Branching-out

SUMMARY

The trend for increasing type 2 diabetes (T2D) has become a major problem worldwide as T2D comes with a profound health burden and possesses significant impact on health expenses. A hallmark of T2D is insulin resistance, a decreased tissue response to insulin stimulations, with disturbed glucose homeostasis as a consequence. Eventually, this will result in elevated glucose levels circulating in the bloodstream, also called hyperglycaemia. Chronic hyperglycaemia has severe, detrimental effects and lead to serious complications, like cardiovascular dysfunction, chronic kidney disease and nerve damage. Therefore, strategies to improve insulin resistance to normalize hyperglycaemia are key to prevent the progression of T2D and its related complications. Despite the availability of current lifestyle and pharmacological therapies, hyperglycaemia remains difficult to manage in patients with T2D, and thus, development of novel treatment strategies is crucial.

Over the last 30 years, branched-chain amino acid (BCAA; leucine, isoleucine and valine) metabolism has been shown to play an emerging role in the development of insulin resistance and T2D. It has been shown that BCAA levels are considerably elevated in plasma and tissues in people with obesity and T2D. Furthermore, elevated BCAA levels in plasma strongly associates with insulin resistance in people with obesity and T2D. Although it is still unknow why BCAA levels are elevated and why BCAA levels correlate positively with insulin resistance, it is clear that dysfunctional BCAA catabolism may be one of the underlying factors. Interestingly, multiple rodent studies have shown that pharmacologically boosting BCAA oxidation improved insulin sensitivity and glucose homeostasis. These studies provide evidence that BCAA catabolism plays an important role in T2D, and that boosting BCAA catabolism could be a potential strategy in the treatment of T2D. Thus far, BCAA metabolism has been rigorously studied in rodent models. Information in humans, however, is rather limited. In this thesis, the role of BCAA catabolism in the pathogenesis of obesity and T2D-related metabolic disturbances and the potential of boosting BCAA catabolism in the treatment of T2D was explored.

In **chapter 2**, we aimed to provide insight into the mechanisms behind elevated plasma BCAA levels in people with insulin resistance and T2D and its role in insulin resistance. Furthermore, we reviewed pharmaceutical and alternative lifestyle intervention strategies in order to lower plasma BCAA levels and its effects on metabolic health. Main findings described in this thesis were that studies in rodents showed that increased levels of BCAA in plasma might be

the results of a dysregulated BCAA catabolism in several tissues. Although only limited knowledge derives from human studies, large differences were observed compared to the results found in rodent tissues. In addition, several rodent studies showed the strength of boosting BCAA catabolism as potential strategy to improve glucose homeostasis, but so far, these findings have not yet been confirmed in humans since a limited number of tools is available to test this concept. The main conclusions of **chapter 2** were that insulin resistance could occur via dysregulated BCAA catabolism or BCAA levels *per se* acting a signaling molecules hampering the insulin signaling pathways. Therefore, exploring intervention strategies to increase BCAA catabolism and/or lower BCAA levels is important to investigate whether this could be a new potential strategy in the treatment of T2D.

In **chapter 3**, BCAA levels and diabetes-related metabolic parameters were investigated in fifteen patients with T2D, thirteen first-degree relatives of patients with T2D (FDR) and seventeen BMI- and age-matched control participants. In the present study, we confirmed the finding of higher plasma BCAA levels in patients with T2D compared to control participants and showed for the first time that plasma BCAA levels significantly correlated with diabetes-related disturbances, like *ex vivo* mitochondrial oxidative capacity and metabolic flexibility. Subsequently, *in vivo* leucine oxidation was measured as reflection of BCAA oxidation and was significantly lower in patients with T2D compared to control participants. Together, these results suggest that a low mitochondrial oxidation of BCAA may contribute to higher plasma BCAA levels and affect metabolic health in people with T2D.

The relationship between elevated BCAA levels and diabetes-related disturbances was further explored in **chapter 4**. In this study, it was shown that BCAA levels were positively associated with intrahepatic lipid (IHL) content, based on data from 1983 individuals from the Netherlands Epidemiology of Obesity (NEO) cohort and a 12-week exercise intervention program, performed in seven patients with T2D, seven individuals with non-alcoholic fatty liver (NAFL) and seven BMI-matched control participants. Together, the results described within this thesis underline that BCAA catabolism relates to insulin resistance and energy metabolism, and that elevated BCAA levels associate with key metabolic disturbances in patients with T2D.

We investigated the effect of boosting BCAA oxidation, by use of exercise intervention (**chapter 4**) and one pharmaceutical strategy (**chapter 5**), on metabolic health in humans. Physical activity and exercise training is well known to improve insulin sensitivity and reduce liver fat content, however, it has never

been investigated whether this effect relates to a reduction in plasma BCAA levels. Based on the strong association between plasma BCAA and liver fat content and considering that physical activity level or exercise training may affects liver fat content, we aimed to investigate whether physical activity and exercise training was able to lower plasma BCAA levels along with alterations in liver fat (**chapter 4**). We found that physically active people had slightly, but significant lower plasma BCAA levels, although the level of physical activity did not alter the positive association between plasma BCAA levels and liver fat content. In addition, we found that a conventional exercise program, including both resistance and endurance training was effective in lowering liver fat content in people with NAFL and control participants. This, however, this drop in liver fat did not coincide with lower plasma BCAA levels. These results indicate that chronic physical activity and exercise training differently affect plasma BCAA levels. Results also show that BCAA do not play a role in mediating the beneficial metabolic effects of exercise training on liver fat content. The mechanism of physical activity leading to lower plasma BCAA levels is, however, unknown and needs further research.

In **chapter 5**, the therapeutic value of boosting BCAA catabolism in type 2 diabetes was considered. We investigated the potential effect of sodium-phenylbutyrate ((NaPB), an inhibitor of the BCKD kinase, and stimulant of BCAA oxidation) on metabolic health in patients with T2D as novel approach. Sixteen participants were administered daily with 4.8 g/m^2/day NaPB or placebo for 2 weeks, whereafter they underwent a comprehensive metabolic evaluation. NaPB was for the first time prescribed 'off-label' to patients with T2D to stimulate the oxidation of BCAA aiming to lower the levels in plasma. In the present study, we showed that 2-weeks treatment of NaPB effectively reduced plasma BCAA levels. This reduction was accompanied by a 27% improved peripheral insulin sensitivity, mainly exerted by enhanced insulin-stimulated glucose oxidation. In addition, NaPB treatment increased *ex vivo* mitochondrial oxidative capacity upon a glycolytic-like substrate in muscle by 10%. We did not find major effects of NaPB treatment on the level of hepatic tissue (hepatic insulin sensitivity, liver fat content and composition), which suggests that insulin resistance in the liver is less responsive to NaPB treatment. Together, these results show that effects of NaPB treatment in patients with T2D for a great part take place in peripheral tissues, mainly muscle, which matches with the observation that skeletal muscle in humans has the highest capacity for BCAA catabolism. From **chapter 5,** we can conclude that pharmacologically boosting BCAA oxidation is able to lower BCAA plasma levels in patients with T2D resulting in beneficial outcomes on patients' glucose metabolism.

The molecular link of BCAA levels with insulin resistance was studied in **chapter 6**. Here, we aimed to investigate whether exposing skeletal muscle cells to elevated BCAA levels could underlie disturbed insulin-stimulated glucose uptake, reflecting insulin resistance, via an inhibition of the insulin signaling pathway. The results of the study showed that supplementation of BCAA to mice skeletal muscle cells impeded the insulin signaling pathway via stimulation of mTORC1 and S6K, although no effects were found on insulin-stimulated glucose uptake. This chapter supports the use of targeting BCAA levels in condition of insulin resistance. Future work in human cells will be required to further increase the insight about the underlying mechanisms of elevated BCAA levels in insulin resistant states, such as T2D.

The role of systemic BCAA levels and T2D development was studied in **chapter 7,** a systematic review and meta-analysis. Here, we aimed to examine the association between BCAA levels and the pathogenesis of T2D through case-control study data of people with overweight, by using systematic search. Furthermore, development of this association over time prior to T2D diagnosis was assessed. The results described in this chapter revealed consistent positive associations between each BCAA level and the eventual development of T2D, irrespective of follow-up period duration. These positive associations between BCAA and T2D at a variable period prior to incident T2D suggest that elevated BCAA levels could potentially predict the onset of T2D years before the clinical T2D symptoms manifest.

In conclusion, the studies outlined in this thesis demonstrate that elevated BCAA levels are linked to insulin resistance and other T2D-related metabolic disturbances. However, the underlying mechanisms of how BCAA links to insulin resistance still require further study. Our data shows that patients with T2D feature compromised BCAA catabolism, which may explain the elevation in BCAA levels measured in these patients. We furthermore, provided evidence that boosting BCAA catabolism could be a new potential strategy to treat T2D. Further evaluations of this potential treatment strategy are needed aiming to prevent the progression towards T2D.