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The role of asprosin in the regulation of energy homeostasis

Introduction

Asprosin, the cleavage product of fibrillin-1, is an orexigenic, glucose-releasing hormone. It is produced in white adipose tissue (WAT) and is praised to be a promising target for treating type 2 diabetes and obesity, as sequestration of asprosin reduces body weight, food intake and blood glucose. The function of asprosin in the liver is particularly important in this regard, as it regulates hepatic glucose homeostasis. However, effects of asprosin deficiency on liver histology and glycogen metabolism have not yet been analyzed. Therefore, the first aim of this thesis is to unravel the encompassing effects of asprosin deficiency on liver function and glycogen metabolism.

Furthermore, profibrillin-1, the origin of asprosin and fibrillin-1, is not exclusively expressed in WAT but also in other connective tissues, such as the skeletal muscle. It also plays an important role in muscle glucose metabolism, however the exact function and origin of asprosin in the musle is still unknown. This is why the second aim of this thesis is to address the role of muscle-derived asprosin in muscular and peripheral metabolism. Additionally, the effects of muscle-derived asprosin on metabolic changes upon diet-induced obesity (DIO) and exercise (RN) are investigated, as these two conditions influence glucose homeostasis in several organs as liver, WAT and the muscle itself.

Methods

As a mouse model, GT8 (green truncated 8) mice were chosen to investigate whole body asprosin deficiency and liver health, as it expresses a truncated form of profibrillin-1 without producing asprosin. Furthermore, muscle-specific asprosin knockout was induced by Cre-Lox recombination (muscle creatin kinase (MCK)-$Cre^{+/-}$;$GT8^{flox/flox}$ mice) in another mouse line.

Mice were characterized regarding body weight, food intake and glucose tolerance. Furthermore, proteome mass spectrometry was performed to generate further hypotheses regarding their metabolic phenotype. These findings were supported and confirmed by western blot and several histological analyses.

Results

Asprosin-deficient GT8 mice displayed reduced body weight and blood glucose on postnatal day (P) 7. Histologically, they have a strong hepatic steatosis as well as elevated hepatic glycogen metabolism indicated by decrease in PhKB (glycogen breakdown) and increase in

GYS2 (glycogen synthesis). Daily asprosin administration from P 1 diminished steatotic conditions in GT8 mice. Muscle-specific GT8 mice (MCK-Cre^{+};GT8$^{flox/flox}$) did not show significant differences in their metabolic phenotype compared to control mice (MCK-Cre^{-};GT8$^{flox/flox}$) upon standard diet. When fed with high sugar and high fat diet (DIO), muscle-specific GT8 mice showed less diet-induced body weight gain and fat mass accumulation compared with control. In addition to that, WAT displayed decreased adipose vacuole size and reduced expression of several adipogenic proteins. Furthermore, insulin and leptin blood levels were increased. In the proteome analysis, DIO-fed muscle-specific GT8 mice showed stronger overall changes in skeletal than in cardiac muscle proteome analysis. There was elevated GYS1 and decreased GSK3B protein levels in the skeletal muscle which indicates reduced breakdown and increased synthesis of glycogen. The cardiac muscle tissue showed contradictory results by increase in fatty acid beta oxidation, but comparable protein changes in glycogen metabolism. In the liver, PAS staining was stronger, indicating higher intracellular carbohydrates as glycogen, and the negative regulation of glycogen synthesis (pPP1) was decreased. Upon voluntary wheel running (RUN), muscle-specific GT8 mice showed increased glucose tolerance compared to control. In the skeletal muscle proteome analysis, proteins of the JAK/STAT signaling were increased. In the cardiac muscle, several ribosomal proteins were decreased. Furthermore, proteins of the electron transport chain were reduced, especially subunits of Complex I. No changes were observed in the liver.

Discussion

Based on the literature asprosin depletion seems to be a promising target to fight obesity and type 2 diabetes. Asprosin-deficient GT8 mice indeed displayed reduced body weight, fat mass and blood glucose. It was shown before that glucose release from the liver is reduced when blocking asprosin's action. However, when taking a deeper look on hepatic histology and proteome, one can see massively unfavorable conditions, which were not described until now. GT8 mice display strong hepatic steatosis and disturbed glycogen storage. Increased GYS2 induces more glycogen storage, which is intensified by decreased PhKB levels, hence glycogen breakdown. These conditions are comparable to glycogen storage disease 0 (GSD0), which is induced by mutation of GYS2 and accompanied by hepatic steatosis. Furthermore, hepatic steatosis can induce mitochondrial dysfunction, as it was shown in non-alcoholic fatty liver disease (NAFLD). GT8 mice seem to have impaired hepatic mitochondria, but the results need to be confirmed in further studies. Muscle-specific asprosin deficiency also appears to affect glycogen, fatty acids and mitochondrial metabolism, but only when mice are metabolically triggered by DIO or RUN. Not only central but also peripheral effects on liver and WAT could be observed. In the skeletal muscle upon DIO, asprosin deficiency increases glycogen synthesis and decreases its breakdown and could therefore be an important

antagonist for insulin. Upon RUN, asprosin deficiency seems to enhance the positive effects by exercise through JAK/STAT signaling. In the cardiac muscle asprosin, deficiency led to similar effects regarding glycogen metabolism but showed an increase in fatty acid beta oxidation. An improvement of negative effects by DIO on cardiac health can be concluded. Upon RUN, missing asprosin seems to have more negative effects as it reduces ribosomal proteins and induces a Complex I deficiency. These effects are connected to less proliferation, hypertrophic cardiomyopathy, and exercise intolerance. Thus, asprosin could be necessary for positive effects induced by RUN in cardiac muscle.

Conclusion

Regarding the literature, asprosin deficiency seem to have positive effects on glucose and fatty acid metabolism, but there are many detrimental effects on liver health as well. Further research should clarify whether blocking asprosin by antibodies induces negative side effects like hepatic steatosis. Furthermore, skeletal and cardiac muscle seem to be an important action and production side for asprosin. A potential autocrine and paracrine mode of action by muscular asprosin seem to be likely and should be focused in future research.