ABSTRACT

Introduction

The global prevalence of type 2 diabetes (T2D) is increasing at an alarming rate, particularly in developing countries such as South Africa (SA). Black African women have a higher risk and T2D prevalence than white European women, but the pathophysiology of T2D appears to differ. Compared to white European women, black African women have lower visceral adipose tissue (VAT) and liver fat accumulation, and greater peripheral adiposity, but paradoxically present with lower peripheral insulin sensitivity and hyperinsulinaemia, often exceeding that required to maintain normoglycaemia. The exact causes and biological mechanisms underlying this phenotype are poorly understood.

Aim

To identify risk factors for T2D and elucidate putative biological mechanisms implicated in the pathophysiology of T2D in middle-aged urban black SA women. This aim was addressed in four parts:

- Using a longitudinal design, the first study **(chapter 2)** aimed to: (i) explore the association between body fat and fat distribution, and the risk for developing T2D 13 years later; and (ii) investigate the independent associations between baseline and/or change in body fat and fat distribution, and measures of glycaemia at follow-up.
- A cross-sectional design was used to (i) examine the relative contributions of insulin secretion and clearance to basal and postprandial hyperinsulinaemia; (ii) and to explore the associations between insulin sensitivity, adiposity and fatty liver index (FLI, as a proxy of liver fat)**,** and insulin secretion and clearance **(chapter 3).**
- The third study **(chapter 4)** used a cross-sectional design to determine whether

dietary-induced inflammation, measured by the dietary inflammatory index (DII), is associated with markers of T2D risk, and if this association is mediated by adiposity and/or inflammatory markers.

- Using metabolomics analysis, the final study of the thesis **(chapter 5)** used a longitudinal design to identify circulating metabolite patterns that predict the development of T2D**.**

Methods

A sample of 221 black SA women were recruited between 2015-2016 as they fulfilled the criteria of being a caregiver from the Birth-to-Twenty plus cohort (BT20+), on whom we have phenotype data and stored biochemical samples from 2002-2003. These criteria included being younger than 65 years of age, HIV negative and willing to be tested for HIV, and had normal fasting glucose (<6.1 mmol/l) at baseline. At both time points the following measurements were collected; basic anthropometry and dual-energy X-ray absorptiometry (DXA)-derived body composition including estimates of VAT and abdominal subcutaneous adipose tissue (SAT). An oral glucose tolerance test (OGTT) was completed at follow-up only and included analysis of glucose, insulin and C-peptide concentrations at 10, 20, 30, 60, 90 and 120 min. At follow-up, fasting bloods were used for additional analyses including the Homeostatic Model Assessment of Insulin Resistance (HOMA2-IR), HbA1c and inflammatory cytokines, as well as liver enzymes from which the FLI was estimated. A validated food frequency questionnaire was collected at follow-up and was used to calculate the energy-adjusted-DII (E-DII). At follow-up, fasting and OGTT-derived samples were used for the analysis of insulin sensitivity (Matsuda Index) and calculation of insulin secretion and clearance using the Mari mathematical model of beta-cell function. The World Health Organisation (WHO) diabetes diagnostic criteria were used for the categorisation of glycaemic groups: normal glucose tolerance (NGT, fasting glucose < 6.1 mmol/l and/or 2-h post glucose load < 7.8 mmol/l), impaired fasting glucose (IFG, fasting glucose 6.1-6.9 mmol/L), impaired glucose tolerance (IGT, 2-h post glucose load 7.8-11.0 mmol/l) and T2D

(fasting glucose ≥ 7.0 mmol/l and/or 2-h post glucose load ≥ 11.1 mmol/l). For statistical analyses the IFG and IGT groups were combined and referred to as impaired glucose metabolism, IGM, as appropriate. Multi-platform mass spectrometry-based metabolomics techniques, followed by multivariate analyses were used to elucidate metabolite signatures that were associated with T2D development at the baseline and follow-up sampling.

Results

Over the 13-year follow-up period, 64% of the participants remained NGT, while 25% developed IGM and 11% developed T2D. Body weight and fat mass (FM) increased by 7.0 ± 9.0kg and 5.8 ± 6.3kg, respectively, with a relative decrease in peripheral adiposity (leg %FM) and increase in central adiposity (trunk %FM and VAT) (all $p < 0.05$). The IGM/T2D group at follow-up had higher trunk %FM and VAT, as well as lower leg %FM at baseline, compared to the NGT group ($p < 0.0001$). Independent of age and body fat, measures of central obesity, baseline trunk FM (odds ratio (OR) (95% confidence interval (CI)) per 1 kg increase, 1.95 (1.43–2.67)), and baseline VAT (OR (95% CI) per 10 cm^2 increase, 1.25 (1.10–1.42)), and the change in VAT (1.12 (1.03–1.23)), were associated with greater odds of developing IGM/T2D at follow-up. In contrast, baseline leg FM (OR (95% CI) per 1 kg increase, 0.55 (0.41–0.73)) was associated with reduced IGM/T2D risk at the 13-year follow-up period. Furthermore, both baseline and change in trunk FM and VAT were inversely associated with insulin sensitivity, while baseline leg FM was positively associated with insulin sensitivity at follow-up.

In the second study of the thesis, it was reported that both insulin secretion and clearance were associated with basal and postprandial hyperinsulinaemia, however, insulin secretion explained more of the variance (47%) than insulin clearance (37%) in the basal state. In contrast, insulin secretion and clearance accounted for a similar proportion of the variance (12 vs 15%) for the total hyperinsulinaemic response to an OGTT. Insulin sensitivity and leg %FM were inversely associated with basal and total

insulin secretion ($p<0.0001$). In contrast, FLI and VAT were positively associated with both measures of insulin secretion ($p<0.05$). Insulin sensitivity was positively, and total adiposity, FLI and VAT were inversely, associated with measures of insulin clearance ($p<0.05$). Furthermore, insulin sensitivity accounted for a greater amount of the variance in both insulin secretion (18 and 38%) and clearance (21 and 26%) in comparison to measures of adiposity (insulin secretion, 8 and 23%; insulin clearance, 7 and 10%) and FLI (insulin secretion, 7 and 23%; insulin clearance, 7 and 9%).

The third study of the thesis showed that E-DII was associated with all markers of T2D risk, namely, fasting glucose and insulin, two-hour glucose, and insulin sensitivity, independent of age (all $p< 0.05$). These associations were mediated by measures of adiposity, but not inflammatory cytokines. Notably, measures of central obesity had proportionally higher (24–100%) mediation effects than total adiposity measures (10–60%).

The results of the final study of this thesis showed that the metabolite profile characterised by phospholipids, branched-chain amino acids (BCAA) and bile acid metabolites, was significantly different between the participants that developed T2D (i.e., NGT-T2D) and those that remained NGT (i.e., NGT-NGT) over the 13-year follow-up period. At baseline, the NGT-T2D group had lower proliferation-related bile acids (ursodeoxycholic acid, UDCA)- and chenodeoxycholic acid, CDCA) and higher lysophosphatidylcholine:lysophosphatidylethanolamine ratio containing linoleic acid (LPC(C18:2):LPE(C18:2)), and higher levels of leucine and its catabolic intermediates (ketoleucine and C5-carnitine), compared to the NGT-NGT group. At follow-up, these metabolites and/or metabolic pathways remained significantly different between the NGT-T2D and NGT-NGT groups. Accordingly, the NGT-T2D group had higher apoptosis-related bile acids (deoxycholic- and glycodeoxycholic acid) and lower LPC(C18:2) levels than the NGT-NGT group. In contrast, BCAAs and their catabolic intermediates remained significantly higher in the NGT-T2D group.

Conclusion

In summary, the findings of this thesis suggest that obesity, in particular central obesity, is a key risk factor for developing IR, and consequently hyperinsulinemia, and T2D in middle-aged black SA women. In addition to BCAA metabolism, which is extensively linked to the development of T2D in white Europeans, the current findings suggest that changes in phospholipid and bile acid metabolism might be important biomarkers for the early detection, prevention and management of the T2D in middle-aged black SA women. It is postulated that the promotion of healthy eating, in particular diets containing foods with low inflammatory properties, may be crucial in addressing the rapid increase in obesity and T2D in black SA women. Intervention and longitudinal studies are needed to determine causality and identify biological mechanisms that predict the development of VAT and liver fat accumulation, insulin resistance and hyperinsulinemia, as well as alterations in BCAA, phospholipid and bile acid metabolic pathways in the African population. This knowledge will assist in formulating intervention programmes to reduce the risk of developing obesity and T2D in black SA women.