Branching-out

IMPACT

(Research) What is the main objective of the research described in the thesis and what are the most important results and conclusions?

The aim of this thesis is to investigate the role of branched-chain amino acid (BCAA) catabolism in insulin resistance and type 2 diabetes (T2D) and if BCAA catabolism can be pharmacologically modulated in patients with T2D to beneficially effect metabolic health.

In **chapter 2**, a review of literature was performed investigating BCAA catabolism in insulin resistance and T2D, and strategies to boost BCAA catabolism. We conclude from this review that dysregulation of BCAA catabolism is closely related to obesity- and T2D related metabolic disturbances. Dysfunctional BCAA catabolism in several tissues could be a plausible explanation for the elevated plasma BCAA levels seen in obesity and T2D, although, an integrated understanding of tissue-specific BCAA catabolism remains largely unknown in humans. Therefore, exploring intervention strategies to increase BCAA oxidation and lower BCAA levels is important to investigate whether this could be a new potential strategy in the treatment of T2D.

We further explored the relation between BCAA metabolism and T2D-related complications in **chapter 3 and 4**. To this end, we observed that individuals with insulin resistance, including patients with T2D and NAFL, featured elevated BCAA levels compared to controls with obesity. We showed for the first time that elevated BCAA levels correlate with other type 2-diabetes related metabolic disturbances. We furthermore showed that patients with T2D have a lower whole-body leucine oxidation compared to healthy obese individuals, and therefore, a low mitochondrial BCAA oxidation may partly explain higher BCAA levels seen in these patients. These results underlined the importance to address BCAA oxidation in the treatment of T2D.

This challenged us to investigate whether BCAA oxidation can be improved by use of several strategies. To specify, we first investigated the potential effect of physical activity and exercise since it is well now for its role in metabolic health and might be an important player in BCAA catabolism. In **chapter 4**, it was tested if physical activity or an exercise training program would affect liver fat content via BCAA levels. We showed that BCAA levels were lower in more active individuals compared to less active individuals. Furthermore, it appeared that by doing

exercise liver fat decreased in people with NAFL, however this was not paralleled by a reduction in BCAA levels. Therefore, we can conclude that physical activity and exercise training differently affect BCAA levels in plasma, and that BCAA do not play a role in mediating the beneficial metabolic effects of exercise training. More research is needed to explore the amount and intensity of exercise that is needed to boost BCAA oxidation and therefore can contribute to the prevention and treatment of obesity and T2D.

In **chapter 5**, we investigated the potential effect of sodium-phenylbutyrate (NaPB), a booster of BCAA catabolism on metabolic health in patients with T2D as novel approach. In rodents, it has been shown that boosting BCAA oxidation has many metabolic benefits including improved glucose metabolism. In this thesis, these effects could be translated to humans for the first time. Although a relative short intervention period was applied in this thesis, the observed effects on metabolic health are promising. This finding may contribute to developing alternative novel strategies to boost BCAA oxidation, and whether they can prevent or treat T2D on the long-term.

In addition to these results, it was shown that exposing mice skeletal muscle cells to elevated extracellular BCAA levels caused impairments in intracellular insulin signaling via the mTOR/S6K pathway, however, insulin-mediated glucose uptake was not affected (**chapter 6**). We used 2-deoxyglucose (2DG) to study insulin-stimulated glucose uptake, which is a powerful tool to investigate the insulin resistant state *in vitro*. Performing cell culture experiments gives us more knowledge on the molecular level to better understand how insulin resistance can be caused by supplementation of BCAA. The results of this study are proof-of-concept the elevated BCAA levels can be a causal factor in insulin resistance and forms the lead to investigate this further in human skeletal muscle cells.

The association between BCAA levels and incident T2D was assessed in a systematic review and meta-analysis in **chapter 7**. In addition, the development of this association over time prior to T2D diagnosis was investigated. Positive associations between BCAA levels and development of T2D were found, irrespective of follow-up durations. These findings suggest the potential utility of BCAA as biomarkers, which reflect early changes in T2D. Detection of changes in BCAA levels years before the onset of physiological symptoms may be crucial for the early diagnosis and development of novel interventions.

(Relevance) What is the (potential contribution) of the results from this research to science, and, if applicable, to social sectors and social challenges?

Worldwide, 4.3 million deaths per year can be attributed to the consequences of T2D, making it the ninth leading cause of mortality (1). T2D is characterized by insulin resistance and metabolic dysfunction in several tissues including skeletal muscle, liver and adipose tissue resulting in hyperglycaemia (2), which can progress to severe complications and comorbidities. These affects patients' functional capacities, leading to significant morbidity and mortality (3). With the rising prevalence of T2D, the rising burden of diabetes forms a major public health problem with great impact on quality of life (4). Therefore, the social impact of T2D is enormous and emphasize the relevance of good treatment strategies. Current classic interventions mostly target one specific mechanism in a specific tissue to lower hyperglycaemia, but is not sufficient to slow down T2D-related comorbidities, that occur with the progression of the disease. This highlights the importance to developing effective treatment strategies working on multiple tissues since they all contribute to hyperglycaemia. In that way, T2D can be prevented or treated, which in turn improves the social burden caused by this disease.

Given the extraordinary healthcare costs to treat T2D and occurring comorbidities, T2D takes a significant part of the health budget around the world. With the increasing prevalence, this health budget is set to increase further (5). Again, this shows the importance of implementing more feasible strategies to prevent the development of T2D and related comorbidities. The knowledge in this thesis contribute considerably to the existing understanding of the pathology and treatment of T2D. This knowledge is important for further development of treatment in the end to delay the onset of diabetes-related comorbidities, thereby reducing health care costs. Even more, prevention of T2D will become increasingly important as this is a cost-effective way to reduce the major economic impact. Since BCAA levels are considered to be a risk factor of T2D, the development of novel potential BCAA-related biomarkers in diabetes can be exploited to intervene at an early stage in the development or even before the onset of T2D.

The research presented in this thesis shows the effects of a potential novel strategy targeting BCAA catabolism, which so far, has not been investigated in patients with T2D. At present, knowledge on BCAA catabolism in patients with insulin resistance and T2D and the health consequences of disturbed BCAA catabolism are limited. The results of the present thesis contribute to better understanding of BCAA catabolism and its importance for metabolic health in humans. Based on the results of this research, development of new treatment strategies can be found to prevent and improve T2D. Because boosting BCAA catabolism act upon several

mechanisms in multiple tissues to improve insulin sensitivity, this treatment is a proposing novel strategy to combat T2D. Taken together, the results obtained in this thesis are of great social and economic relevance since they could contribute to the development of new treatment options and treatment strategies, which are feasible and can be implemented in daily life, for people with prediabetes and T2D. Ultimately, this will contribute to better quality of life, less healthcare costs and relieving the pressure on the health care system.

(Target group) To whom are the research results interesting and/or relevant? And why?

The results of the studies described in this thesis add an extra piece to the complex puzzle of BCAA metabolism. This can be of great interest for other researchers in this field to design new studies to fill the current knowledge gap in human physiology regarding BCAA metabolism and insulin resistance. In the end, this will result in a better understanding of the aetiology of T2D and development of better treatment strategies. Next to this, future studies can extend the findings demonstrated in this thesis by exploring if BCAA catabolism can be stimulated by several approaches, including diet, exercise, medication or change in microbiota and the consequences on metabolic health in people with insulin resistance and T2D on the long-term. Therefore, knowledge from future studies based on our results could lead to the development of new drugs for the treatment of T2D, thereby reducing the risk of progressed diabetes-related complications or related diseases.

Next, the knowledge obtained in this thesis is beneficial for patients with T2D. If we understand how BCAA is compromised and can be modulated, we could develop strategies to boost BCAA catabolism in patients with T2D. Since BCAA catabolism is involved in multiple tissues, metabolic health can be targeted by multiple mechanisms, which might reduce their symptoms and improve the quality of life. In terms of prevention, the results of the studies described in this thesis might also be relevant for the increasing number of people at risk of T2D and classified as having prediabetes. If we can identify people with a compromised BCAA catabolism before the onset of T2D with specific markers, it is possible prevent the onset of the disease. To translate those results to a broader population, e.g., people with prediabetes, more research is needed.

Furthermore, health care professionals involved in treating patients with T2D, including general practitioners, endocrinologist en dieticians could use the findings in this thesis to understand the consequence of a compromised BCAA catabolism in the development of T2D. The results of this thesis support the development

of strategies to boost BCAA catabolism as potential novel treatment of diabetes. Additionally, lack of knowledge is a huge barrier to self-care for patients with T2D (6), and therefore, they can help to inform and aware patients about the aetiology of their disease and the advantage of using this new strategy to improve their disease and delay T2D-related symptoms.

(Activity) In what way can these target groups be involved in and informed about the research results, so that the knowledge gained can be used in the future?

The conducted research and obtained results in this thesis have been communicated to other researchers and health care professionals through oral presentations and posters at national and international conferences. Additionally, most of the results presented in this thesis are and other results will be published in the future as original scientific articles in international well-recognized peer-reviewed journals. The scientific articles are available online and are shared on websites and social media. In this way, the acquired knowledge in this thesis was assessable worldwide for researchers, health care professionals and other people interested.

Next to this, this thesis has strengthened the collaboration between our lab and other external labs. The lab of Prof. Z. Arany at the Perelman School of Medicine (Pennsylvania, US) provided us to use metabolomics to obtain more insight in our valuable plasma samples. The partnership with the research group at the University of Nottingham (UK), headed by Prof. P. Atherton, shared their expertise in several methodologies in cell culture studies, which has given a lot of new opportunities to our lab. Future metabolic analysis on plasma and human skeletal muscle samples already obtained from patients with T2D following interventions known to boost BCAA catabolism will be established by Lilly Corporate Centre (Indianapolis, US). This cooperation will contribute to a molecular understanding of the link between BCAA catabolism and insulin sensitivity and a further evaluation of a potential new treatment strategy to combat T2D. Together, these collaborations make it possible to exchange ideas and share knowledge, which will stimulate future research.

All study participants received a document including their individual and group study results in layman terms. To transfer the knowledge obtained in this thesis to the society, the results were also communicated at participant information events. In that way, it was possible to inform patient with T2D and people at risk for the development of T2D or related diseases.

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