Hunting for the SNARK in metabolic disease

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DYSREGULATION OF ENERGY BALANCE is a primary constituent in the etiology of obesity and type 2 diabetes mellitus, which is manifested by altered metabolic homeostasis and insulin resistance in a variety of tissues, including brain, liver, and skeletal muscle. The discovery of the AMP-activated protein kinase (AMPK), an evolutionarily conserved serine/threonine kinase that acts as a master sensor and regulator of energy balance at the cellular level (8, 31), has been critical to our understanding of whole body energy homeostasis. Modulation of AMPK activity in various metabolic tissues is a feature of therapeutic strategies, such as exercise (1) and metformin (27), known to improve metabolic homeostasis in type 2 diabetes and insulin resistance. Metabolic regulation by AMPK has been extensively studied, but little is known of the role of AMPK-related kinases in metabolic regulation. Twelve protein kinases (BRSK1, BRSK2, NUAK1, NUAK2, QIK, QSK, SIK, MARK1, MARK2, MARK3, MARK4 and MELK) in the human kinome are closely related to AMPKα1 and AMPKα2 (18), thus forming a 14 kinase phylogenetic tree known as “AMPK-related kinases” (Fig. 1A). In this issue of the Journal, Ichinoseki-Sekine et al. (9) have explored the role of NUAK2, also known as SNARK [SNF (sucrose, nonfermenting) 1/AMPK-related kinase], in whole body energy homeostasis in sedentary and physically active animals. They provide evidence for a robust effect on whole body metabolism by hemi allelic Snark deficiency (Fig. 1B), suggesting that this AMPK-related kinase is a previously unrecognized regulator of whole-body metabolism.

Thorough reviews of the structure, regulation, and metabolic effects of AMPK have been published elsewhere (8, 17, 31); some aspects are briefly discussed here. AMPK was originally identified as a kinase responsible for inhibitory effects of 5′-AMP on both HMG-CoA reductase and acetyl-CoA carboxylase activity (3), although earlier workers had implicated AMP sensing in cellular metabolism (5, 33). A wide range of cellular stresses that deplete ATP (such as metabolic poisons) or increase the cellular AMP/ATP ratio (such as glucose deprivation or muscle contraction) activate AMPK (8). Consequently, AMPK acts as a master regulator of cellular metabolism in response to alterations in energy charge in the cell (8). The generalized effects of AMPK activation occur in such a manner as to conserve ATP by inhibiting biosynthetic pathways and anabolic pathways while stimulating catabolic pathways that generate ATP in a control mechanism that acts to restore cellular energy (ATP) stores (8). In the context of skeletal muscle, this is observed acutely as a suppressive effect of AMPK on glycogen synthesis (11) and protein synthesis (2) and a permissive effect on glucose transport (19) and fatty acid oxidation (28). The effects of chronic or constitutive AMPK activation lead to alterations in metabolic gene expression and mitochondrial biogenesis (6, 34), which enhance the ability of the cell to rapidly replenish ATP or resist metabolic perturbation. However, several experiments now demonstrate that AMPK is dispensable in modulating the effects of contraction or pharmacological activation on fuel metabolism and gene expression, raising the possibility that AMPK-independent pathways may regulate glucose and lipid metabolism. Contractile-mediated glucose uptake is unaltered or slightly impaired in AMPKα2 knockout (KO) and AMPKα2 kinase-dead mice (11, 22), whereas AMPK is dispensable for fat oxidation during contraction or AICAR stimulation (4, 21). Similarly, the mitochondrial adaptation to exercise training is preserved in these mice (10, 24). Despite the apparent capacity of AMPK to increase mitochondrial biogenesis, it is not required for classical exercise-induced training responses.

The tumor suppressor LKB1 kinase is a master kinase regulating activity of 13 of the 14 kinases of the AMPK-related kinase subfamily (16). In muscle-specific LKB1-KO mice, AMPK activation during contraction is prevented and glucose uptake is blunted (26). Similarly, the contraction-induced increase in acetyl-CoA carboxylase (ACC) phosphorylation and decline in malonyl-CoA levels were attenuated in these animals, although fat oxidation was not measured directly (26, 29). However, these observations do not necessarily mean that AMPK is solely responsible for LKB1 effects on metabolism, but rather suggest a role in cellular metabolism for other AMPK-related pathways (25) or alternative signaling pathways such as calcium-dependent protein kinases (23, 32). A pertinent point here is that results obtained in transgenic animals may be affected by compensatory alterations in AMPK isofrom activity or alternative signaling pathways. However, the possibility that AMPK-related kinases play a permissive role in contraction or pharmacological effects on fuel metabolism represents an attractive and worthy hypothesis.

Ichinoseki-Sekine et al. have investigated the in vivo effects of altering expression of SNARK by using hemi allelic loss of SNARK (Snark+/-) on whole body metabolic homeostasis and physical activity behavior. Prior to this publication, little was known about the metabolic role of SNARK, but earlier reports from in vitro studies have identified remarkably similar aspects of regulation and activity to AMPK (13, 14, 16). Homozygous Snark-deficient mice have a high incidence of embryonic lethality, whereas the heterozygous Snark-deficient mice have an obvious metabolic phenotype with mature-onset obesity and increased white adipose tissue mass evident after 4 mo of age (9, 30). These changes are accompanied by liver fat accumulation and increased serum triglyceride concentration, as well as hyperinsulinemia, hyperglycemia, glucose intolerance, and enhanced tumorigenesis (30). Further published characterization of this phenotype is eagerly awaited, but fat synthesis and deposition are reported to be enhanced, along with a reduction in total body temperature and daily energy expenditure, as assessed by oxygen uptake (30). Habitual...
food intake between Snark-deficient and wild-type animals in the sedentary state does not differ (9).

SNARK was originally cloned in 2001, being encoded by the NUAK2 locus and was identified as the fourth AMPK-related kinase (13, 14). AMPK and most AMPK-related kinases are directly activated by an increased AMP/ATP ratio through an allosteric effect and/or indirectly activated by phosphorylation at a conserved threonine residue in the activation loop by upstream kinases such as LKB1 (16). Activation of SNARK by LKB1 occurs by phosphorylation of Thr208, a residue equivalent in position to Thr 172 within the activation loop of AMPKα2 (16). LKB1 is attractive as a regulator of SNARK activity by virtue of its nuclear localization, which is coincident with the predominant nuclear localization of SNARK (12), but SNARK may also directly mediate some physiological effects of LKB1. Several aspects of SNARK regulation and activity are broadly similar to those of AMPK, which can be summarized as follows. First, SNARK possesses AMPK-like phosphotransferase activity (13). Second, activation of SNARK is AMP responsive (13, 14). Third, SNARK activity is increased by AICAR (12, 14, 16), albeit in a cell-specific manner. Fourth, SNARK is activated by treatments known to increase AMP/ATP ratio or disrupt ATP production, including glucose deprivation and chemical ATP depletion among others (12, 14, 16). Possible AMPK activation of SNARK, secondary to the activation of AMPK by these treatments, has not been investigated but raises the possibility that one or more activities previously attributed to the AMPK-signaling cascade may be attributable, in part, to SNARK activation. In addition, similarities between AMPK and SNARK regulation do not necessarily infer that SNARK activity directly mirrors AMPK activity in the context of cellular metabolism. Cell-specific differences are reported between SNARK and AMPK activity (14) and pharmacological activation (16) as well as in the relative rates of phosphorylation and peptide substrates phosphorylated (16).

Examining metabolic and anthropometric effects of Snark deficiency, the core finding in the present paper is that the provision of voluntary exercise opportunities to Snark-/- mice results in habitually increased daily physical activity (∼2-fold) compared with Snark+/- mice, commensurate with the prevention of mature-onset obesity to which these animals are genetically predisposed. Physical activity resulted in a reduction in total body mass, liver mass, and white and brown adipose tissue mass in both exercise groups compared with sedentary controls, but the magnitude of this decrease in body mass was ∼10% and 25% for the Snark-/- and Snark+/- mice, respectively. At termination of the study, body mass was similar between genotypes in the physically active mice. The prevention of weight gain in the active Snark-/- mice occurred despite a 10% increase in food intake. Differences in physical activity were not attributable to sex, age, or disrupted circadian rhythm, nor were they attributable to any intrinsic deficit in forced exercise capacity/muscle energetics associated with Snark deficiency.

The data presented in this paper and the phenotype described elsewhere (30) pose many questions on the role of SNARK in whole body energy balance. More data will be required to draw any clear conclusions on the phenomenology of this phenotype. The reported reduction in basal energy expenditure is ostensibly the cause of the increase in fat deposition and weight gain. How reduced SNARK expression mediates this phenomenon is more speculative. Direct SNARK-dependent modulation of whole body metabolism, similar to AMPK effects in the context of carbohydrate and lipid metabolism, has not been demonstrated. However, SNARK is predominantly and consti-
tutively localized to the nucleus, where it is likely to be regulated by LKB1 or other unidentified kinases (12). Overexpression of SNARK in human liver hepatoma cells results in the upregulation (>2.0-fold) of 76 mRNA targets and down-regulation (>2.0-fold) of 32 mRNA targets, suggesting that SNARK can work as a stress-responsive transcriptional modulator in the nucleus (12). Protein expression of SNARK in rodent tissues is highest in the brain and adrenal tissues but undetectable in skeletal muscle (14, 25). The highest basal SNARK activity was reported in the brain and liver, although basal kinase activity was ~100-fold less that AMPKα2 activity in most tissues (14). Therefore, SNARK gene expression and kinase activity is tissue specific, and its activity profile differs significantly from the AMPKα2 activity profile. Therefore, targeting SNARK could potentially affect whole body metabolic homeostasis, and a more thorough examination of the physiological role of SNARK is warranted.

In light of the high basal SNARK activity in brain and liver, and the absence of detectable protein in skeletal muscle, it is tempting to speculate that this phenotype is regulated centrally rather than at the periphery in skeletal muscle despite the active phenotype. The absence of a basal muscle energetic deficit, an as yet unexplained increased motivation/drive to exercise, and altered food intake in Snark−/− mice supports this contention. Determining the exact metabolic role for SNARK is somewhat complicated by employing a whole body Snark deficiency. For example, in the case of the transcriptional coactivator PGC-1α, whole body PGC-1α-KO mice are resistant to diet-induced insulin resistance due to a confounding hyperactivity phenotype (15), whereas muscle-specific PGC-1α-KO mice exhibit marked dysregulation of glucose homeostasis (7). In the context of AMPK-related kinases, AMPK regulates energy balance not only by altering fuel metabolism in skeletal muscle, but also by responding to nutritional and hormonal signals at the hypothalamus to govern food intake (20). These points raise another important caveat for the interpretation of Snark deficiency: the role of AMPK in whole body energy metabolism is both at the level of a specific tissue effect(s) and through interorgan communication, a regulation that may be recapitulated by SNARK on the basis of its similarity to AMPK. In the case of a whole body gene deficiency, novel systemic compensatory mechanisms cannot be excluded as effectors of alterations in energy homeostasis.

Many questions remain at present, and we await the publication of further metabolic characterization of this phenotype in earnest. Does SNARK acutely affect carbohydrate and lipid metabolism in vivo? Does the established role of SNARK in regulating gene expression affect metabolic processes, which in turn enhance the Snark−/− phenotype through, for example, enhanced lipid synthesis or storage? SNARK expression and activity in adipose tissue is presently unknown, and whether this plays any role in the accumulation of adipose tissue observed remains to be seen. If SNARK regulates ACC in a similar manner to AMPK, reduced SNARK activity could conceivably account for the increased lipid synthesis, as inferred from this phenotype. However, the pertinent questions remain: 1) how does Snark deficiency reduce basal energy expenditure, and 2) why are Snark-deficient mice intrinsically more physically active when presented with adequate opportunity? In general terms, Snark deficiency predisposes mice toward mature-onset obesity. However, when presented with exercise opportunities, Snark deficiency predisposes mice toward increased physical activity (with consequent reduction in fat mass), which presumably represents some compensatory behavior to restore homeostasis based on a gene environment interaction. Despite the many unanswered questions, SNARK appears on the scene (9) as a novel regulator of whole-body metabolic homeostasis and highlights yet another protein kinase as an exciting new addition to the already extensive paradigm of homeostatic regulation by cellular energy sensors.

REFERENCES


