Response to Letter Regarding Article, “Myostatin Regulates Energy Homeostasis in the Heart and Prevents Heart Failure”

In the article referred to by the letter of Neumann et al., we found that inactivation of myostatin in the adult heart caused a metabolic switch toward glycolysis and glycogen accumulation, cardiac hypertrophy, and heart failure, which was associated with massive activation of AMP-activated kinase (AMPK). Because pharmacological inhibition of AMPK in vitro and in vivo reversed several aspects of this phenotype, we concluded that myostatin-mediated repression of AMPK is required to maintain a mature aerobic energy metabolism in the heart and to prevent cardiac hypertrophy.

Neumann et al challenge this conclusion arguing that activation of AMPK might represent the reaction to metabolic alterations rather than the cause. Furthermore, they postulate that increased transphosphorylation of protein kinase D observed in our study after inactivation of myostatin in the adult heart might explain increases in glucose utilization and uptake and cardiac hypertrophy by phosphorylation of HDAC5 (histone deacetylase 5) and subsequent dephosphorylation of myocyte enhancer factor 2 (MEF2).

We share the skepticism expressed by Neumann et al on the use of pharmacological inhibitors such as the AMPK-inhibitor compound C, because many inhibitors exert off-target effects. To cope with this problem, we took advantage of a second inhibitor Ara-A, which is structurally and mechanistically different from compound C. Ara-A had exactly the same effect as compound C in rescuing the metabolic phenotype after myostatin deletion. In addition, we found that the treatment of primary cardiomyocytes with compound C followed by real-time respirometry leads to decreased glycolytic capacity, whereas AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), an AMPK activator, increased the glycolytic capacity. We also demonstrated that either inhibitor blocked increased phosphorylation of AMPK in the heart and skeletal muscle.8,9 Moreover, the expression of a kinase-dead version of AMPK-α2 mice in compound-heterozygous mice carrying the Prkag2 allele N488I, which increases AMPK activity, blocks cardiac hypertrophy and decreases enhanced glycogen storage.7 In further support of the prohypertrophic role of aberrant AMPK activity, we identified several new interaction partners of AMPK involved in hypertrophic signaling by coimmunoprecipitation. Therefore, a link seems to exist between myostatin, AMPK, and cardiac hypertrophy, which would fit to the well-known role of myostatin as inhibitor of Akt and mTOR signaling in heart and skeletal muscle.4

Neumann et al correctly state that most of the Prkag2 mutations render AMPK insensitive to AMP, but cause increased AMPK activity, glycogen storage, and cardiac hypertrophy10 (see also paragraph above). We cannot see that these facts collide with our notion that Prkag2 “mimics aspects of the myostatin phenotype,” which is characterized by increased AMPK activity, glycogen storage, and cardiac hypertrophy. Instead, the rescue of the Prkag2 N488I phenotype associated with increased AMPK activity by kinase-dead AMPK-α2 seems to phenocopy the effects of pharmacological inhibitors of AMPK in hearts of myostatin mutants with increased AMPK activity.

Neumann et al reason that activation of AMPK after inactivation of myostatin in the heart might represent the reaction to metabolic alterations rather than the cause, which is a reasonable concern. However, we demonstrated several direct effects of myostatin signaling on AMPK: (1) we showed that cardipecific overexpression of myostatin reduces AMPK activity in vivo and (2) we demonstrated that treatment of adult primary cardiomyocytes with recombinant myostatin blocks AMPK phosphorylation after 10 minutes in vitro.1 Moreover, other groups also observed activation of AMPK in skeletal muscle and fat after inactivation of myostatin.11,12 Regulation of AMPK activity is complex and does not solely depend on the cellular energy status as indicated by the activation of AMPK via CaMKKβ (calcium/calmodulin-dependent protein kinase kinase beta).13 Similarly, we are just at the beginning of understanding the pleiotropic effects of AMPK in the heart in physiological and pathological conditions. Neumann et al point out that the stimulation of AMPK in cardiomyocytes upregulates glucose and fatty acid uptake as well as their utilization but did not mention that AMPK activation in left-ventricular hypertrophy activates glucose uptake and in parallel decreases β-oxidation.14 In our article, we described decreased interaction of AMPK with all major enzymes involved in cardiac hypertrophy in our experimental setting. We are aware of the fact that AMPK is generally seen as inhibitor of protein synthesis, which seems to contradict a role of AMPK activation in promoting cardiac hypertrophy. However, point mutations in the nucleotide-binding region of the AMPK γ2-subunit, encoded by the Prkag2 gene (eg, the N488I allele) resulting in aberrant increase of kinase activity, cause cardiac hypertrophy probably via mechanistic target of rapamycin (mTOR) and FoxO (forkhead box O) signaling pathways independent of glycogen storage. Moreover, the expression of a kinase-dead version of AMPK-α2 mice in compound-heterozygous mice carrying the Prkag2 allele N488I, which increases AMPK activity, blocks cardiac hypertrophy and decreases enhanced glycogen storage. In further support of the prohypertrophic role of aberrant AMPK activity, we identified several new interaction partners of AMPK involved in hypertrophic signaling by coimmunoprecipitation. Therefore, a link seems to exist between myostatin, AMPK, and cardiac hypertrophy, which would fit to the well-known role of myostatin as inhibitor of Akt and mTOR signaling in heart and skeletal muscle.4

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in β-oxidation after inactivation of myostatin, whereas interaction with glycolytic proteins was increased.1 Taken together, our genetic, proteomic, and respiratory data argue against a simultaneous activation of glycolysis and β-oxidation after myostatin inactivation and subsequent AMPK activation but because we did not measure β-oxidation directly, we cannot tell whether increased AMPK activity after inactivation of myostatin significantly represses β-oxidation.

In our article, we extensively discussed the controversial findings concerning the regulation of AMPK by TGF-beta activated kinase 1 (TAK1). Previous publications described decreased phosphorylation and activation of AMPK after expression of a dominant-negative version of TAK1 in neonatal cardiomyocytes,13 which seem to be at odds with our finding that TAK1 directly or indirectly inhibits AMPK. However, the same group failed to show that wild-type TAK1 overexpression activates AMPK in neonatal cardiomyocytes.14 In this context, it should be remembered that cardiac metabolism dramatically changes during early postnatal stages from a preference to glycolysis toward fatty acids in adult hearts,16 which might be a reason for the conflicting findings. We agree that additional studies are necessary to clarify the impact of TAK1 on the regulation of AMPK in the heart at different developmental stages characterized by distinct metabolic states.

Finally, Neumann et al speculate that the increased transphosphorylation of protein kinase D observed in our study might provide a suitable explanation for enhanced glucose utilization and cardiac hypertrophy after inactivation of myostatin in the adult heart. We cannot exclude a contribution of protein kinase D but many of the observed metabolic effects such as glycogen accumulation, increased glycolytic capacity, inhibition of acetyl-CoA carboxylase, and increased lactate content are difficult to reconcile with protein kinase D activation. Moreover, cardiac-specific overexpression of PKD1 leads to systolic dysfunction and dilated cardiomyopathy17 and MEF2a transgenic mice do not develop cardiac hypertrophy but show signs of dilated cardiomyopathy and mechanical dysfunction,18 supporting our hypothesis that the Rgs2-PKD-MEF2 axis is involved in the systolic dysfunction after acute deletion of myostatin but not in development of the metabolic phenotype.

We agree that our study raised several exciting questions about the regulation of AMPK by myostatin and its impact on cardiac metabolism, which should be addressed in future experiments. However, we stand by our hypothesis that excessive activation of AMPK as a result of myostatin inactivation in the adult heart is currently the most plausible explanation for the increased glycogen accumulation, increased glycolysis, and cardiac hypertrophy in myostatin mutants. Even a safeguard might sometimes turn into a culprit.

Sources of Funding

Dr Braun is supported by the Max-Planck-Society, the Deutsche Forschungsgemeinschaft (Excellence Cluster Cardio-Pulmonary System, and Sondierforschungsbereich TR81), the Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Excellenz Center for Cell and Gene Therapy, and the German Center for Cardiovascular Research.

Disclosures

None.

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References

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Circ Res. 2015;116:e97-e98
doi: 10.1161/CIRCRESAHA.115.306486

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