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Lipotoxicity and type 2 diabetes in South Asians

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General Discussion

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To date, it remains hard to explain the high T2D prevalence among populations of South Asian compared to European background. Since development of T2D is usually of polygenic nature many pathways may play a role. However, many hypotheses have already been refuted while evidence for the ultimate explanatory variable(s) is lacking[1]. A promising cue points towards a relatively high fat deposition in ectopic compartments. Ectopic fat deposition may lead to lipotoxicity, which is a major contributor to T2D prevalence in the general population. Therefore, this thesis set out to investigate whether the high T2D prevalence among South Asians compared to other populations may be explained by a higher susceptibility to lipotoxicity, with the ultimate aim to identify potential targets for prevention of T2D in South Asians. Evidence was obtained at various levels of the lipotoxicity model. The evidence included comparisons of levels of metabolite profiles reflecting both dietary intake and (disturbed) endogenous metabolism, and an exploration of inflammation as a possible mediating pathway between disturbed metabolite profiles and T2D.

The studies included in this thesis confirmed that lipotoxicity is, in general, indeed an underlying mechanism leading to T2D, since both dietary fat intake and metabolic profiles reflecting increased use of non-oxidative pathways were associated with increased risk for T2D. This thesis added to that knowledge that the lipotoxicity mechanism leading to T2D is likely to be similar in South Asians and Europeans, since no evidence of multiplicative interactions between ethnicity and one of the parameters under study with T2D were identified. Moreover, parameters reflective of lipotoxicity and associated with T2D risk were not more prevalent among South Asians compared to Europeans. Therefore, there is no evidence to suggest that lipotoxicity accounts for the higher T2D risk among South Asians compared to other populations. Although lipotoxicity is not likely the mechanism underlying the high T2D risk among South Asians compared to Europeans, this thesis did identify some cues that may be further explored in future studies. These include amino acid patterns that point towards reduced liver function. Despite mechanisms underlying the high T2D risk are still not identified, these small cues are of major importance in this quest, especially since decades of previous research has not lead to identification of the underlying causes.

In this final chapter the overarching findings, limitations and suggestions for future research are discussed. First, an overview of the methodological considerations is given to enhance the interpretability of the results. Second, lipotoxicity-related mechanisms underlying T2D risk (**chapters 2, 3, 4** and **5**) and implications of the findings are discussed (**chapters 6** and **7**). Finally, suggestions for future research are provided.

Methodological considerations

Although it can be argued whether cohorts need to be representative of the entire population[2], insight in the generalizability of the included study population benefits the interpretations of the results. The original studies in this thesis were nested within the HELIUS study. The HELIUS study included people living in Amsterdam, the Netherlands and was set-up in a way that selection bias was minimized. This was done by taking a random sample from the population through the municipality register. The random sample was stratified by ethnicity. This approach allows for a minimal introduction of bias since almost all people legally living in Amsterdam are registered with the municipality register. The main source of selection bias within the HELIUS cohort was introduced by non-response of those invited to the study. The HELIUS cohort had a relative low response rate, with 31% of the invited Surinamese and 33% of the invited Dutch finally participating in the study. Non-response analyses, though limited to age, sex and some socioeconomic characteristics, showed only very small differences between participants and non-participants[3]. Selection-bias is therefore unlikely, although not completely excluded for other variables.

Generalizability may also have been limited since the included population is solely reflective of the population living in Amsterdam, the Netherlands. Dutch and South-Asian Surinamese living in Amsterdam, may not represent all European and South Asian populations respectively. South Asian populations, for instance, consist of a very heterogeneous group of people with a wide variety of population characteristics. The ancestral background of South Asian populations differs since they originated from various areas[4], and heterogeneity exists both in genetic aetiology and in lifestyle factors. This heterogeneity may impact on absolute T2D risk, but all South Asian populations have the communality of a relatively high susceptibility to T2D compared to other populations. The impact on generalizability of study results will, thus, likely be limited. Although absolute differences may differ between populations, relative differences of included subpopulations will be comparable to that of other European and South Asian populations. However, when preventive programs are developed, these should consider the heterogeneity within subpopulations and adapt the intervention to fit different population characteristics, including sociocultural practices[5].

Further selections of participants that may have led to limited generalizability were made in individual studies included in this thesis. In **chapter 2** data from the HELIUS-Dietary Patterns sub-study was used, dietary patterns were determined for a subpopulation of the HELIUS study and included approximately 1000 participants per ethnic group, while blood measurements were done for a random sub-sample of approximately 200 participants per ethnic group. Non-response comparisons showed that a somewhat more healthy sub-population of participants was willing to participate in the dietary patterns sub-study[6]. Since differences in characteristics were similar across ethnic groups this will likely not have affected comparisons across ethnic groups. **Chapter 3** was based on a

random sample of participants from the HELIUS study, which will likely not have affected the representativeness of participants any further than discussed above. In **chapter 4** and **5** follow-up data was used. Participants may have selectively dropped-out, e.g. due to decease or emigration. Since data from insurance companies was used to obtain follow-up data, bias could have been introduced if some participants were not registered with such a company. Since all inhabitants of the Netherlands are obliged to be registered with an insurance company, the effects of participants not being registered with an insurance company may have had limited impact on the results.

The included studies in this thesis were of observational nature, which implies that the studies may be prone to confounding biases. Causal inferences from observation data should be drawn with care, since there may be alternative explanations at stake. **Chapter 2** and **3** were cross-sectional. The observed association between CEFA and T2D in **chapter 2** may for instance also be caused by changed dietary habits in participants who were diagnosed with T2D. However, people diagnosed with a disease such as T2D only report minimal lifestyle changes, and generally tend to change their lifestyle in a more healthy direction[7]. In **chapter 4** and **5** follow-up data was used. Therefore, reverse-causality is less likely to have affected the results. For more certainty landmark analysis could have been used, but we refrained from these analyses since follow-up duration was limited. In future studies with a longer follow-up, landmark analyses could also reveal existing associations since participants that did not develop T2D in the excluded time period could have an unfair survival advantage compared to the participants that did develop T2D, known as immortal time bias[8].

For the interpretation of the results, the definition of the T2D diagnoses is also of importance. Commonly used methods to establish a diagnosis with T2D in clinical practice are based on repeated measurements of HbA1c, fasting plasma glucose, or 2-h plasma glucose after an oral glucose tolerance test (OGTT)[9]. Due to constraints of resources, T2D was based on one instead of repeated measurements. This might have led to a slightly higher prevalence of T2D. Participants who were classified to have T2D according to this measurement, might have had HbA1c and glucose values that fall within normal ranges at a second measurement. These participants would not have been diagnosed with T2D in the clinic. In addition, the chosen instrument of diagnoses may be important for the interpretation of results since the various diagnostic tools do not perfectly correlate[10]. This may have consequences for both the estimate of T2D prevalence within groups, and for relative differences between groups, since the correlation may differ by ethnicity. In **chapter 2** prevalence of T2D was based on self-reported physician diagnosis, anti-diabetic medication use, fasting blood glucose levels and HbA1c levels. Results of studies which use HbA1c diagnostic tools should be interpreted with special care. Diagnostics by HbA1c has advantages concerning less day-to-day variability and convenience for the participants since no fasting or glucose loaded drink is required, but many factors may influence the correlation between HbA1c levels and plasma glucose levels[11]. These factors include variants of haemoglobin and turnaround time of

haemoglobin and, importantly, may vary by ethnicity. To illustrate, at similar fasting plasma glucose levels, HbA1c concentrations are higher among Blacks, Hispanics and Asians than among Caucasians[9, 12]. Results of HbA1c tests may also differ among South Asians compared to Europeans, especially since hemoglobinopathies (e.g. Haemoglobin E) are common among South Asians[13]. Incorporating HbA1c levels in the definition may thus have affected the results. This bias was kept to a minimum by excluding HbA1c levels of participants who were homozygotes for known hemoglobinopathies or heterozygotes for variants that may disturb reliable measurements. The outcome variable in **chapter 4** and **5** was incident T2D. Therefore, participants with T2D at baseline were excluded from the analyses. In these chapters, prevalent T2D was based on self-reported physician diagnosis, anti-diabetic medication use and fasting blood glucose levels. The concerns related to HbA1c measurements were thereby anticipated. Consideration of self-reported physician diagnosis may have caused bias in case participants did not recall their diagnosis, or did not want to report it. This bias may be of special importance in case self-report was more accurate in one ethnic group than another. Due to differences in prevalence of T2D, some ethnic groups may have better awareness of the disease and higher screening rates. However, studies on the validity of self-reported physician diagnosis showed both excellent positive and negative predictive value across ethnic groups[14]. Consideration of fasting blood glucose levels besides self-reported physician diagnosis will have further decreased possible bias due to different screening rates between ethnic groups.

Although statistical methods are chosen as objectively as possible, there is always unavoidable subjectivity involved. In addition, multiple perspectives may be relevant to each statistical discussion which may lead to different choices. In **chapters 2**, **4** and **5** we aimed to unravel the mechanisms underlying the high T2D risk among South Asians compared to Europeans by considering various mediators. In all these chapters different statistical approaches were used. In **chapter 2** a formal causal mediation analysis was used according to the counterfactual method[15, 16]. Causality was assumed in this model, although this may not necessarily be the truth. An advantage of the formal use of mediation analysis is that this is a powerful technique to assess the contribution of a variable to the total effect of an exposure. The use of mediation analysis largely avoids the bias that might be introduced by traditional methods, that were used in **chapter 5.** Standard ordinary least squares regression models adjusting for a confounder were here used to estimate the contribution of the mediating variable to the total effect of ethnicity on T2D risk[17]. In **chapter 4** no formal mediation analysis was performed, but the implications of the observed concentrations and associations was described. This approach avoids the strong underlying assumptions that need to be met for mediation analysis to be reliable, but are most of the time untestable. For instance, mediation hypothesis is always based on a causal assumption and the model will assume that the causal modelling of the variables is correct, while it is generally difficult to prove a causal relationship and even impossible using observational data.

Interpretation of the results

Lipotoxicity was studied as a possible cause for the high T2D risk among South Asians. The mechanism presumes that storage of fatty acids in non-adipose tissue has detrimental effects, and may ultimately lead to T2D[18]. First, collected evidence regarding development of T2D as a cause of lipotoxicity is discussed. Thereafter, ethnic differences in prevalence of lipotoxicity related components are discussed. *Table 1* provides an overview of the evidence obtained in this thesis.

Table 1: Association of lipotoxicity related markers with T2D.

Lipotoxicity marker	Association T2D	Multiplicative interaction by ethnicity*	Concentration South Asians compared to Europeans	Likely contributor high T2D risk South Asians
Saturated fatty acids	↑[a]	No	↓	No
Mono-unsaturated fatty acids	↑[a]	No	↓	No
Polyunsaturated fatty acids	↓[a]	No	↑	No
Acylcarnitines	↓[a]	No	↑	No
Amino acids	↑[a]	No	↑	Yes
Ceramides	↑	No	↓	No
Complex sphingolipids	↓	No	↓	Yes[c]
C-reactive protein	↑[b]	No	↑	No

*↑ A positive association between the lipotoxicity marker and T2D was identified. ↓ A negative association between the lipotoxicity marker and T2D was identified. [a] Associations of some individual metabolites within this group of metabolites showed different directionality in the association between metabolite and T2D. [b] The positive association between C-reactive protein and T2D was only observed in analyses that were unadjusted for markers of adiposity. The association is almost fully explained by adiposity levels, and high C-reactive protein levels may thus directly be a result of an imbalance in energy intake and expenditure rather than amplified by lipotoxicity related mechanisms. [c] The lower levels of complex sphingolipids among South Asians compared to Europeans may be directly related to the lower levels of ceramides. Since low levels of ceramides are protective of T2D, intervening in levels of complex sphingolipids might have counter effective results and should be considered with care. *Based on p-value <0.05.*

T2D as a cause of lipotoxicity

As was shown in the introduction, lipotoxicity is usually induced by an imbalance in energy intake and expenditure, but specific types of macronutrients may promote the use of non-oxidative pathways[19]. **Chapter 2** examined the association of a specific macronutrient, namely dietary fat intake, with T2D. Dietary fat intake was quantified by cholesteryl ester fatty acids (CEFA) which reflect dietary intake in the past weeks, although it reflects some endogenous fatty acid metabolism as well. Consistent with previous literature[20], higher proportions of poly-unsaturated fatty acids were associated with lower prevalence of T2D, while higher proportions of saturated fatty acids were associated with a higher prevalence of T2D. Studies on absolute, rather than proportionate values of fatty acids showed positive associations with insulin resistance independent of the type of fatty acid[21]. This is consistent with the idea that lipotoxicity is mainly the result of an energy imbalance. Fat

is a highly energy-dense macronutrient[22], higher intakes of fat thus increase the energy intake, which may lead to dysregulated fatty acid metabolism and T2D. This suggests that although some types of fat may be preferred over others, the total amount of fat intake needs to be limited.

It is thought that one of the lipotoxicity related pathways leading to T2D is mitochondrial dysfunction. Low mitochondrial oxidative capacity may lead to high levels of lipid intermediates and may interfere with insulin signalling. Metabolites that mark disturbed long-chain fatty acid metabolism are acylcarnitines. Up to date remained unclear whether acylcarnitines were in the causal pathway to T2D, or rather were a consequence of T2D[23]. **Chapter 4** showed negative associations between acylcarnitines and T2D incidence. This suggests that the observed disturbed long-chain fatty acid metabolism in prevalent T2D patients in other studies is a *consequence* of T2D rather than indicative of a causal link between e.g. an oversupply of energy and T2D. On the other hand, plasma carnitine concentrations may not well represent tissue specific concentrations and carnitine concentrations in liver and muscle tissue may better reflect the mitochondrial dysfunction and accumulation of fatty acids, this could be unravelled further in future studies[24].

Oversupply of fatty acids may also lead to the use of non-oxidative pathways. Bioactive lipid metabolites that may accumulate and distort insulin signalling include diacylglycerol and fatty acyl-CoA[25]. But also ceramides, a subfamily of sphingolipids, were hypothesized to be involved[19,26]. Moreover, ceramides may induce apoptosis of β-cells and distort signalling pathways involved in glucose metabolism. **Chapter 4** confirmed the associations of ceramides with T2D. This chapter, however, also showed that more complex sphingolipids are associated with decreased T2D risk. Future studies may investigate whether these types of sphingolipids are protective against T2D, or whether expression of enzymes that convert ceramides to more complex sphingolipids is lowered in those at risk for T2D[27].

Closely linked to lipid metabolism is amino acid metabolism. Both palmitoyl-CoA and amino acids (mainly serine) are, for instance, rate limiting substrates in *de novo* formation of sphingolipids. **Chapter 4** showed that circulating amino acids were, consistently with other studies[28,29], associated with T2D. Studies on different types of protein intake, e.g. casein versus soy intake, showed that type of protein may interact with the lipotoxicity mechanisms. Type of dietary protein for instance influences lipogenesis in general and the *de novo* production of ceramides specifically[30]. Soy protein may have beneficial effects e.g. reducing hepatic lipotoxicity due to beneficial amino acid profiles[30].

Lipotoxicity may partly lead to T2D via an inflammatory pathway, e.g. via signalling pathways in which ceramides play a role[19]. **Chapter 5** showed that, indeed, low-grade inflammation was associated with T2D. However, high levels of CRP were mainly related to increased levels of adiposity, and may thus be directly related to an imbalance in energy intake and expenditure rather than amplified by lipotoxicity related pathways. **Chapter 5** was, however, limited to CRP and other inflammatory markers may show different results.

Lipotoxicity in relation to higher risk of T2D in South Asians: no indications for heterogeneity in associations

Chapters 2, **4** and **5** examined whether the lipotoxicity mechanism played a different role among people of South Asian than European background, by checking the multiplicative interaction between ethnicity and the lipotoxicity related marker with T2D as the outcome. No evidence of a different association between the markers and T2D by ethnicity was found. Therefore, it is likely that the lipotoxicity mechanism biologically plays a similar role in the development of T2D in both Europeans and South Asians. This contrasts our hypothesis that because of the observed Asian Indian phenotype storage capacity for fat tissue among South Asian may be lesser, leading to a predisposition of lipotoxicity related damage.

Lipotoxicity in relation to increased risk of T2D in South Asians: weak indications for the contribution of a higher prevalence of circulating amino acids

For each of the lipotoxicity related markers prevalence among Europeans and South Asians was compared, in order to examine whether lipotoxicity explained some of the ethnic differences in T2D risk. **Chapter 2** suggests that proportions of dietary fat intake may be more beneficial among South-Asian Surinamese than Dutch. This contrasts the hypothesis in the introduction, where was proposed that consumed products and food preparation habits among South Asians may lead to relatively high intakes of saturated fats[31, 32]. The findings do, however, confirm opposite suggestions that South Asian diets may be relatively healthy, also in terms of dietary fat intake[33]. This implies that dietary intake may not explain the excess T2D risk among South Asians compared to other populations. The contrasting hypothesis based on dietary observations also implies that it might be recommended not to speculate on nutrient intake of various subpopulations based on observations of commonly used food items and preparation techniques, but to objectively measure dietary intake. In **chapter 2** proportion data was reported, but results were similar when absolute amounts of fatty acids are compared between ethnic groups in other studies[21]. Absolute amounts of most saturated and mono-saturated fatty acids were, consistently with proportion data, reported to be lower among South Asians than Caucasians, while absolute amounts of poly-unsaturated fatty acids were higher[21]. Importantly, we did not consider trans-fats, nor did any other study to date, although these have been consistently associated with T2D[34]. South Asians may have considerably different levels of trans-fat intake since both dietary products and food preparation habits are culturally determined. Products such as trans-fats may be formed during food preparation, and especially during high-heat cooking practices commonly used among South Asians[32]. However, since formation of such intermediates are difficult to predict on observations only future studies could compare objectively measured trans-fat concentrations between ethnic groups.

Chapter 3 and **4** showed that markers of disturbed long-chain fatty acid metabolism, acylcarnitines, were comparable between European and South Asian women, but somewhat higher among South Asian men than European men. As discussed above, we

infer that increased levels of acylcarnitines may be a consequence of T2D rather than induce T2D. Therefore, higher acylcarnitines concentrations among South Asians do not likely explain the high T2D risk among South Asian compared to other populations.

Chapter 4 showed that sphingolipid concentrations, markers of non-oxidative pathways, were lower among South Asians than Europeans. This might directly be related to the observed lower plasma concentrations of saturated fatty acids in **chapter 2**, since saturated fatty acids are among the rate-limiting substrates for de-novo synthesis of sphingolipids[35]. The lower availability of saturated fatty acids among South-Asian Surinamese might thus result in lower sphingolipid concentrations. This also suggests that for non-oxidative pathways to play a role in lipotoxicity and T2D risk, types of macronutrient intake and especially type of dietary fat intake plays an important role. Both plasma fatty acid and ceramide concentrations, thus, suggest that dietary fat intake together with endogenous metabolism is currently more beneficial among South Asians compared to Europeans. If these more beneficial profiles are not maintained in the future, T2D risk may even further increase among South Asian compared to European populations.

Chapter 3 and **4** showed that amino acid concentrations were higher among South Asians than Europeans. Circulating levels of amino acids may both reflect dietary intake and amino acid metabolism in the body. Although the different amino acid levels between populations may thus reflect different dietary intake, the observed pattern of elevated levels of methionine, alanine, phenylalanine, tyrosine and lysine in South Asians compared to Europeans, resembles the pattern observed in patients with reduced liver function[36]. The liver is one of the compartments in which South Asians tend to store disproportional amounts of ectopic fat, including in the liver, compared to Europeans. Previous studies have shown that it is not likely that differences in body composition account for the observed differences in T2D risk[37]. If, however, ectopic fat deposition in the liver leads to reduced liver function among South Asians, this may impact on T2D risk, since reduced liver function has been associated with T2D. However, the complex relationship between liver fat deposition, insulin resistance and T2D needs disentanglement, especially among South Asians. A recent Mendelian randomization study showed that insulin resistance may increase non-alcoholic liver disease risk, which then increases T2D risk[38]. This suggests that reduced liver function among South Asians may also *result* from the higher levels of insulin resistance. Nonetheless, the observed amino acid patterns forms a clue that needs further investigation and may help to identify why South Asians have such a high predisposition for T2D.

Finally, this thesis assessed one of the pathways of how lipotoxicity, and more specifically, non-oxidative lipid metabolism may lead to T2D. **Chapter 5** showed that markers of low-grade inflammation (C-reactive protein) were elevated among South Asians compared to Europeans. But because of above described associations with T2D, these are not likely to explain the high T2D risk among South Asians compared to Europeans above different distributions of adiposity levels.

Conclusion, discussion of implications and future research

In conclusion, no evidence was found to suggest that lipotoxicity accounts for the higher T2D risk among South Asians compared to other populations, but lipotoxicity does underlie T2D risk in general. These findings suggest that lipotoxicity-related targets, including those related to energy balance and type of dietary fat intake, may be included in interventions and guidelines to prevent T2D, as is currently the case. However, including such targets will not decrease the relative ethnic differences in T2D risk. Although lipotoxicity is not likely to explain the ethnic differences in T2D risk, this thesis found some clues that merit further exploration in future studies. Disturbances in amino acid metabolism resemble those seen in patients with reduced liver function. In addition, we may investigate why the metabolic disturbances may exist from a young age.

Implications
Population-based primary preventive strategies are used to minimize the manifestation or to delay the onset of T2D. Intervention programmes using lifestyle modification strategies were first developed and shown to be effective for, mostly, European-based populations[39]. Recent evidence showed that these kind of interventions could also effectively reduce T2D risk among South Asian populations[40]. Interventions that target specific high-risk populations may focus on determinants that are more prevalent or are more strongly associated with the disease.

Chapter 7 showed that recommendations to prevent T2D among South Asians to date have been similar to those for general, mainly European-based, populations. Moreover, recommendations have only been culturally targeted by adapting language, culture and context. To enhance effectiveness of future interventions and guidelines to prevent T2D among South Asian populations, it may be advisable to evaluate current and emerging components on its biological effectiveness among the predisposed South Asian populations. If, for example, liver function is reduced among South Asians compared to Europeans, specific recommendations that spare the liver may be incorporated to prevent T2D among South Asians. The studies included in this thesis showed that lipotoxicity is similarly associated with T2D among South Asians and Europeans, and prevalence of lipotoxicity related markers is similar or lower. There is, thus, no evidence that lipotoxicity related recommendations for prevention of T2D need to differ between European and South Asian populations based on biological mechanisms. However, they may need targeting regarding e.g. dietary habits.

Although the high T2D risk among South Asian compared to European populations may not be related to lipotoxicity related mechanisms, this thesis did provide some leads that may help to unravel the causes of the high T2D risk among South Asians in the future. First, **chapter 3** showed that metabolic disturbances among South Asians compared to Europeans may already exist at the start of adulthood. To illustrate, metabolites reflective of disturbed long-chain fatty acid and amino acid metabolism increase at similar rates

during adulthood in South Asian and Europeans (**chapter 3**), while the difference in concentration already existed at young age. This may thus also underlie the observed lower age at which South Asians develop T2D. Other studies have also shown that South Asians were already more insulin resistant in childhood than Europeans[41]. Studies that examined cord-blood suggested higher insulin resistance among South Asians than Europeans at birth, but these may be directly related to higher maternal fasting glucose levels[42, 43]. Future studies thus need to examine mechanisms underlying T2D risk at young age. The findings of these studies may also implicate that interventions need to start early in life. Second, **chapter 3** and **4** indicated that ethnic differences in amino acid metabolism, possibly related to diminished liver function among South Asians, may be important. If confirmed, this may implicate that interventions are needed that include recommendations which spare the overloaded amino acid metabolism and/or reduced liver function.

A different way to decrease ethnic differences in T2D risk, if no novel mechanisms or mechanisms that play a larger role among South Asians than among Europeans are identified, is to set more stringent thresholds regarding existing strategies. The current preventive strategies do work in both European and South Asian populations, and can thus be used to decrease ethnic differences if targets are set higher among South Asians. For example, a study evaluating recommended minutes of physical activity showed that South Asians need to undertake approximately 230 instead of 150 minutes of moderate intensity physical activity per week for a similar cardio-metabolic risk profile as Europeans[44]. Dietary strategies may be adjusted in similar ways, e.g. by decreasing recommendations for energy intake specifically for South Asian populations.

In the meantime, it is important that cultural habits that protect South Asian populations from T2D are maintained. Counter-intuitive, given the high T2D prevalence among South Asians, native South Asian diets may be more healthy regarding fat intake than European diets. **Chapter 2** showed the type of fat intake may be more beneficial among South Asians than Europeans, while another study showed a low adherence to a high-sugar high-fat dietary pattern[45]. Therefore, current South Asian dietary intakes may in this regard be protective regarding T2D risk compared to European intake[46]. Studies showed that migration to countries with populations that consume more unhealthy diets may lead to replacement of native dietary components by less healthy alternatives that are ubiquitously available in the country of migration[46], but this needs to be countered to prevent a further rise in T2D prevalence among South Asian migrants. In addition, this finding suggests that a focus on prevention of an increase in T2D prevalence in South Asian countries may be needed as well. Due to a more affluent population, unhealthy dietary options become more available and affordable in the South Asian subcontinent as well[47, 48].

Finally, when developing interventions and guidelines specifically aimed at certain populations, such as in this case South Asian populations, ethnic specific considerations regarding the form or meaning of specific components rather than content need to be taken into account. Even if mechanisms leading to T2D are similar, and risk factors are similarly

associated with T2D in the various populations, these cultural specific determinants may play a larger role among one population compared to others. Some risk factors that are not mentioned in general guidelines for the majority population may need to be incorporated when targeting a specific high risk population, such as for instance the use of ghee or Vanaspati among South Asian populations. And importantly, when interventions and guidelines are developed, the target population needs to be consulted to guarantee that the mode of delivery is culturally sensitive and will be picked-up by the target population.

Suggestions for future research

This thesis has led to some insights for future research. These are both directly related to research aimed to unravel the underlying causes for the high susceptibility to T2D among South Asians and to medical research in general. **Chapter 3** showed that metabolite patterns that were compared between South Asians and Europeans differed already from young age. However, since the HELIUS cohort had not included participants aged under 18 years, it was not possible to determine at what age these differences manifested. Future studies could identify both when and why these metabolic differences occur. The metabolic disturbances could both develop *in utero* or during childhood. They could occur both due to (epi)-genetic factors, early-life nutrition, or perhaps other early-life determinants. Birth cohorts, such as the South Asian Birth Cohort (START) could help to unravel when and why metabolic disturbances occur[49].

Since the lipotoxicity mechanisms did not explain the high T2D risk among South Asians, other targets for the reduction of ethnic differences in T2D risk need to be considered. **Chapter 3** and **4** indicated that amino acid metabolism, and more specifically amino acid pattern that indicate reduced liver function may be important. Future studies may study whether liver function is indeed diminished among South Asians and in the causal pathway to T2D.

Populations that are at considerably high risk for T2D compared to others, such as South Asians, may be used as a sort of magnifying glass for the general mechanisms that lead to T2D, especially when the underlying biological processes leading to the disease are comparable. Since this thesis did not provide evidence for different mechanisms, especially regarding lipotoxicity related mechanisms, studies investigating the processes leading to T2D may consider including participants of South Asian background in future investigations unravelling lipotoxicity related mechanisms underlying T2D. Inclusion of high-risk populations will increase efficiency in research since the disease is more likely to manifest, and less participants or shorter follow-up may be needed to identify underlying causes of the disease. Especially the inclusion of both low-risk and high-risk populations will allow for extreme comparisons between groups since the variation in exposure variables will be larger.

Many studies are centralized around one disease outcome, as was also the case in this thesis. However, many diseases may have a common cause. Lipotoxicity may for instance not only underlie T2D, but also other diseases, including but not limited to cardiovascular disease, renal disease and non-alcoholic liver disease[50, 51]. All these diseases are known to have a high prevalence among South Asians compared to other populations, which further points towards a probable common cause. Therefore, the focus of research projects might need to shift from a specific disease outcomes focus to a focus on the potentially shared underlying mechanisms of diseases that co-occur among high risk populations. This may also help in the development of guidelines with preventive strategies aimed to reduce the burden of disease among the general public[52]. Intervention studies that are developed to reduce one disease may be more practical if also proven efficient to prevent other diseases and to promote overall health.

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