK activities contribute to the autoregulation of glycogen synthesis in the muscle (109).

Paradoxically, in contracting muscle despite stimulated glycogenolysis and decreasing glycogen stores – glycogen synthesis is stimulated (108, 110). On the other hand, depressed glycogen stores and exercise per se elevate AMPK activity, so glycogen synthase may be potentially phosphorylated and deactivated.

However, it could not be excluded that a potent effect of contraction on glucose uptake, and phosphorylation and massive elevation in glucose-6-phosphate – allosteric GS activator, play more important role than AMPK-induced GS deactivation. It seems feasible, since it has been demonstrated that during exercise muscle glucose pool is mostly regulated by hexokinase II activity and the rate of glucose phosphorylation (111).

In this context, the contribution AMPK-induced GS phosphorylation and deactivation to overall glycogen metabolism in contracting muscle remain elusive and needs further studies.

The associations between AMPK and glycogen phosphorylase

Data concerning AMPK action on glycogen phosphorylase (GP), a key enzyme of glycogen breakdown are still fragmentary and equivocal. No effect of in vitro AMPK activation on GP activity has been demonstrated (101, 112,113). Suprisingly, *in vivo* the elevation in glycogen phosphorylase activity has been found in both red and white muscles of the rat. In addition, using purified enzymes preparations it has been found that phophorylase kinase, the immediate activator of GP is a potential target of AMPK (114). Moreover, in isolated rat muscle glycogen phosphorylase activity and net lactate release were elevated in response to AICAR treatment (115). However, muscle glycogen reserves in the presence of AICAR and AMPK activation are not reduced and this raised the question concerning AMPK action on GP activity. It could not be excluded that AMPK activation does not affect GP activity per se but rather increases glycolytic flux during exercise activating a key glycolytic enzyme – phosphofructokinase (PFK). This assumption has to be interpreted with caution since the stimulation of PFK-2 due to increased AMPK activity was studied in the heart but not in the striated muscle (63, 116).

It is not established if AMPK activate PFK-2 in the skeletal muscle, however rat hindquarter perfusion with AICAR has been shown to reduce (by approximately 50%) glucose oxidation. But this finding may also suggest that depressed glucose oxidation was rather due to pronounced stimulation of free fatty acids oxidation in the presence of AICAR (117).

This raises the possibility that AMPK action in the contracting muscle is due primarily to promotion of fatty acids oxidation, while the effects on carbohydrate metabolism is rather secondary and due to to AMPK-activated glucose uptake (118).

In the context of all above mentioned doubts and often contradictory findings, AMPK contribution to the regulation of carbohydrate metabolism in contracting skeletal muscle need further studies with precisely controlled muscle glycogen stores, glucose uptake, phosphorylation as well as evaluation of activity of a key enzymes of glycogen metabolism – glyco-

gen synthase and glycogen phosphorylase. To further explore the issue intramuscular triacyloglycerol stores and their contribution to fuel homeostasis in contracting muscle have to be carefully analyzed.

AMPK affects the expression of genes involved in energetic processes

The regulation of metabolic pathway in response to reduced cellular energy charge is one of the primary function of AMPK in different tissues. However, it is postulated that in addition to its well-characterized function in the cytoplasm, AMPK also directly phosphorylates and regulates proteins involved in gene transcription and expression (119). Although these AMPK effects are not a topic of present review they have to be mention to underline a special role of AMPK in the living systems.

Mitochondrial uncoupling protein (UCP) and AMPK

In isolated rat extensor digitorum muscle (EDL) the incubation with 2 mM of AICAR caused threefold increase in UCP-3 mRNA and 1.5-fold increase in UCP-3 protein compared with untreated muscle. Similar induction in UCP-3 protein was noted in response to running or swimming exercise (120). These data are consistent with the notion that activation of AMPK, presumably as a result of fuel depletion, rapidly regulates UCP-3 gene expression in the muscle. The contribution of AMPK to the regulation of UCP-3 expression in rat muscle was confirmed by Stoppani et al. (121) and Putman et al. (122).

A close link between UCP-3 expression and AMPK activity in the muscle indicated that AMPK regulation of energetic processes include transcriptional regulation of important components of energy-sensing systems (123).

Uncoupling protein (UCP) is a transporter family present in the mitochondrial inner memebrane and as its name suggests, it uncouples respiration from ATP synthesis by dissipating the transmembrane proton gradient as a heat.

UCP is now recognized as a key molecule in metabolic thermogenesis such as cold- and diet-induced heat production. Among the UCP family UCP-1 is expressed exclusively in brown adipose tissue (BAT), while UCP-2 is present in many organs and UCP-3 in skeletal muscle (124).

At present it is well documented that UCP-3 expression in the muscle is upregulated by elevated plasma free fatty acid concentrations (125). It is proposed that in skeletal muscle both UCP-2 and UCP-3 act in concert in the overall regulation of lipid oxidation probably exporting of non-metabolizable free fatty acid out of mitochondria (126,127).

However, it should be pointed out that increased UCP-3 mRNA expression in human muscle in response to AMPK stimulation may be secondary to well-known AMPK-induced elevation in free fatty acid oxidation rather than to the direct AMPK effects on UCP-3 mRNA expression. This assumption seems to be supported by Schrauwen et al. (128) who have indicated that exercise-induced UCP-3 mRNA expression is abolished when free fatty acid availability and oxidation is inhibited by glucose ingestion. Therefore, the relationship between UCP-3 expression and AMPK activity in the muscle and its contribution to overall energy metabolism under stress conditions need further studies.

Peroxisome proliferator-activated receptors (PPARs) association with AMPK cascade

Peroxisomes are cell organells with monolayer membrane. In humans they play a crucial role in ß-oxidation of free fatty acids containing more that 18 carbon molecules (129). Peroxisome proliferator-

activated receptors are members of the nuclear receptor superfamily that act as ligand-dependent transcription factors regulating gene expression and playing a critical role in endocrine signaling. Until know three distinct PPARs have been isolated termed α, δ, and γ. PPAR-α regulates the transcription of multiple genes encoding enzymes involved in the oxidation of fatty acid and the energy expenditure by fatty acid oxidation in the liver, adipose tissue and skeletal muscle, PPAR-γ is involved in glucose homeostasis and PPAR-δ contributes to adipocyte differentiation (130-133).

Data concerning the association between PPARs and AMPK activity in the muscle are fragmentary. However, it has been noted that the PGC-1 – peroxisome proliferator-activated γ coactivator -1 m-RNA expression in isolated rat muscle incubated for 18 h with AICAR was approximately 3-fold elevated in comparison with control muscle with concomitant 4-fold increase in AMPK activity (134). Additionally, the PGC-1 m-RNA expression and AMPK activity in the muscle was markedly elevated following prolonged exercise bout and *in vitro* electrical stimulation. These results may suggest that a single exercise directly enhances the PGC-1 m-RNA expression at least partially due to AMPK-related mechanism. Similarly, Suwa et al. (135) have demonstrated that chronic AICAR treatment for 14 days results in elevated PGC-1 protein levels in rat muscle.

Additionally, AMPK has been found to regulate HNF4-α - a nuclear receptor that regulates the expression of the genes involved in energy metabolism in the liver, intestine and endocrine pancreas (136). Furthermore it has been found that AMPK is involved in the regulation of glucose-activated gene expression in hepatocytes and endocrine pancreas (137,138).

The above data suggest that there is a direct link between AMPK - sensing the energy status of the cell and gene expression, however much more studies are needed to establish all details concerning in vivo AMPK- gene expression associations.

AMPK cascade and mitochondrial biogenesis in the muscle

Bergeron et al. (139) have noted that chronic deprivation of phosphocreatine, and ATP and decreased ATP/AMP ratio in rat muscle due to ß-guanadinopropionic acid (ß-GPA) ingestion brings about the activation of AMPK, increased mitochondrial density and the elevation in nuclear respiratory factor-1 (NRF-1) binding activity and cytochrome c content. The contribution of AMPK to mitochondrial biogenesis was further supported by Zhong et al (140) who have demonstrated that in transgenic mouse expressing a dominant-negative mutant of AMPK in the muscle and treated with ß-GPA neither AMPK activation nor increased mitochondrial density is observed. On the contrary, ß-GPA treatment of wild animals induces both AMPK activation and mitochondrial biogenesis. The above data indicated that AMPK - a sensitive marker of energy status of the muscle is a critical regulator of mitochondrial biogenesis

In the face of the above findings it seems feasible that chronic AMPK activation (e.g. during endurance training) is responsible for elevated mitochondrial density and increased aerobic capacity of endurance-trained muscle. Moreover, chronic AMPK activation due to 4 weeks AICAR treatment has been found to increase citrate synthase, succinate dehydrogenase and malate dehydrogenase activities in white quadriceps and soleus rat muscles (141). In consequence, it could not be excluded that chronic AMPK activation may be at least partially responsible for in vivo skeletal muscle adaptational response to endurance training.

In summary, numerous studies of the last decade indicated that the regulation of energetic processes in skeletal muscle is more precise than that presented in the academic books. Creatine-phosphocreatine system, anaerobic carbohydrate metabolism, aerobic carbohydrate and lipid pathway and their mutual interaction (e.g. glucose-free fatty acid cycle) did not depict all complicated interaction observed in the face of increasing energetic demands during contraction. Apart from well documented ATP/ADP role in the regulation of cellular metabolism, ATP/AMP ratio seems to be of special importance especially in the context of 5'-AMP-activated kinase role in the regulation of free fatty acid oxidation, glucose transport into the muscle, glycogen synthase and possibly creatine kinase activity as well as in mitochondrial biogenesis.

In consequence, 5'-AMP-producing adenylate kinase reaction, usually recognized as simple way of ATP resynthesis from ADP has to focused more attention as an important tool in energy-providing processes.

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