Maastricht University

Regulation of skeletal muscle mitochondrial biogenesis by GSK-3β

Citation for published version (APA):

Theeuwes, W. F. (2020). Regulation of skeletal muscle mitochondrial biogenesis by GSK-3β. [Doctoral Thesis, Maastricht University]. ProefschriftMaken Maastricht. https://doi.org/10.26481/dis.20200403wt

Document status and date:
Published: 01/01/2020

DOI:
10.26481/dis.20200403wt

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 19 Apr. 2024

Valorization

Societal relevance

Prevalence and burden of skeletal muscle dysfunction

Aging as well as several chronic diseases, such as chronic obstructive pulmonary disease (COPD), chronic kidney disease, type II diabetes and chronic heart failure, are characterized by a decline in peripheral muscle mass and a reduced number and impaired functionality of skeletal muscle mitochondria, primarily in the lower limbs [1-4]. The latter is associated with a reduced capacity of the muscle to produce energy. These skeletal muscle abnormalities ultimately dramatically affect the quality of life of these patients and are associated with increased morbidity and even higher mortality [5-7]. Indeed, good physical performance and muscle health strongly correlate with the ability to perform daily life activities, which is positively associated with quality of life and represses healthcare costs [8]. Indicative of the colossal impact of the abovementioned muscle abnormalities, sarcopenia defined as the loss of muscle mass and muscle function as a result of ageing, is highly prevalent in healthy aged individuals (10% [9]). Alarmingly, sarcopenia is even more prevalent in patients suffering from chronic diseases, such as type II diabetes and COPD [10-12] and numbers are predicted to rise further in the near future [13]. Sarcopenia is thus highly prevalent and, as stated above, correlates with an inability to adequately perform daily life tasks which ultimately leads to a reduced social status and increased hospitalization [14]. In 2014, the costs of sarcopenia-related hospitalization in the United States was estimated to be $40.4 billion [15]. Although Bruyère *et al.* could not make definitive conclusions regarding the economic burden of sarcopenia, because the studies used were heterogeneous in the assessment of sarcopenia and the type of costs evaluated, their systematic review gives a recent overview of several papers indicating elevated healthcare-cost in sarcopenic individuals. In addition, already in 2000, Janssen *et al.* estimated that a 10% reduction in the prevalence of sarcopenia in the United States alone would save $1.1 billion in healthcare-related expenses per year [16]. Treatment or even prevention of skeletal muscle impairments can therefore contribute significantly to increasing quality of life of large groups of patients and can help with decreasing the massive healthcare costs associated with (the management of) chronic diseases [17, 18]. Besides the high prevalence of skeletal muscle abnormalities, the above-mentioned diseases are also often associated with a high cardiometabolic risk. For example, COPD patients have an increased risk for the development of cardiovascular disease [19]. Interestingly, reduced skeletal muscle oxidative phenotype (OXPHEN) has been suggested as a major driver of increased cardiometabolic risk in COPD [20] and type II diabetes [21, 22]. This further stresses the need to minimize skeletal muscle abnormalities and

thereby reduce cardiometabolic disease-related healthcare costs in these patient populations [23].

Future perspective for GSK-3β and TFEB to alleviate muscle abnormalities

In this thesis, we describe a previously unknown role for glycogen synthase kinase (GSK)-3β in the molecular regulation of mitochondrial biogenesis and oxidative energy metabolism in skeletal muscle. We unraveled that inactivation of GSK-3β activated peroxisome proliferator-activated receptor-γ co-activator-1 (PGC-1)α gene expression via transcription factor EB (TFEB), which resulted in enhanced mitochondrial biogenesis and oxidative substrate use in skeletal muscle. This indicates that both GSK-3β as well as TFEB may serve as potential targets for therapeutic intervention to alleviate abnormalities at the level of the mitochondrion in skeletal muscle during ageing or in chronic disease. However, the relevance or efficacy of specific GSK-3β inhibitors or TFEB agonists in this context remains to be explored in more detail. Moreover, it has been reported that the inhibitory phosphorylation of GSK-3β and nuclear abundance of TFEB increase during physical activity. Combined with the data presented in this thesis, this indicates that improving muscle OXPHEN via modulation of the GSK-3β-TFEB-PGC-1α pathway can be achieved by adopting a healthier and more active lifestyle. As a long-term perspective, the knowledge gained by research described in this thesis could aid in the development of GSK-3β inhibitors, TFEB agonists or lifestyle interventions aiming to alleviate muscle abnormalities in the above-mentioned conditions.

Treatment strategies for increasing muscle oxidative phenotype

Exercise and exercise mimetics

Physical exercise is the most common and effective method to increase skeletal muscle health. However, exercise training is not always feasible in humans due to disease-related symptoms or age-related frailty. In addition, lifestyle changes are particularly hard to accomplish in elderly [24]. Nonetheless, exercise training or increased loading of skeletal muscle are highly effective evidence-based intervention methods for improving skeletal muscle function in several chronic conditions, including COPD [25]. However, the anabolic and mitochondrial response to exercise training is often blunted in COPD patients [26] as well as during aging [27, 28]. Alterations in the abundance or activity of TFEB in these conditions might underlie this

mal-adaptability, because TFEB knock-out mice have been shown to have a reduced ability to increase mitochondrial function and maintain the same exercise capacity compared to control animals. This makes them unable to fully benefit from exercise in terms mitochondrial regeneration [29], which speculatively could result in a blunted anabolic response. In this context, pharmacological activation of the pathways controlling muscle OXPHEN and muscle mass (so called 'exercise-mimetics') may prove beneficial to alleviate skeletal muscle abnormalities in the above-mentioned disorders and the aging population [30]. The mechanistic insight in the regulation of skeletal muscle PGC-1α expression gained in this thesis suggests a potential role for specific GSK-3β inhibitors or TFEB agonists as exercise-mimetics. Although the effects of inactivation of GSK-3β on mitochondrial biogenesis and oxidative energy metabolism and the involvement of TFEB herein needs to be studied in humans, the promising potential of GSK-3β inhibitors for clinical implication is clear.

Pharmacological potential of GSK-3β inhibitors

In order to verify the importance of GSK-3β in the (dys)regulation of skeletal muscle mitochondrial biogenesis and energy metabolism, it needs to be elucidated whether or not GSK-3β abundance or activity is altered in skeletal muscle in the above-mentioned human conditions associated with loss of muscle OXPHEN. In this context, it has been shown that the abundance and activity of GSK-3β is higher in skeletal muscle of obese type II diabetic patients compared with lean and weight-matched non-diabetic subjects [31]. In addition, GSK-3β phosphorylation was found to be reduced (indicating increased GSK-3β activity) in skeletal muscle of pregnancy-associated diabetes mellitus patients [32]. If GSK-3β activity is also increased in skeletal muscle of COPD patients is unclear. Several studies reported contradicting evidence on the phosphorylation status of GSK-3β in skeletal muscle of COPD patients. Indeed, unaltered [33, 34] and increased [35] levels of phosphorylated/inactivated (Ser^{9}) GSK-3β have both been reported in skeletal muscle of COPD patients. Literature on the activity of GSK-3β in skeletal muscle in chronic human diseases is thus scarce and contradictory and needs to be investigated in closer detail. Furthermore, it needs to be elucidated if structural absence of GSK-3β (in ablation modalities) or short-term acute inactivation of GSK-3β exert similar effects on TFEB nuclear translocation. To date, several experimental studies reveal that inactivation of GSK-3β in skeletal muscle is sufficient for nuclear translocation and activation of TFEB. Besides the effect of inactivation of GSK-3β on TFEB nuclear translocation in healthy conditions, these effects have not been verified in experimental models of chronic disease associated with reduced mitochondrial biogenesis. In order to fully exploit the benefits of exercise on muscle function, specific inhibition of GSK-3β

(or activation of TFEB) combined with an exercise protocol may be a potential novel treatment strategy [29]. Indicative of the therapeutic potential of GSK-3β inhibition in augmenting muscle functionality, lithium chloride (LiCl; an inhibitor of GSK-3) improved muscle strength and muscle mass in a murine muscle dystrophy model [36]. However, whether or not pharmacological inhibition of GSK-3β can rescue mitochondrial abnormalities in skeletal muscle remain to be investigated. Therefore, new studies should focus on the effect of exercise and pharmacological inhibition of GSK-3β on TFEB nuclear translocation in skeletal muscle in health and disease. In order to accomplish this, placebo-controlled pilot experiments aiming to investigate the effect of GSK-3β inhibitors in combination with or without exercise training on skeletal muscle mitochondrial biogenesis should reveal the clinical potential of GSK-3β inhibitors. Interestingly, lithium is clinically approved and often used to treat bipolar disorder [37]. However, some minor side effects of lithium treatment have been reported [38]. The clinical use of lithium indicates that lithium as well as more specific GSK-3β inhibitor are likely safe for human application. In this thesis, we report that GSK-3β inactivation enhanced TFEB nuclear translocation. In addition, TFEB was required for the inactivation of GSK-3β-mediated induction of PGC-1α, hence TFEB agonist might also be a promising pharmacological intervention aiming to induce muscle PGC-1α levels and thereby enhance muscle mitochondrial biogenesis.

Pharmacological potential for TFEB agonists

The role of TFEB in the regulation of PGC-1α and the subsequent control over mitochondrial biogenesis and oxidative energy metabolism was only recently discovered [29]. Despite this, several studies using multiple cell-types now support that nuclear translocation of TFEB, or other microphthalmia/TFE (MiT) family members, increases the expression of PGC-1α and enhance mitochondrial biogenesis [29, 39, 40]. Interestingly, TFEB agonists that stimulate nuclear translocation are being developed and tested [41]. However, if pharmacological activation of TFEB can rescue mitochondrial abnormalities in skeletal muscle remains to be investigated. In order to accomplish this, placebo-controlled pilot experiments aiming to investigate the effect of these compounds on mitochondrial function can be the first step to elucidate potential pharmacological potential of TFEB agonist in humans. Interestingly, digoxin (chemical TFEB agonist) is clinically approved and thus found to be safe for human application. The above-mentioned trials could increase our understanding of mitochondrial abnormalities in skeletal muscle within human disease and ultimately could reverse aging- or chronic disease-associated skeletal muscle mitochondrial deficits.

A potential role for GSK-3β and TFEB in neurodegenerative disorders

Next to skeletal muscle, mitochondria are highly important for the function of a myriad of other tissues, for example the brain. Growing lines of evidence suggest that mitochondrial dysfunction is involved in neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's disease. Interestingly, it has been proposed that inactivation of GSK-3β, aiming to prevent mitochondrial dysfunction, is a potential treatment of neurodegenerative disorders. Pre-clinical data showed that pharmacological inhibition of GSK-3β restored mitochondrial biogenesis in a mouse model of Parkinson's disease [42]. Interestingly, Alzheimer's disease is linked to increased activity of GSK-3β in the cortex. Furthermore, reduced levels or decreased activity of PGC-1α and TFEB have been associated with Alzheimer's disease progression [43, 44]. This suggest that GSK-3β inhibitors or TFEB agonists might also be beneficial for patients with Alzheimer's disease. Intriguingly, pre-clinical data revealed that inhibition of GSK-3β using LiCl reduced both β-amyloid as well as tau protein (the two major protein aggregates involved in Alzheimer's disease progression) formation [45, 46]. LiCl treatment was previously reported to reduce cognitive decline in Alzheimer's disease [47]. This indicates that inhibition of GSK-3β indeed might be suitable to alleviate the burden of Alzheimer's disease [48].

Conclusion

The data in this thesis convincingly shows that inactivation of GSK-3β in skeletal muscle enhances PGC-1α-mediated mitochondrial biogenesis and oxidative substrate metabolism via TFEB. This suggests that both GSK-3β inhibitors as well as TFEB agonists are potential therapeutic strategies to treat or even prevent deficits in skeletal muscle OXPHEN associated with chronic diseases and aging. In addition, as outlined in the general discussion (**Chapter 6**), GSK-3β is involved in both the regulation of muscle mass as well as the regulation of muscle mitochondrial biogenesis and oxidative energy metabolism. Thus, specific GSK-3β inhibitors might be beneficial in alleviating both abnormalities in muscle mass and muscle OXPHEN that often coincide in chronic diseases. To date however it remains unclear if pharmacological inhibition of GSK-3β or activation of TFEB can induce skeletal muscle mitochondrial biogenesis or enhance oxidative substrate metabolism in a clinical setting. In the future, new randomized placebo-controlled human trials should shed light on these remaining questions. In addition, particular care should be given to confirm safety of novel pharmaceuticals for human application. Furthermore, in order to minimize side

effects, the possibility to use specific drug-delivery techniques should be explored to transport the medication to the targeted tissue, for example the skeletal muscle [49]. Moreover, the beneficial effects of inhibition of GSK-3β on the brain, as discussed above, indicates a broader perspective for the pharmacological inhibition of GSK-3β in human diseases. Although speculative in nature, the data presented in this thesis thus may be applied in several research disciplines beyond skeletal muscle pathology.