AMPK

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5′AMP-activated protein kinase (AMPK) is known as metabolic sensor in mammalian cells that becomes activated by an increasing adenosine monophosphate (AMP)/adenosine triphosphate (ATP) ratio. The heterotrimeric AMPK protein comprises three subunits, each of which has multiple phosphorylation sites, playing an important role in the regulation of essential molecular pathways. By phosphorylation of downstream proteins and modulation of gene transcription AMPK functions as a master switch of energy homeostasis in tissues with high metabolic turnover, such as the liver, skeletal muscle, and adipose tissue.

Keywords: lipotoxicity ; insulin resistance ; free fatty acids ; steatohepatitis ; type 2 diabetes ; skeletal muscle ; adipose tissue ; diacylglycerol

1. Introduction

The healthy liver displays remarkable metabolic plasticity with hepatocytes in concert with a variety of other cell types, readily switching between various metabolic tasks. These transitions are regulated by numerous factors including hormones, nutrients, and further influences. However, with the onset of non-alcoholic fatty liver disease (NAFLD), this highly balanced physiological homeostasis becomes compromised [1].

It was shown in vivo that de novo lipogenesis (DNL) contributes substantially to liver fat storage in humans [2]. One hallmark finding in that matter has been the discovery of the regulation of lipid metabolism by 5′AMP-activated protein kinase (AMPK) via phosphorylation and inactivation of acetyl CoA carboxylase (ACC), the enzyme catalyzing the first and rate limiting step in DNL [3][4][5][6]. Moreover, a close association of ectopic lipid deposition with insulin resistance is well recognized. This supports the hypothesis of a crucial role of AMPK as molecular node between the epidemic pathologies obesity, type 2 diabetes (T2D), and NAFLD [7][8][9][10][11][12][13][14].

2. Structure and Physiological Regulation of AMPK

Eukaryotic cells adapt to environmental energetic fluctuations by constantly managing energy consumption and the capacity to produce adenosine triphosphate (ATP). ATP is broken down into adenosine diphosphate (ADP) and can be further converted to adenosine monophosphate (AMP). When intracellular ATP levels decrease while AMP levels rise, cellular metabolism must shift towards ATP-producing catabolic pathways [15]. AMPK was recognized as mammalian homolog of a stress sensing kinase in yeast that is critical for cellular survival under conditions of marginal energy supply [5][16][17]). AMPK becomes allosterically activated by binding adenine nucleotides and thereby functions as a sensor of intracellular energy homeostasis [18][19].

From a biochemical perspective AMPK is a phylogenetically highly conserved heterotrimeric serine/threonine protein kinase complex consisting of one catalytic (α) and two regulatory subunits (β, γ). Various isoforms of each subunit are encoded by individual genes [18][20][21][22]. They are expressed tissue specific and the resulting AMPK subunits can either exert overlapping or distinct functions [23][24][25][26][27][28][29][30]. Depending on isoform expression and combination, twelve heterotrimeric holoenzyme-variants can theoretically be deduced [22]. Excellent reviews on structure, tissue related subunit expression, the multitude of phosphorylation sites, and subcellular localization are available, to which the specifically interested reader is referred [18][22][30][31][32][33].

Multiple endocrine, (patho-) physiological, and pharmacological stimuli are known to modulate AMPK . The phosphorylation of a conserved threonine-172 (Thr172) residue in the activation loop of the N-terminal region of the α-subunit is known to be required for full AMPK activation by upstream kinases in mammalian cells (reviewed in [30]). Using purified recombinant enzyme preparations, Suter et al. have shown that upstream kinase activation in the presence of saturating AMP effects resulted in a 1000-fold increased activation of α1-β1-γ1- and α2-β2-γ1 AMPK heterotrimers [34]. Interestingly, AMP did not enforce kinase dependent phosphorylation, at least under the research conditions chosen by

the authors, but protein phosphatase mediated deactivation by site specific dephosphorylation was prevented [34]. Meanwhile, these findings have been challenged and complemented by a broad body of further research, and AMP binding is suggested to modify the AMPK protein by allowing more effective upstream stimulation. Moreover, augmented allosteric activation of still Thr172 phosphorylated AMPK by AMP and further mechanisms have been discussed [20][35][36][37]. Together, available evidence generally supports the view that for full enzyme activation adenine nucleotide (AMP, ADP) binding is essential for Thr172 phosphorylation in the activation loop of the α-catalytic domain [18][36]. Adenine nucleotide binding is realized by three adenine nucleotide binding sites (ANBS), which are localized in three out of four cystathionine-β-synthase repeats (CBS1-4) in the AMPK γ-subunit [30]. CBS4 was shown to exclusively and permanently bind AMP [38][39], while CBS1 and CBS3 competitively bind AMP, ADP, or ATP, depending on their respective cellular abundance [18]. This elegantly enables the AMPK protein complex to rapidly sense changes in AMP:ATP ratio and immediately balance cellular energy supply [35]. Another structural key element in the AMPK γ-subunit is CBS2. CBS2 is discussed to be closely related to ACC phosphorylation [40]. The β-subunit is of extraordinary importance for mediating metabolic effects and contrasting with humans, the rodent liver predominately expresses β1, while the β2-subunit appears to be of minor importance in this species [20][24][41][42][43][44]. The β-subunit enables AMPK to bind glycogen via a carbohydrate binding module (β-CBM), augmenting its energy sensitizing properties [35]. Moreover, it was actually shown by Steinberg's group that activated long chain fatty acid esters can allosterically activate β1-subunit containing isoforms and thereby increase lipid oxidation through phosphorylation of ACC [45].

Due to the core functional position in metabolism, AMPK was recognized as a promising pharmacologic target structure. Metfomin, a drug widely used in the therapy of T2D, exerts some of its main effects by AMPK activation in the liver and skeletal muscle [46][47]. Increasing AMPK activity is widely viewed as viable therapeutic strategy [48]. Stimulation of AMPK not alone inhibits ACC1, but further leads to concomitant phosphorylation of ACC2 and activation of malonyl CoA decarboxylase (MCD), resulting in an acute reduction of cytosolic malonyl CoA [49]. Malonyl CoA is well known as negative regulator of fatty acid oxidation by virtue of its ability to inhibit carnitine palmitoyl-transferase 1 (CPT1). Thus, when malonyl CoA levels decrease, CPT1 remains uninhibited and fatty acid oxidation becomes accelerated [50][51][52][53].

3. The Role of AMPK in Human Fat Depots

Based on the close association of NAFLD and obesity, AT expansion is common in fatty liver patients. The shift of fat storage away from subcutaneous (SCAT) towards visceral white adipose tissue (VAT) is considered a predictor of increased risk profile [54][55].

AMPK is known to be expressed in AT and to exert relevant metabolic functions (reviewed in [48]). Under physiological conditions AMPK is primarily discussed to contribute to AT insulin sensitivity [48]. Expression appears to be higher in human SCAT compared to VAT, at least in morbid obesity (BMI ≥ 40 kg/m^2; n = 17), and after short term starvation [56]. Reduced AMPK activity, as estimated by Thr172 phosphorylation of αAMPK in relation to total protein, positively correlated with insulin resistance in obese humans [57][58]. However, the number of studied subjects was limited, all were morbidly obese, and the homeostasis model of insulin resistance (HOMA-IR) as a relatively rough estimate was used to evaluate the efficacy of insulin signaling [57][58]. Our group has studied AMPK expression and activation in VAT and SCAT of NAFLD patients . However, we were unable to observe any significant differences between groups, yet subjects were matched for main confounding factors. Contrasting with the studies of Ruderman's group [57][58], our patients were not morbidly obese, and NAFLD was evaluated by gold standard methods. However, we did also use the HOMA-IR for estimating insulin resistance and did not perform functional analyses. Moreover, our control subjects were overweight and we cannot exclude that this could have biased the results. Grisouard et al. differentiated human preadipocytes in vitro and studied the effects of metformin stimulation on glucose uptake [59]. Metformin increased glucose uptake more than twofold, accompanied by a rise in GLUT4 and glucose oxidation. Silencing of AMPKα1 counteracted the effects [59]. Available evidence therefore supports the hypothesis that AMPK contributes to peripheral insulin sensitivity. Pharmacological AMPK activation in a manner supporting cellular glucose uptake with consecutively enforced FFA esterification could help to control for hyperlipolysis. Insulin mediated lipolysis suppression showed a continuous worsening with rising liver fat accumulation [60] and hyperlipolysis is considered a major contributor of NAFLD (reviewed in [61][62]). Hyperlipolysis is further aggravated when adipocytes under conditions of a repetitively positive energy balance become hypertrophic. This can result in systemic low-grade inflammation, as promoted by macrophages residing in AT with worsening of insulin resistance [14][61][63][64][65][66]. However, according to numerous experimental and animal models, the role of AMPK on AT lipolysis remains inconclusive, with existing evidence supporting both enhanced and suppressed lipolysis by AMPK [30][67][68][69][70][71][72][73][74]. We are aware of no research data specifically addressing this issue in human NAFLD. However, indirect evidence to support the hypothesis of suppressed lipolysis (and/or elevated fat oxidation) in humans via AMPK comes from a study using an AICAR stimulation protocol [75]. Given the percentage of AT

in relation to total body mass, further research on this principle could provide interesting insights on the role of AMPK, particularly regarding peripheral insulin resistance in various AT depots [76]. This principle is also supported by animal data showing that abolishing AMPK in AT results in decreased whole body insulin sensitivity and enhanced liver triacylglycerol accumulation [77]. Furthermore, human adipocytes in principal have the capability of DNL [78]. However, in contrast to rodents it is known that AT is not a significant site of DNL in humans [79][80].

4. Skeletal Muscle Is a Predominant Target Tissue of Insulin and Sensitive to AMPK Activation

Skeletal muscle is the most important site of insulin stimulated glucose disposal in human body (reviewed in [62][81]). Liver triacylglycerol content can be interpreted as direct barometer of whole-body insulin resistance, with maximally impaired insulin signaling in skeletal muscle being reached when hepatic triacylglycerol levels range about 6% [60]. AMPK is expressed at high levels in skeletal muscle, and under physiological conditions the kinase contributes to insulin sensitivity and insulin independent exercise related peripheral glucose uptake.

A number of studies have addressed the role of AMPK in human skeletal muscle. The majority of trials were of short duration and included limited subject numbers. Only one study considered NAFLD [82]. The studies can be categorized in (i) descriptive studies focusing basal AMPK activity in obesity/insulin resistance [83][84][85], (ii) interventional studies using AMPK mimetics under in vivo or ex vivo conditions [75][82][84][86][87][88], and (iii) exercise studies (e.g., [87][89][90][91][92]). It was shown that after 12–18 h of starvation basal AMPK activity and expression remained comparable in muscle of lean and obese subjects [84]. This was also reported for T2D patients and controls [83][85][86]. However, patients in several of these studies were under antidiabetic medication including metformin, with no relevant drug free interval before intervention [86][88]. Thus, the results must be interpreted carefully.

5. Dysregulation of Hepatic AMPK in Humans

DNL is a main contributor to hepatic triacylglycerol accumulation under conditions of caloric oversupply, with glucose representing the main DNL substrate. The quantitatively only relevant site of DNL in human metabolism is the liver [79]. Therefore, attempts to modulate DNL in a therapeutic manner by means of AMPK activation should primarily address hepatic isoforms. Under physiological conditions AMPK is considered to balance hepatocellular energy demands, with major influence on liver insulin sensitivity and lipid homeostasis [29].

The main predictor of NAFLD is hepatic insulin resistance, defined by the failure of insulin to suppress endogenous glucose production, e.g., gluconeogenesis [61][93]. Under physiological conditions DNL, like gluconeogenesis, is under slow but potent transcriptional control by an insulin dependent mechanism [62]. When hepatocellular insulin signaling becomes attenuated DNL activation remains paradoxically uncompromised. This is referred to as selective insulin resistance, resulting in fasting hyperglycemia and uncontrolled DNL at the same time [94]. Corresponding to the human study of Lambert et al. [95], fasting hyperglycemia is a significant driver of DNL since glucose independently from insulin can enter hepatocytes in large amounts via GLUT2, and thereby enter glycolysis .

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