Model Based Analysis of Ethnic Differences in Type 2 Diabetes

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Preface

This thesis was prepared at the Department of Informatics and Mathematical Modelling (IMM) at the Technical University of Denmark (DTU) in fulfillment of the requirements for acquiring the PhD degree in engineering. The PhD thesis was created as an industrial PhD with collaboration between DTU Informatics and Quantitative Clinical Pharmacology (QCP) at Novo Nordisk A/S (NN).

Main supervisors have been Henrik Madsen (IMM), Rune Viig Overgaard (NN), Steen H. Ingwersen (NN), and Claudio Cobelli from Universita di Padova. Furthermore Søren Klim (NN), Niels Rode (NN), and Christoffer Tornøe (NN) have participated in the project.

The project deals with application of population PK/PD modeling in describing the glucose homeostatic system and mathematical methods to analyse the possible differences between Japanese and Caucasians related to the development of type 2 diabetes. The thesis consist of a summary report and 4 scientific papers written during the PhD study and published/submitted/prepared for international journals.

Lyngby, Oct 2011

Jonas Bech Møller

Abstract

The present thesis deals with different aspects of population pharmacokinetic / pharmacodynamic (PK/PD) modelling of the glucose homeostatic system. The thesis consist of a summary report and four scientific research papers.

A description of the main topics covered in the thesis is given in the summary report. This includes a short introduction to the mathematical methods applied in the thesis, followed by an outline of the physiological and pathological aspects of the glucose homeostatic system and how to obtain diagnostic indices for characterising the condition of the system. Finally an overview of ethnic differences in type 2 diabetes (T2D) is given, which relates to the subject of the last 2 papers included in the thesis.

One of the main objectives of the thesis was to investigate possible ethnic differences between development of T2D in Caucasian and Japanese and investigate the applicability of stochastic differential equations (SDEs) and non-linear mixed effects (NLME) models for such an assessment. One way to perform such an investigation is to characterise the pathophysiology of the two groups at different stages of disease progression. For T2D this involves a characterisation of the glucose homeostatic system, which is a complex feedback system mainly involving mainly organs such as the liver and the pancreas, the hormones insulin and glucagon, and the carbohydrate glucose.

As for any other dynamical system, a proper characterisation at non-steady state, requires a proper input to the system. This input must reflect the circumstances in which one wants to draw conclusions. In this thesis the intake of oral glucose, which closely resembles the intake of food under daily living has been applied.

Mathematical modelling of such complex physiologal phenomenas as the glucose homeostatic system will usually be based on both insight into the system and experimental data. Through estimation techniques, free parameters in the models are estimated and can be related directly to behaviour of the system. These semi-physical (grey box) models are well suited for understanding the system, although in many cases they are not able to fully describe the systematic behaviour observed in the applied data sets. This issue can be adressed through an inspection of the autocorrelation function (ACF) of residuals and the description can be improved by switching to the use of stochastic differential equations (SDEs) or another improved description of residuals.

For characterising disease progression in Caucasian and Japanese, established models that include parameters for insulin sensitivity and beta-cell function were implemented in a non-linear mixed-effects setting with ODEs. Based on the ACF of residuals it was clear that the two models provide a good, although not perfect, description of the systematic variation in the analysed data sets. Based on this the models were extended to SDE models for improved description of residuals. Using the SDE models it was not possible to obtain convergence with the full covariate models so the results presented in the thesis mainly originate from the ODE models. This also caused a more fair comparison with the well-established single-subject models implemented using ODEs.

Previous research have stated the importance of the gut hormone glucagon-like peptide-1 (GLP-1) as determinant for normal beta-cell function. Based on this a population PK/PD model for secretion of (GLP-1) following an oral glucose tolerance test (OGTT) was developed. This model can be used as a tool to analyse potential differences in the secretion capabilities of GLP-1 between subjects. ACF of residuals did not show any signs of strong serial correlation, and the model was thus not implemented using SDEs.

Assessment of simple and model-based measures for insulin sensitivity and beta-cell function in Japanese and Caucasian subjects stratified according to normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and T2D showed that Japanese in general have higher insulin sensitivity and lower beta-cell function compared to Caucasians. In spite of this, the pattern going from NGT to T2D appeared similar in the two cohorts and the majority of the difference in insulin sensitivity and beta-cell function, measured by simple insulin based measures, could be explained by difference in body size (BMI). This was supported by Forest plots of covariate effects obtained from population models, in general indicating that race had no clinical relevant effect on either the insulin sensitivity or the beta-cell function when measures for obesity (android fat mass or BMI) was taken into account.

KEYWORDS: Pharmacokinetic/pharmacodynamic (PK/PD), type 2 diabetes (T2D), autocorrelation function (ACF), stochastic differential equations (SDEs), oral glucose tolerance test (OGTT), glucagon-like-peptide 1 (GLP-1), disease progression, ethnic differences

Resumé

Denne afhandling omhandler forskellige aspekter af populations pharmakokinetik / pharmakodynamik (PK/PD) modellering af glukose homeostase systemet. Afhandlingen indeholder en sammenfatning af projektet og fire videnskabelige artikler.

I sammenfatningen gives en generel beskrivelse af hovedemnerne i afhandlingen. Dette inkluderer en kort introduktion til de matematiske metoder der er anvendt i forbindelse med projektet efterfulgt af en beskrivelse af de fysiologiske og patologiske aspekter af glukose homeostase systemet, samt et kapitel om hvorledes diagnostiske index der kan karakterisere systemet kan beregnes. Til sidst gives en oversigt over etniske forskelle indenfor type 2 diabetes (T2D), hvilket relaterer sig til de to sidste artikler i afhandlingen.

En af hovedemnerne i afhandlingen var at undersøge mulige etniske forskellige mellem udvikling af T2D i kaukasere og japanere og undersøge anvendelsen af stokastiske differentialligninger og ikke-lineær mixed-effects modeller i en sådan undersøgelse. En måde at undersøge forskellen på, er at karakterisere patofysiologien i de to grupper ved forskellige stadier af sygdomsudvikling. For T2D involverer dette en karakterisering af glukose homeostase systemet, der hovedsageligt involverer organer såsom lever og bugspytkirtel, hormonerne insulin og glucagon, og sukkerstoffet glukose.

Ligesom for ethvert andet dynamisk system, kræver en korrekt karakterisering af systemet udenfor ligevægt, et fornuftigt input til systemet. Dette input skal reflektere de betingelser for hvilke der skal drages konklusioner om systemet. I denne afhandling er der anvendt oral glukose indgift, hvilket minder om normalt indtag af føde.

Matematisk modelling af et komplekst fysiologisk fænomen som glukose homeostase systemet, vil, hvis ikke altid, i de fleste tilfælde blive baseret på en forhåndsviden om systemet samt eksperimentielle data. Parametre i de matematiske modeller, som fortæller noget om hvorledes systemet opfører sig, kan estimateres ved hjælp af statistiske estimeringsmetoder. Sådanne semi-fysiologiske (grey box) modeller kan hjælpe med at opnå en forståelse for systemet, selvom disse ofte ikke er i stand til at beskrive hele den systematiske variation i data. Dette kan undersøges vha. autokorrelations funktionen (AKF) for prediktions residualer og forklaringsgraden kan blive forbedret enten ved at udvide modellen til en model med stokastiske differentialligninger (SDE) eller en anden forbedret beskrivning af residualer.

For at karakterisere sygdomsudvikling i kaukasere og japanere, er der blevet implementeret etablerede modeller som inkluderer parametre for insulin sensitivitet og beta-celle funktion i et ikke-lineært mixed-effects setup med ordinære differentialligninger (ODE). Udfra ACF af prediktionsresidualer var det klart at de 2 modeller giver en god, men ikke perfekt beskrivelse af den systematiske variation i det analyserede data set. Baseret på dette, blev modellerne udvidet til SDE modeller for forbedret beskrivelse af residualer. Anvendelsen af SDEer gjorde at modellerne med fuld kovariat model ikke konvergerede, så resultaterne i denne afhandling afspejler hovedsageligt de resultater der er opnået med ODE modellerne. Dette gjorde også at sammenligningen med vel etablerede single-subject modeller som er implementeret med ODEer blev mere retfærdig.

Tidligere forskning har vist at tarmhormonet glukagon-lignende peptide-1 er vigtig for normal beta-cell function. Baseret på; dette blev der udviklet en populations PK/PD model der beskriver sekretionen af glukagon-lignende-peptid 1 (GLP-1) under en oral glukose test (OGTT). Denne model kan anvendes som et værktøj til at analysere potentielle forskelle i sekretionsevnen af GLP-1 mellem individer. ACF af residualer viste ingen tegn på seriel korrelation, og modellen blev derfor ikke udvidet til at inkludere SDEer.

Baseret på beregninger af simple og model-baserede mål for insulin sensitivitet og beta-cell funktion i japanere og caucasere stratificeret mht. normal glukose tolerance (NGT), nedsat glukose tolerance (IGT), og T2D konkluderes det at japanere generelt har højere insulin sensitivitet og lavere beta-celle funktion i forhold til kaukausere. På trods af dette, var mønstret for at gå fra NGT til T2D stort set ens i de to kohorter og størstedelen af forskelle i insulin sensitivitet og beta-celle funktion, målt ved simple index baseret på; insulin, kunne forklares ved forskelle i kropsstørrelse (BMI). Dette blev understøttet af Forest plots af resultater fra populationsmodellen, hvor race ikke kom ud som en klinisk relevant faktor for hverken insulin sensitivitet eller beta-celle funktion når der var taget højde for graden af fedme utrykt hhv. android fedt masse eller BMI.

STIKORD: Pharmakokinetic / Pharmakodynamik (PK/PD), Type 2 diabetes (T2D), Autokorrelationsfunktion (AKF), stokastiske differential ligninger (SDE), oral glucose tolerance test (OGTT), glukagon-lignende-peptid 1 (GLP-1), sygdomsudvikling, etniske forskelle

Papers included in the thesis

[A] Møller, J, Overgaard R, Madsen H, Hansen T, Pedersen O, Ingwersen S.H. Predictive performance for population models using stochastic differential equations applied on data from an oral glucose tolerance test.
Published in *Journal of Pharmacokinetics and Pharmacodynamics 37(1):85-98*, 2010.

[B] Møller J, Jusko W, Gao W, Hansen T, Pedersen O, Holst J, Overgaard R, Madsen H, Ingwersen S.H. Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following and oral glucose tolerance test.
Published in *Journal of Pharmacokinetics and Pharmacodynamics 38(6):713-25*, 2011.

[C] Møller J*, Maria Pedersen*, Haruhiko Tanaka*, Mitsuru Ohsugi*, Rune V. Overgaard, Jan Lynge, Katrine Almind, Nina-Maria Vasconcelos, Pernille Poulsen, Charlotte Keller, Kohjiro Ueki, Steen H. Ingwersen, Bente K. Pedersen, Takashi Kadowaki. Pathophysiology of Type 2 diabetes in Japanese versus Caucasians: A Direct Comparative Study
*Authors contributed equally.
Submitted

[D] Møller J, Chiara Dalla-man, Rune V. Overgaard, Steen H. Ingwersen., Maria Pedersen, Haruhiko Tanaka, Mitsuru Ohsugi, Bente K. Pedersen, Jan Lynge, Katrine Almind, Nina-Maria Vasconcelos, Charlotte Keller, Cobelli C. Disease Progression to Type 2 Diabetes in Japanese and Caucasians: An Oral Minimal Model Analysis
Manuscript

Abbreviations and Symbols

Articles and sources are presented with identifications of first author(s) followed by publication year. Bibliography is sorted according to last name. References to formulas are made by curved parentheses. Description of abbreviations and symbols applied in the thesis is presented below.

Abbreviations

ACF	Autocorrelation function
AIR	Acute insulin response from IVGTT
AUC	Area under curve
AR	Auto-Regressive
BMI	Body mass index
BSA	Body surface area
BW	Body weight
FDA	Food and drug administration
FOCE	First-order conditional estimation
GLU	Glucose concentration
IGT	Impaired glucose tolerance
IIV	Inter-individual variability
INS	Insulin concentration
IV	Intravenous
IVGTT	Intravenous glucose tolerance test
ISR	Insulin secretion rate
LR	Likelihood ratio
LDF	Lag dependent function
LRT	Likelihood ratio test
ML	Maximum likelihood
MTT	Meal tolerance test
NGT	Normal glucose tolerance test
NL	Non-linear
NLME	Non-linear mixed-effects
ODE	Ordinary differential equation
OFV	Objective function value
OGTT	Oral glucose tolerance test
OU	Ornstein-Uhlenbeck
PD	Pharmacodynamic
PK	Pharmacokinetic
SDE	Stochastic differential equation
T2D	Type 2 diabetes
WHO	World Health Organization

Symbols

$\boldsymbol{\epsilon}$	Residual error
$\boldsymbol{\eta}$	Inter-individual random-effects
γ	Parameter in OU-process
Λ	Likelihood ratio
μ	Parameter in OU-process
σ	Standard deviation on measurement error
$\boldsymbol{\sigma}_w$	Diffusion term
τ	Time delay between glucose and insulin

A_i	Amount in compartment i
C_b	C-peptide baseline
$\mathbf{e}$	Measurement error
G_b	Baseline glucose parameter
I_b	Insulin baseline
j	Subject
k_i	Kinetic parameter
k_e	Elimination constant
ϕ_d	Dynamic insulin secretion parameter
ϕ_s	Static insulin secretion parameter
S_I	Minimal model based insulin sensitivity

Contents

Part I

Summary report

Chapter 1

Introduction

1.1 Background

Type 2 diabetes (T2D) is a global health problem leading to illness and death of millions of people each year. The total number of patients is expected to rise to alarming 366 million in 2030 [Wild et al., 2004]. This rise is mainly caused by increasing incidence in countries with large shifts in lifestyle due to urbanisation, as is the case in many asian countries [Zimmet et al., 2001], [Yoon et al., 2006], [Hayashino and Fukuhara, 2010].

Unfortunately, at present there is no direct cure for T2D, but in most cases the disease can be managed. A healthy lifestyle and proper diet is first line of treatment [Tuomilehto et al., 2001], although at more severe stages pharmacological intervention is inevitable [Chiasson and Rabasa-Lhoret, 2004]. For optimal development of such interventions, a deep insight into the underlying pathophysiology of the disease is essential .

The two main components in the pathophysiology of T2D is insulin resistance and beta-cell dysfunction, and the disease is believed to be triggered by insulin resistance in liver and peripheral tissues, and at a later stage dysfunction of the beta-cells in pancreas to effectively compensate by increasing insulin secretion [Bergman, 2002], [DeFronzo, 2004], [Lyssenko et al., 2005]. Characterisation of

T2D disease state thus typically involve an assessment of these two components. This can be performed using so-called diagnostic tests, in which an input to the glucose homeostatic system is given. Generally, the goal with these diagnostic tests is to obtain an understanding of how the system performs under daily living conditions such as intake of a meal. In epidemiological studies, especially the oral glucose tolerance test (OGTT), where the subject receives an oral administration of glucose, has been shown to have great value due to its simplicity and close relation to daily living conditions [Cobelli et al., 2007]. Furthermore mathematical models for assessment of insulin sensitivity and beta-cell function using data from the OGTT has been developed and applied in many different settings such as comparing pathogenesis to T2D in young vs. elderly [Basu et al., 2006], and hispanic whites vs. african american [Cali et al., 2009]. In line with this, the majority of the data analysed in this thesis originates from OGTTs performed in subjects spanning the range from healthy volunteers to patients with T2D.

T2D is known as a so-called multifactorial polygenetic disease for which ethnic differences have been reported [Takeuchi et al., 2008], [Torréns et al., 2004]. In line with this, the response to a given anti-diabetic treatment regimen has appeared different between ethnicities [Herman et al., 2007], [Davidson et al., 2010]. It is thus clear that optimal treatment for one race, might not be the optimal treatment in another race and understanding potential differences in pathogenesis in different races is needed. For T2D, specifically the pathogenesis in Japanese and Caucasian has been identified as being different [Fukushima et al., 2004]. In spite of this few studies have compared the progression and pathophysiology of the disease in these two ethnicities. One of the main aims of the current project is to conduct such a comparison, which in the future can provide a knowledge base for facilitating drug development in Japan, which at present is the worlds second largest pharmaceutical drug market.

Pharmacokinetic/Pharmacodynamic (PK/PD) modelling is becoming an important decision support tool for faster and more efficient drug development [Lalonde et al., 2007]. These types of models apply prior knowledge of physiological systems, in combination with data obtained from input and output of the systems. Compared to more black-box models such as the neural networks, it is of key importance that parameters obtained from the models can be related to physiological concepts such as clearance, volume of distribution, or even beta-cell function, and insulin sensitivity. In this thesis PK/PD models will be applied to investigate the feedback mechanisms between glucose, insulin, C-peptide, and GLP-1.

The PK/PD models applied in the pharmaceutical industry are generally formulated using ordinary differential equations (ODEs). An unfortunate property with modelling using ODEs is that all unmodelled dynamics is assigned

to measurement noise. Contrary the application of stochastic differential equations (SDEs) allows for decomposition of the residual error into a system noise term representing unknown or incorrectly specified dynamics and a measurement noise term accounting for uncorrelated errors such as assay errors [Overgaard et al., 2005], [Kristensen et al., 2005]. Although the SDEs are thus preferred against ODEs from a theoretical point of view [Nielsen et al., 2000], only few have shown benefit in PK/PD modelling [Tornøe et al., 2004], [Kristensen et al., 2005], [Røge, 2011]. More practical examples identifying where PK/PD modelling can benefit from SDEs are thus needed.

1.2 Goals and contributions of the thesis

The overall goal of the work presented in this thesis were to assess possible ethnic difference between development of T2D in Caucasian and Japanese and to investigate the applicability of different mathematical modelling methods for this assessment. Related to the fulfillment of this goal, the contribution of the thesis more specifically include:

- An assessment of beta-cell function and insulin sensitivity in a Caucasian and Japanese cohort spanning the range from normal glucose tolerance (NGT) to T2D
- A characterisation of differences in beta-cell function and insulin sensitivity between Caucasian and Japanese at glucose tolerance states (NGT, IGT, and T2D) and an analysis on whether such differences can be explained by demographic/genetic factors
- A comparison between single-subject and population based approach for such an analysis
- An investigation on the use of SDEs vs. ODEs for model based estimation of beta-cell function
- Development of a mechanism-based population model that can describe the dynamics of GLP-1 secretion following an OGTT

1.3 Outline

The following chapters of this thesis will be structured as follows:

In **Chapter 2** the methodological concepts behind the models applied in the thesis will be introduced. This includes description of PK/PD models, single subject vs. population modelling, and an introduction to modelling using SDEs.

Chapter 3 briefly describes the physiology behind glucose homeostasis and some of the pathophysiological concepts related to disease development of T2D.

A presentation of indices for quantifying T2D development is presented in **Chapter 4**. These indices are presented in parallel with the mathematical models from which these indices are derived.

Chapter 5 consist of a brief review on the present knowledge about ethnic differences in T2D followed by a section specifically focusing on the difference in disease development between Caucasian and Japanese.

The main results and analysis of the study focusing on T2D disease development in Caucasian and Japanese are presented in **Chapter 6**.

A general discussion and perspectives related to the results obtained through the PhD is provided in **Chapter 7**, and finally in **Chapter 8** the overall conclusions according to the thesis objectives are presented.

Chapter 2

Modelling methodology

The following chapter will deal with different aspects of pharmacokinetic (PK) and pharmacodynamic (PD) modelling. A brief introduction to the subject will be given, followed by a section discussing the use of single-subject and population approaches for estimation. The last section elaborate on the use of SDE vs. ODEs in PK/PD modelling.

2.1 PK/PD modelling

PK defines the time course of the concentration of a given substance in the body, normally in blood or plasma, whereas PD aims at describing the time course of the drug effect. By linking PK and PD one can establish a dose-exposure-effect relationship. The field of PK/PD modelling is widely applied in the pharmaceutical industry, but the concept and procedures can also be applied in research settings as the ones covered in this thesis. Here the PK part is defined by the concentration of glucose or insulin and the PD part, the response to the glucose intake. This response can eg. be concentration of insulin, C-peptide, or GLP-1, which all are increased under administration of oral glucose. In general PK/PD models can be classified according to what level they operate at:

- **Empirical** - Mainly based on mathematical descriptions that can provide a good fit of a given data set. These models can be useful for describing for example a delay between concentration and drug effect [J.Gabrielsson and D.Weiner, 1997], but in general have poor predictive performance outside the scope of the data applied for estimation.
- **Mechanistic** - Aim to include known physiological mechanisms so that the model provides a reliable description of the system. Thus, these models are usually build by combining different known mechanisms. Typically they have better predictive performance outside the data used for estimation, but can be difficult to estimate either due to lack of data to support estimation of all parameters or simply lack of observability [Quaiser and Monnigmann, 2009], [H.Madsen and J.Holst, 2007].

The goal with performing PK/PD modelling can be very different from one analysis to another. Thus, a specific analysis must be aligned with the questions, the analysis is sought to answer. Stated in another way it is highly dependent on the goals and objectives. A typical PK/PD analysis is usually done in order to fulfill at least one of the following objectives [Gieschke and Steimer, 2000]:

- Describe and summarize trial/study data and obtain an increased understanding of the system in place
- Obtain understanding for important factors influencing the PK or the PD part
- Extrapolation to other conditions eg. different dosing regimens or different species

Based on these statements it is clear that PK/PD modelling can have a positive contribution to clinical drug development. In this thesis, the PK/PD modelling techniques will be used to derive information on the glucose homeostatic system, describe data originating from an oral glucose tolerance test (OGTT), and study the importance of ethnicity on development of type 2 daibetes. Thus the goals for the analyses performed in this thesis mainly relates to the first two bullet points.

The frequency of blood sampling from a test such as the OGTT can vary significantly, although for research purposes where modelling of the data is applied, usually > 10 samples are drawn in an interval of 300 min. Based on this, both

SS and population methods can be applied when parameters from such data are estimated. The theory behind these methods will be presented below and will be related to the application on modelling of the glucose/insulin system.

2.2 Single-subject vs. population approach

Study of drug effects or physiological mechanisms are typically applied on more than one subject/animal in order to assess uncertainty and sources of variability in findings. In case a mathematical model is sought for description of data, this can be done either by deriving parameters for one subject at a time (single-subject analysis) or for all subjects simultaneously (population approach).

In a SS analysis, parameters for one subject are estimated using data obtained only from that given subject. This is typically performed using a least-squares technique for minimization of distance between model prediction and observations [Aldrich, 1998]. The SS estimation technique makes it easy to follow the estimation procedure, and is well-suited for studies including frequent sampling. In the case this method is used for studies with sparse sampling, it can be difficult to estimate parameters as the only data to support estimation is originating from that given subject. Another unfortunate property is the overestimation of variability between subjects (inter-individual variability (IIV)) [Sun et al., 1999], [Denti et al., 2009]. In spite of this, the method is still applied for estimation of metabolic indices from OGTTs due to its simplicity and interpretability [Basu et al., 2009], [Chandler-Laney et al., 2010].

In contrast to the SS procedure, the application of a population model provides parameters for all subjects in the analysis simultaneously. The model is described as a mixed-effects model referring to the fact that fixed effects are mixed with random effects. In a mixed effects models, both intra-subject variability and IIV (See Fig. 2.1) is assessed in one model estimation. Due to inherent non-linearities in many physiological/pharmacological phenomena, these models are often generalised to handle non-linearities leading to non-linear mixed-effects (NLME) models.

The NLME model is a setup for a model that developed from a recognition that, if PK and PD were to be investigated in patients, pragmatic considerations dictated that data may be collected under less stringent and restricted design conditions. The approach considers the population study as a unit of analysis for the estimation of the distribution of parameters and their relationship with covariates within the population [He et al., 1999].

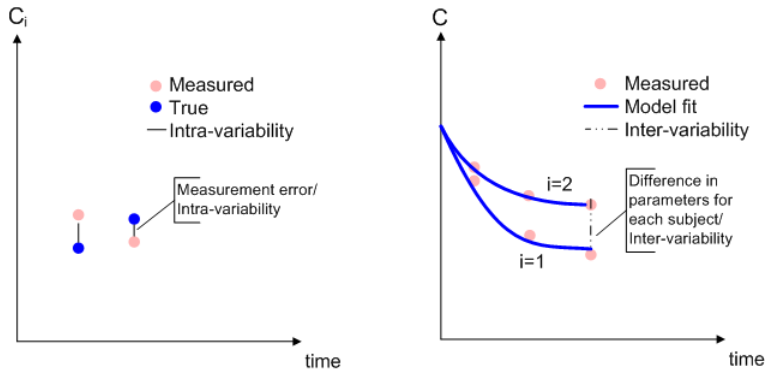

Figure 2.1: Illustration of intra-individual variability and IIV. **Left**: Representation of data for a single subject. Intra-individual variability stems from measurement error. **Right**: Measurements from two subject and corresponding model fits. IIV due to difference between response of different subjects. The intra-individual variability is mixed with the IIV in an NLME.

Analysis according to an NLME model thus provides estimates of distribution of the PK and/or PD parameters in the population [Beal and L.B., 1982]. Clearly, a mixed-effects modelling approach to population analysis of PK/PD data, therefore consist of estimating directly the parameters of the population from the full set of individual concentration and effect values.

The NMLE models can be thought of as a hierachical model structure consisting of two stages. At the first stage the data of a particular individual is modelled whilst at the second stage relationships between individuals are modelled. At this stage, covariates are handled through a regression model which relates the parameter values to the covariates by means of a regression equation as presented below:

$$\theta = g(z, \beta) + \eta \tag{2.1}$$

where θ is a vector of individual parameters, z a vector of covariates, g a parametric function expressing the second-stage model, β a vector of the parameters in the regression model, and η the residual variability which is assumed to be independt on z and has a zero mean. Thus, in a population approach both the individual parameters and their relation to covariates can simultaneously be estimated, in contrast to a SS analysis where estimation of covariate effects is based on post-hoc estimates of individual parameters.

2.2.1 Covariate effects in SS vs. population approach

The model-based analysis of ethnic differences in T2D has been carried out both using a SS approach (paper D) and a population approach (Chapter 6). From each approach individual estimates of early and - late phase beta-cell function (Φ_d, Φ_s) and insulin sensitivity (S_I) have been obtained (See Chapter 4 for further description). Table 2.1 shows the correlation between these estimates obtained either using the SS or the population approach, and covariates such as BMI and age [1]. Compared to the correlation obtained using the SS approach, the correlation between indices (Φ_d, Φ_s, S_I) and covariates (BMI, age) is higher for the population approach. This indicates that the individual estimates from the population approach are less noisy than the ones obtained from SS approach[2]. The parameters in the population PK/PD models applied

Correlation	$log(\Phi_d)$		$log(\Phi_s)$		$log(S_I)$	
Method	SS	Population	SS	Population	SS	Population
BMI	0.13	0.25	0.07	0.21	-0.24	-0.29
Age	-0.19	-0.26	-0.21	-0.25	-0.14	-0.26

Table 2.1: Correlation coefficients between metabolic indices and covariates. SS is individual estimation from single-subject approach.

in this thesis (See Chapter 4) have been estimated using the software package NONMEM VII. Estimation was performed according to a minimisation of the objective function value (OFV). This minimisation corresponds to a maximisation of the likelihood function, which is approximated using a first-order conditional estimation (FOCE) approximation [Beal and L.B.Sheiner, 1994].

2.3 ODE vs. SDE modelling

Besides comparing the SS and the population approach for estimating parameters for beta-cell function and insulin sensitivity, the effect of extending basic ODE models to SDE models, have been investigated. Main driver for performing this analysis has been the inability of the model for beta-cell function to fully descibe the systematic behaviour in data obtained from an OGTT (Paper A). The structure of the minimal model for estimation of beta-cell function will be descibed in Chapter 4.

[1] A similar trend was observed also for other covariates, but for simplicity only BMI and age is presented

[2] 4 extremely low values of Φ_d were removed from single-subject analysis to make fair comparison

The application of stochastic differential equations (SDE) is found in a lot of different areas, and were introduced to PK/PD modelling in 2002 [Tornøe, 2002]. It has been shown that compared to ODEs, the introduction of SDEs can give a more realistic description of the measured physiological data by allowing information about unmodelled dynamics of the system to be extracted from data. This is another motivation to introduce SDEs in the PK/PD models applied in this thesis. Below an ODE and the mathematical structure of a corresponding SDE is presented. For a thorough description of SDEs the reader is referred to [Øksendal, 2005].

$$\begin{aligned} d\mathbf{x}_t &= \mathbf{g}(\mathbf{x}_t)dt & (ODE) \\ d\mathbf{x}_t &= \mathbf{g}(\mathbf{x}_t)dt + \sigma_w d\mathbf{w}_t & (SDE) \end{aligned} \tag{2.2}$$

The diffusion term ($\sigma_w d\mathbf{w}_t$) in (2.2) consists of a magnitude defined by σ_w and $\mathbf{w}_t$ which is a standard Wiener process also known as a random walk with increments which are Gaussian distributed with mean zero and covariance defined by difference in time ($|t_2 - t_1|\mathbf{I}$). By adding this diffusion term it is possible to describe phenomenas that follow dynamics which are combined by a deterministic and a stochastic behaviour such as stock prices or in the present case, a physiological system.

In the following subsection the setup of a stochastic state space model (SSSM) will be presented. In contrast to a basic state space model based on ODEs, in a SSSM based on SDEs, the system noise (originated from $\mathbf{w}_t$) influences the evolution of the states causing them to be stochastic processes instead of deterministic processes (See Fig. 2.2 for graphical presentation of a solution to an SDE)

2.3.1 Stochastic state space models

SDEs for modelling are best introduced as the system equation of a state space model which represents a mathematical model of a physical (in this case physiological) system consisting of input, output and state variables linked by differential and algebraic equations. Inputs, outputs, and states are normally expressed as vectors whereas the whole state space can be written in matrix form. The state space representation thus provides a compact way to model and analyse systems with multiple inputs and outputs. A general way of representing a SSSM is

$$\begin{aligned} d\mathbf{x}_{it} &= \mathbf{g}(\mathbf{x}_{it}, \mathbf{u}_{it}, \boldsymbol{\phi}_i)dt + \mathbf{h}(\mathbf{x}_{it}, \mathbf{u}_{it}, \boldsymbol{\phi}_i)d\mathbf{w}_{it}, \\ \mathbf{y}_{ij} &= \mathbf{f}(\mathbf{x}_{ij}, \mathbf{u}_{ij}, \boldsymbol{\phi}_i) + \mathbf{e}_{ij}, \qquad \mathbf{e}_{ij} \sim N(0, \Sigma) \end{aligned} \tag{2.3}$$

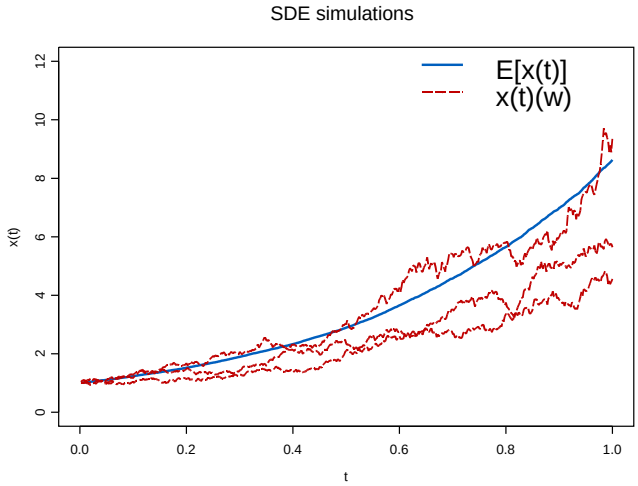

Figure 2.2: Three simulations of a solution to an SDE (red) and the corresponding mean of 1000 simulations (blue).

where $\mathbf{x}_{it}$ is the state vector for the i'th subject at time t, and $\mathbf{u}_{it}$ the input to the system for that given subject at time t. The vector $\boldsymbol{\phi}_i$ is the parameter vector for subject i, and $\mathbf{w}_{it}$ a standard Wiener process as defined above. The lower equation in (2.3) defines the measurement equation, where $\mathbf{y}_{ij}$ is the measurement vector for subject i at measurement j, and $\mathbf{e}_{ij}$ is the residual measurement error for the subject at timepoint j with mean zero and covariance Σ. The unknown parameter vector defined by $\boldsymbol{\phi}_i$ can, provided identifiability, be estimated using maximum likelihood techniques. The likelihood function is evaluated using the extended Kalman filter [Kristensen et al., 2004], [Overgaard et al., 2005], and the optimization of parameters are obtained using an iterative FOCE method [Beal and L.B.Sheiner, 1994]. In the case that the diffusion term ($\mathbf{h}$) depends on the state, the extended Kalman filter, which is applied for estimation in SSSM both in CTSM [Kristensen et al., 2003] and PSM [Klim et al., 2009] has difficulties as it requires higher (than 1) order terms to make filter approximations sufficiently accurate. One way to work around this issue is to transform the state space such that the diffusion term is independent on the state [Iacus, 2008], [Madsen and Møller, 2010]. Adding a restriction on $\mathbf{h}(\cdot)$, not to be dependent on the state, the SSSM can be written

$$\begin{aligned} d\mathbf{x}_{it} &= \mathbf{g}(\mathbf{x}_{it}, \mathbf{u}_{it}, \boldsymbol{\phi}_i)dt + \mathbf{h}(\mathbf{u}_{it}, \boldsymbol{\phi}_i)d\mathbf{w}_{it} \\ \mathbf{y}_{ij} &= \mathbf{f}(\mathbf{x}_{ij}, \mathbf{u}_{ij}, \boldsymbol{\phi}_i) + \mathbf{e}_{ij} \end{aligned} \tag{2.4}$$

2.3.2 Ornstein-Uhlenbeck(Langevin equation)

A general property of SDEs compared to ODEs is the possibility of describing residuals correlated in time. Inspired by [Karlsson et al., 1995], and [Overgaard, 2006] a reasonable way to account for time-correlated residuals in a PK/PD model is to introduce a continuous time version of the so-called AR(1) process presented in [Madsen, 2007]. The continuous version can be defined as a process x_t (one-dimensional) with a correlation decay rate γ, i.e. $COV(x_{t_1}, x_{t_2})$ $= \exp(-\gamma|t_1 - t_2|)$. Such a process could also be formulated as the steady state solution to an SDE written in the form (one-dimensional)

$$dx_t = \gamma x_t dt + \sigma_w dw_t \tag{2.5}$$

This process has the properties of being stationary, Gaussian, Markovian, and continuous in both time and probability [Uhlenbeck and Ornstein, 1930],[Wang and Uhlenbeck, 1945]. As stated above, the process will be used as a tool to improve description of noise in implemented insulin and C-peptide models and will be denoted the OU-process[3]. This is the main subject of Paper A.

2.3.3 SDEs in glucose/insulin modelling (Paper A)

Modelling the glucose/insulin system using SDEs have previously been performed based on data from an euglycemic clamp or from an intravenous glucose tolerance test (IVGTT) [Tornøe et al., 2004]. In paper A a study was conducted in the use of SDEs for modelling the data from an OGTT. The classical oral minimal model for estimation of beta-cell function index [Breda et al., 2001] and a modified version to use insulin instead of C-peptide was extended to include SDEs. As a first approach, the ODE models were extended to SDE models with additive system noise [Overgaard et al., 2005]. Using this implementation it was observed that the description of insulin concentrations at high levels was still inadequate. It is speculated that this was caused by the use of non-state dependent inclusion of the system noise. In order to overcome this problem, an extra state was added to the measurement equation with dynamics as described by the Ornstein-Uhlenbeck process above. With this approach, the correlation between prediction errors from the original model was accounted for also at high insulin concentrations (See Figure 2.3).

One drawback using this approach is that it can not be related to any physiological concept. It is rather a mathematical technique to handle correlated prediction residuals. A better solution would thus be to implement the model

[3]Due the fact that it is a special case of the general Ornstein-Uhlenbeck process with $\mu = 0$

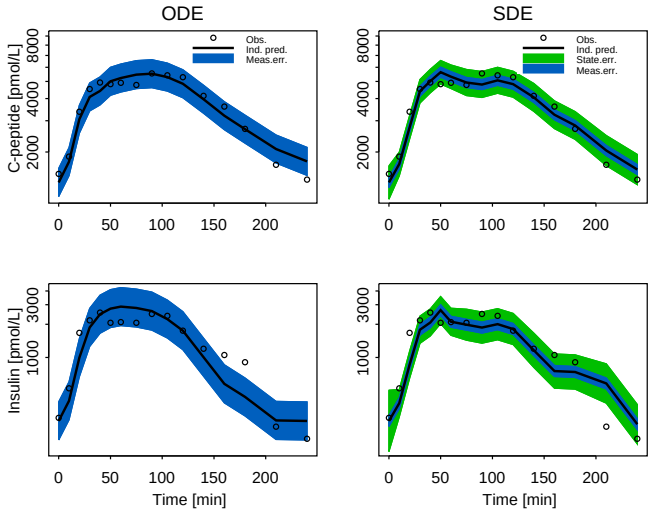

Figure 2.3: Individual predictions for implemented OGTT models of C-peptide and insulin using ODEs and SDEs. Dark band identifies measurement error whereas light band identifies the state error obtained from the extended Kalman filter.

using state-dependent noise as stated earlier. The problem using SDEs with state-dependent noise is that an accurate solution can not be directly obtained using the EKF. Thus it is needed to transform the SDE back to be independent of the state, using the so-called Lamperti transformation [Iacus, 2008], [Madsen and Møller, 2010]. From a simulation study using basic PK/PD models extended to SDEs, this approach seems to provide reliable estimates both in population and in SS estimation, also in the cases where the true model includes state-dependent system noise [Røge, 2011]. The reason for not implementing the glucose/insulin models presented in this thesis using the approach with state-dependent diffusion term is that no software can easily handle parameter estimation in such models , and the Lamperti transformation gets fairly complicated when having more than one state.

Chapter 3

The Glucose Homeostasis and Pathophysiology of Type 2 diabetes

The mathematical models applied in this thesis all build on the principles of the glucose homeostatic system and the pathophysiology related to development of T2D. In order to obtain understanding of these models, a fundamental knowledge in this area is thus essential and will be presented below, starting with a short review on the basic glucose homeostasis.

3.1 Glucose Homeostasis

Glucose is used by many organisms as fuel, and it is vital that glucose levels are tightly regulated. Too low glucose will lead to loss of consciousness, while too much is toxic. Glucose homeostasis is accomplished through complex mechanisms involving many different molecules, cell types, and organs but is generally regulated by the hormones insulin and glucagon. Insulin downregulates the blood glucose whereas glucagon upregulates. Both hormones are secreted from the pancreas, which therefore is the central player in keeping the blood sugar at the right level. The insulin and glucagon production can thus be used as

a good estimator in detecting if a patient has diabetes, insulin resistance, or hypoglycemia.

When glucose enters the blood stream (eg. after digestion of food), it is detected by specialized cells in the pancreas, placed in the islets of Langerhans, called beta-cells. These cells respond to the rising blood-glucose concentration by releasing the hormone, insulin. The release of insulin is highly dependent on two other hormones identified as gastic inhibitory polypeptide (GIP) and glucagon-like-peptide 1 (GLP-1) [Vilsboll et al., 2003], [Holst et al., 2009], [Tolhurst et al., 2009], and GLP-1 has in itself proved to increase the glucose-stimulated insulin secretory dose-response curve [Brandt et al., 2001]. In general this means that in the presence of GLP-1, the beta-cells have a higher response to increased glucose levels. In spite of this, it is still not clear how the release of this hormone is changed during transition from normal glucose tolerance (NGT) to T2D. The model developed in Paper B was made for future investigations of the effect of demographic factors and diabetes duration on GLP-1 secretion dynamics and the results from the model development will be summarised in the following chapter.

Following secretion, approximately 50% of the newly secreted insulin is degraded in the liver [Vølund et al., 1987], although this number can vary between individuals and whether measured in steady-state or under glucose provocation [Campioni et al., 2009]. It is thus clear that not only the ability of insulin secretion, but also the hepatic extraction determines how much insulin can reach the tissues. When reaching the tissues (i.e. muscle cells and adipose tissue), insulin signals to take up glucose to be used as energy (in muscle cells) or stored for later use (in adipose tissue). The result is a lowering of blood sugar concentration to non-toxic levels.

In times of low glucose intake (between meals or in cases of starvation) the alpha-cells of the pancreas can release glucagon. This hormone directs the liver to break down stored glycogen into glucose and release this glucose into the bloodstream, thereby raising blood glucose concentration to a desired level [Matsuda and Defronzo, 1999]. The glucose transporters expressed in the beta- and alpha cells that bind glucose are the receptors of this homeostatic system. The beta- and alpha cells, themselves, are the control centers. They process information from the receptors and respond to it in a way that will maintain a constant internal environment in terms of blood sugar level.

3.2 Pathophysiology of Type 2 diabetes

From the previous section, it should be clear that the whole-body glucose homeostasis is controlled primarily by three tightly coupled regulators: 1) Hepatic glucose production, 2) insulin and glucagon secretion from pancreatic beta- and alpha cells, and 3) glucose uptake by three main target tissues of insulin: liver, muscle, and adipose tissue.

T2D is characterised by two major abnormalities defined by a relative dysfynction in insulin release from the beta-cells causing insufficient insulin levels to maintain normoglycemia, and impaired whole-body sensitivity to insulin [DeFronzo, 2004] (See also Fig. 3.1). The ability of the beta-cells to secrete

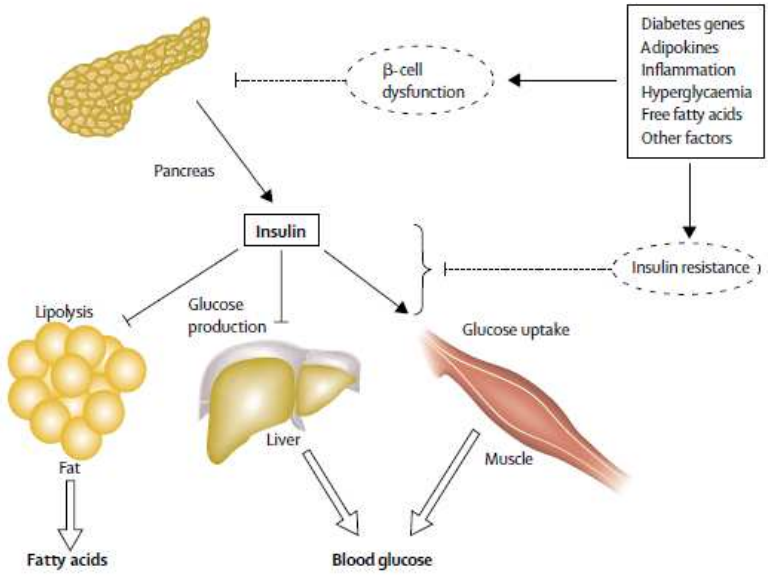

Figure 3.1: The role of beta-cell dysfunction and insulin resistance in the pathophysiology of T2D [Stumvoll et al., 2005].

insulin relative to the level of glucose concentration sensed by the pancreas, is characterised as the beta-cell function. Measuring the beta-cell function under conditions where the glucose/insulin system is in steady-state gives an indication of how well the subject can control the basal insulin secretion to prevent high fasting plasma glucose (FPG). Contrary, when one wants to capture the subjects ability to secrete insulin under varying levels of glucose (eg. after a meal) a glucose load is administered as input to the system.

Both muscles, adipose tissue and liver can take up glucose. The insulin sensitivity defines to what degree a certain amount of insulin causes glucose to be taken up by these tissues. In the fasting state, the muscles account for less than 20% of the overall glucose disposal whereas the endogenous glucose production is totally responsible for the glucose entering the plasma [Stumvoll et al., 2005]. Measures of insulin sensitivity in the fasting state, is thus suggested to mainly reflect the hepatic insulin resistance [Abdul-Ghani et al., 2006]. In the fed state or under exercise, the overall glucose uptake is dominated by uptake to muscles and to a smaller extent adipose tissue. Estimating separately the insulin sensitivity in liver and in peripheral tissues (muscle and adipose) is not trivial since glucose concentration is affected by both of these factors. One solution is to add a tracer to the ingested glucose and from that determine what part of glucose is produced in liver (endogenous) and what stems from ingestion (exogeneous). These concentrations can then be applied to assess the hepatic and peripheral insulin sensitivity [Man et al., 2008]. Another way is to determine what part of the glucose curve following an OGTT, that mostly reflects the hepatic and the peripheral sensitivities and from this derive approximate estimates for the two [Abdul-Ghani et al., 2007].

The typical characteristic of T2D is an increase in FPG and/or a higher glucose profile following a standard oral glucose tolerance test (OGTT). From the glucose profile, the glucose measurement at 2 hours (G2H) is selected as indicator for glucose control following a meal[1]. The FPG indicates how well the body handles glucose in the fasting state, whereas the G2H describes the ability of the body to remove glucose from blood following a meal. These two measures are thus used to classify the development of T2D, which is usually separated in various pathophysiological stages. The classification is based on criterias from the World Health Organization (WHO) as presented below, assuming glucose concentration is measured in mmol/L[2].

- NGT(normal glucose tolerance), FPG$<$7.0 and G2H$<$7.8
- IFG (impaired fasting glucose), 6.1$\geq$FPG$<$7.0 and G2H$<$7.8
- IGT(impaired glucose tolerance), FPG$<$7.0 and 7.8$\geq$G2H$\geq$11.0
- T2D(screen detected diabetics), FPG$\geq$7.0 or G2H$>$11.0

The pathogenesis from NGT to T2D is generally understood to involve increased insulin resistance [LeRoith, 2002]. Insulin resistance signifies that the effects of

[1] At around 2 hours post oral glucose administration, the glucose is returned to baseline in normal individuals

[2] In the study comparing Japanese and Caucasian, the classification of IFG have not been applied in order to have more subjects at each disease state

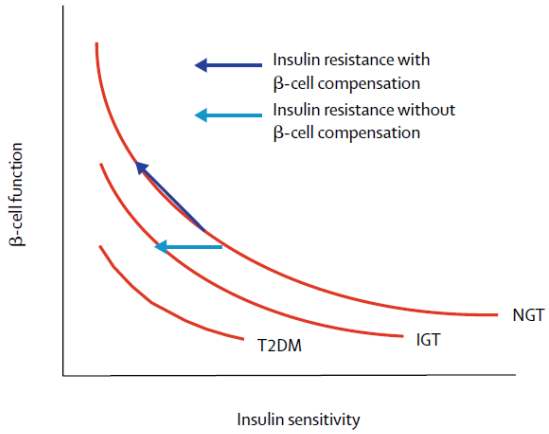

Figure 3.2: Compensation with increased insulin secretion when insulin sensitivity is decreasing. In cases of no compensation a transition from NGT to IGT is apparent. (NGT=Normal glucose tolerance, IGT=Impaired glucose tolerance, T2=Type 2 diabetes mellitus). [Stumvoll et al., 2005]

insulin are less than expected for removal of glucose and suppression of the endogenous hepatic glucose production [Dinneen et al., 1992]. As stated above, at fasting state, the glucose level is mainly governed by the liver and early signs of diabetes is thus expected to involve hepatic insulin resistance [Stumvoll et al., 2005]. Many factors are known to cause insulin resistance and one of the most important factors is obesity, for which the mechanisms are reasonably well described [Kahn et al., 2006].

In case of decreasing insulin sensitivity, the normal beta-cells can compensate by producing more insulin, thus maintaining normoglycemia (Fig. 3.2). This leads to hyperinsulinemia in prediabetic subjects (See Fig. 3.3), but as long as the beta-cells can provide sufficient insulin, decreased insulin sensitivity can last for decades without leading to the diagnosis of T2D (See insulin resistance with beta-cell compensation in Fig. 3.2). However, when beta-cells do not function adequately to provide enough insulin, perhaps due to genetic factors, uncontrolled hyperglycemia occurs. The key element in the development of T2D is thus the product of insulin sensitivity and beta-cell function, also known as the disposition index [Bergman et al., 2002]. An overall picture of the pathogenesis is presented in Fig. 3.3[3]. In the case, where subjects can not compensate increasing insulin resistance with increasing insulin secretion, the glucose is slowly

[3]The exact relationship between the beta-cell function and insulin sensitivity can vary among individuals and ethnicity, so Figure 3.3 is drawn for illustrative purpose but is highly simplified compared to reality and shows one way to progress to T2D

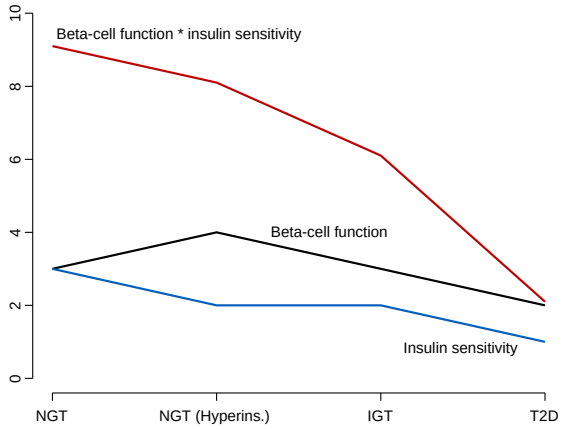

Figure 3.3: Involvement of insulin sensitivity and beta-cell function in development of T2D. (NGT=Normal glucose tolerance, IGT=Impaired glucose tolerance, T2D=Type 2 diabetes). The beta-cells can compensate decreased insulin sensitivity with increased insulin production causing the subject to be hyperinsulinaemic, although still in NGT state. The product of beta-cell function and insulin sensitivity (disposition index) is a good predictor of diabetes state. Inspired by [DeFronzo, 2004]

cleared from the blood and diagnosis of T2D is inevitable either based on high FPG or on high G2H (See criterias above). In case the disease is not treated properly, long-term complications develop. Typical long-term complications involve nerve damages (neuropathies) and vascular damages (microvascular and macrovascular diseases) causing the patient to develop reduced vision, numbness and severe kidney problems. Both the micro - and the macrovascular diseases are known to be caused by a thickening of the basal membrane in the capillaries/arteries and changed permeability properties, although other factors are also expected to play an important role [Hansen et al., 2004].

Chapter 4

Indices for characterising pathophysiology of Type 2 diabetes

This chapter deals with the application of indices providing information on the pathophysiology and disease state of T2D. Such indices are typically derived directly from measurements obtained from a glucose tolerance test or represented as parameters in mathematical models applied on the measurements. The chapter is introduced with a discussion of the underlying requirements for a diagnostic index to be applicable dependent on the purpose of the study.

4.1 What characterises a good index ?

The use of indices for describing disease state and disease progression is motivated by the fact that many diseases are characterised by different disease processes in different organs which are difficult to measure *in vivo*. Typically these indices can provide information relating to the functional state of a specific/several organ(s), while in the same time avoiding the need of invasive procedures such as surgical intervention. In most cases these indices are calculated based on concentrations of specific chemical substances as is the case with creati-

nine for calculation of renal clearance rate, or glucose and insulin for calculation of pancreatic beta-cell function.

In general, indices for description of disease physiology are both applied for diagnostic and research purposes. The indices used for diagnostic purposes must be easy obtainable, and must provide necessary information for the physician to provide correct medication for the patient. Contrary in a research setting, usually involving much less patients, the requirements for simplicity are not as restrictive. In such settings it is of higher importance, that the indices can provide information relating to the pathophysiology behind the disease state. In spite of these differences, it is evident that indices in general must be robust and precise, reflected in a low coefficient of variation combined with a high discrimination ratio [Katz et al., 2000]. In the following sections we will discuss the application of indices related to characterization of disease state of T2D in a research setting as the one applied in this project.

4.2 Indices for beta-cell function

Abnormalities in insulin secretion are important determinants of T2D. However, due to the complex feedback between glucose and insulin, assessment of the ability of the pancreas to secrete insulin in response to glucose (beta-cell function) under physiological conditions, has always been a challenge. Especially because the use of insulin measurements can be misleading due to the confounding effect of hepatic extraction.

One of the most applied indices for beta-cell function is the HOMA-B [Matthews et al., 1985] index, which is based on basal concentrations of insulin and glucose as described in the formula below.

$$\frac{20 \cdot FPI}{FPG - 3.5} \tag{4.1}$$

where FPI is the fasting insulin level measured in mU/L, and FPG the fasting glucose level measured in mmol/L. The index is thus based only on a single point of the dose-response curve between glucose and insulin and cannot provide insight regarding the ability of the beta-cells to respond to varying levels of glucose. In order to get further insight into characterisation of beta-cell function, alternative approaches have been introduced, where a glucose load is administered intravenously, such as the clamp technique [Defronzo et al., 1979] or the intravenous glucose tolerance test (IVGTT). From the IVGTT insulin response, one can calculate indices for first-phase secretion (up to $\approx$ 8 min, dependent on the individual profiles) and second-phase (simply following first-phase). The

first-phase insulin secretion is generally accepted as a good predictor of diabetes and a reduced first-phase secretion is one of the earliest signs of a progression towards disease [Prato et al., 2002] [Prato et al., 2005], although some controversy exists regarding a similar importance of the second-phase [Gerich, 2002]. An index for first-phase secretion can be obtained from the IVGTT either by calculating the incremental area under the insulin curve from 0-8 min, classified as acute insulin response (AIR_{0-8}), or using a mathematical model on glucose and insulin data [Bergman et al., 1981]. One drawback with the use of glucose and insulin data for estimation of insulin secretion ability is that a major part of the insulin secreted from the pancreas is degraded in liver before reaching the systemic circulation. Thus, other methods have been proposed based on C-peptide, which is not degraded in liver to the same extent as insulin and thus has a much longer half-life [Toffolo et al., 1995].

Although the intravenous glucose tests can provide information on the glucose/insulin system in non-steady state, it does not reflect what happens in real life. During an intake of a meal, glucose is absorbed through the gastrointestinal (GI) tract which causes the plasma concentration to increase much slower than is the case for an intravenous administration. Also, when glucose passes the GI, various incretin hormones such as GLP-1 cause the insulin response to be significantly higher than the corresponding intravenous response [Holst et al., 2009] [Holst and Gromada, 2004]. Another problem with the intravenous methods is that they are fairly laborious and are difficult to perform in large clinical studies. This has lead to the use of oral methods such as the oral glucose tolerance test (OGTT), where the subject is given a standard dose of 75g glucose orally. One of the mostly applied beta-cell indices from this test is the Insulinogenic index which applies glucose and insulin samples at baseline and 30 min. after administration of glucose [Phillips et al., 1994] and is calculated using the formula below

$$\frac{I_{30} - FPI}{G_{30} - FPG} \tag{4.2}$$

where $I_{30} and G_{30}$ are the insulin and glucose level 30 min. after administration of oral glucose. This index has shown to have a strong correlation with AIR both in subjects with NGT, IGT, and T2D [Hanson et al., 2000] and is well accepted to be a good marker of first-phase insulin secretion. In paper C the Insulinogenic and the HOMA-B indices have been used to analyse the transition from NGT to T2D in Japanese and Caucasians. Both indices are well established in litterature and are easy to calculate.

Besides being based on insulin, another unfortunate property with indices such as the HOMA-B and Insulinogenic index is that they are both based on few samples from the OGTT. In general that causes them to be highly dependent on the accuracy of those samples. In contrast, using a mathematical model to describe the concentration curves, all samples can be taken into account and

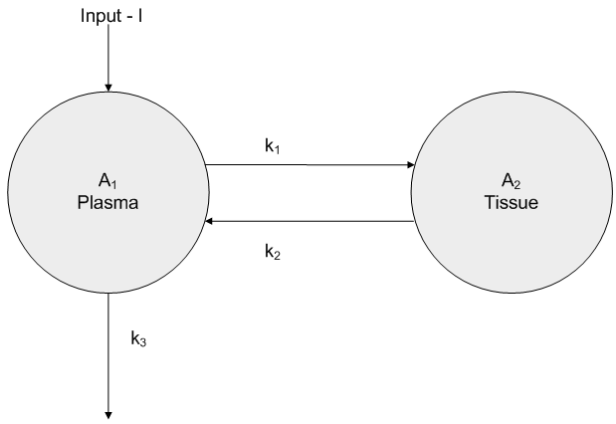

Figure 4.1: 2-Compartment model.

the estimates for indices (parameters in the mathematical models) are less dependent on the accuracy of each of the measurements compared to the simples indices presented above.

One of the most applied models, for analysing data from an OGTT is the oral beta-cell minimal model [Breda et al., 2001], which has been used in a single-subject analysis in paper C and in a population analysis in Chapter 6 to assess beta-cell function in the studied subjects. The theory behind the model will be outlined below.

4.2.1 Model for estimation of beta-cell function

The oral minimal model is based on C-peptide instead of insulin in order to take into account the hepatic extraction. The relation between insulin secretion and C-peptide kinetics is established through the use of a compartment model [Eaton et al., 1980] [Van et al., 1992]. The model includes two compartments, a central compartment representing plasma and tissues in rapid equilibration with plasma, and a peripheral compartment representing extravascular space. By inspecting Figure 4.1 it is clear that the 2-compartment model describes the distribution of a drug into tissue and back into plasma. The following equations

model the amount of C-peptide in each compartment

$$\frac{dA_1}{dt} = SR - k_1 \cdot A_1 + k_2 \cdot A_2 - k_3 \cdot A_1 \tag{4.3}$$

$$\frac{dA_2}{dt} = k_1 \cdot A_1 - k_2 \cdot A_2 \tag{4.4}$$

$$\tag{4.5}$$

where A_1 represents amount of drug in central compartment (plasma) and A_2 the amount of drug in tissue (muscles, adipose tissue etc.) The insulin secretion estimates based on kinetic analysis of C-peptide concentration alone, involve multiple experimental protocols or *a priori* assumption of C-peptide kinetic parameters [Vølund et al., 1987]. The values of these kinetic rate constants (k_1, k_2, k_3) are obtained using standard kinetic parameters calculated using the age and body surface area (BSA), k_1, k_2, k_3 [Van et al., 1992]. BSA is estimated according to the Mosteller formula, which is the standard in clinical research [Verbraecken et al., 2006] [Mosteller, 1987].

The OMM relates C-peptide kinetics to insulin secretion rate above basal (SR) from the OGTT. The secretion of insulin is stimulated through a static component proportional to the glucose level above baseline, and a dynamic component presented by the derivative of glucose when glucose concentration is rising. This approach is presented in [Breda et al., 2001], and [Lim et al., 2009] and a reformulated version is

$$SR = [A_3 + SR_d]^+ \tag{4.6}$$

where

$$\frac{dA_3}{dt} = -\tau^{-1}(A_3 - \phi_s[GLU - G_b]^+) \tag{4.7}$$

and

$$SR_d = \begin{cases} \phi_d \frac{dGLU}{dt} & \text{if } \frac{dGLU}{dt} > 0 \\ 0 & \text{if } \frac{dGLU}{dt} \leq 0 \end{cases}$$

where SR is the insulin secretion rate above basal caused by the glucose uptake and A_3 the secretion based on stimulation signal from glucose level above baseline. GLU is the interpolated version of the glucose curve and G_b the baseline glucose concentration. SR_d is the secretion rate originating from the dynamic part, and the parameter ϕ_d is the secretory response to the rate of change of glucose. The derivative of glucose $\frac{dGLU}{dt}$ is obtained from the interpolated glucose curve. The parameter τ is the time delay between glucose sensing and insulin production, and ϕ_s determines the magnitude of the static response. In summary, the parameters ϕ_s and ϕ_d provide static and dynamic indices for beta-cell function, relating to first - and second phase secretion. The observation equation

relating observation, error model, and prediction is defined by

$$Y = (\frac{A_1}{V} + C_b)(1 + \epsilon_2) + \epsilon_1 \quad (4.8)$$

where V is the volume of distribution of C-peptide, C_b the basal C-peptide concentration and ϵ_1, ϵ_2 additive and proportional error term, respectively.

In Paper A it was shown that ϕ_d has a significant correlation with AIR_{0-8}, which to a certain extent partly evaluates the index as a marker of first-phase secretion. In spite of this, based on the data obtained in Japanese and Caucasian subjects, it was found that the index has fairly poor correlation with covariates such as age and BMI compared to the simple indices for first-phase secretion such as the Insulinogenic index. Furthermore as will be shown in Chapter 6, it was found that the dynamic part only constitutes a minor part of the secretion contribution to the C-peptide curve, which causes the estimation of ϕ_d to be less accurate than ϕ_s.

In summary, although ϕ_d is mathematically sound as a marker for first-phase secretion, a complete evaluation of predictive performance relating to disease state of the pancreas is still subject for future research.

4.2.2 SDEs in estimation of dynamic index (Paper A)

In paper A, the minimal model for estimation of beta-cell function was extended to include an SDE for description of correlated prediction residuals. Besides investigating the effect on description of data, also the relation between ϕ_d and the *gold standard* measure for first-phase secretion (AIR from IVGTT) was investigated. As stated above, the parameter ϕ_d is intended to be a marker of first-phase secretion and thus a significant correlation between ϕ_d and AIR is expected. Both the correlation to AIR (not shown) and the similarity in covariate relations were improved using the SDE approach for estimation of individual ϕ_d values.

The relation between ϕ_d and AIR and covariates is presented in Table 4.1. Values in the second column were calculated as the slope between $\log(AIR_{0-8})$ and the corresponding covariate indicated in the first column. Last four columns present parameter values obtained for relation between the given covariate and ϕ_d estimated by the ODE or SDE approach using either the C-peptide version or an insulin version of the OMM (See Paper A). The value 0.273 in first row

[1] This is the observation equation used in the population approach and is slightly different from the one used in single-subject estimation which is formulated based on prior estimations results

thus indicates that the slope between BMI (normalized by subtracting mean and dividing by SD) and $\log(\phi_d)$ in the basic C-peptide model equals 0.273. Similarity between slopes obtained for the $\log(AIR_{0-8})$ and $\log(\phi_d)$ indicates that the index obtained using the OGTT model has similar relation as $\log(AIR_{0-8})$ to the given covariate. In all cases the extended error structure based on the OU stochastic process caused the slopes for the covariates to better reflect the ones observed for the $\log(AIR_{0-8})$. This supports the fact, that covariate relations can be inaccurate when the correlation between residuals is not fully described [Silber et al., 2009].

Covariate relation	$log(AIR_{0-8})$	$log(\Phi_d)$			
Data		**C-peptide**		**Insulin**	
Model		**ODE**	**SDE**	**ODE**	**SDE**
BMI	0.176(0.052)	0.273	0.106	0.490	0.196
AGE	-0.017(0.053)	0.030	-0.017	0.207	0.033
FPG	-0.199(0.052)	0.030	-0.105	0.177	-0.050
$I_{0'}$	0.159(0.052)	0.287	0.147	0.543	0.271

Table 4.1: Relationship between beta-cell indices and selected covariates for C-peptide and insulin models built using ODEs and SDEs in NONMEM. (.) Indicate standard error of estimate (SEE).

4.3 Indices for insulin sensitivity

As stated in Chapter 3, the development of T2D involves a complex interplay between beta-cell function and insulin sensitivity. Estimates for beta-cell function is thus usually followed by further assessment of insulin sensitivity. Especially in epidemiological studies, the HOMA-IR [Matthews et al., 1985] has been widely applied for such purpose. It reflects the inverse of the insulin sensitivity, characterised as insulin resistance, based on basal levels of glucose and insulin and is given by

$$\frac{22.5 \cdot FPG}{FPI} \tag{4.9}$$

where FPG and FPI is the fasting plasma concentrations of glucose and insulin as above. This index has been suggested mainly to reflect hepatic insulin sensitivity due to the use of only basal values [O'Rahilly et al., 1994]. Contrary, for assessment of whole-body insulin sensitivity it is suggested to use glucose provocation tests as also described above for beta-cell function. One widely applied index is the Matsuda Composite Index that applies insulin and glucose samples up to 120 min. It has shown to correlate well with insulin sensitiv-

ity derived from the euglycemic clamp [Matsuda and Defronzo, 1999] and is calculated according to

$$\frac{10000}{\sqrt{FPG \cdot FPI \cdot \bar{G}_{0-120} \cdot \bar{I}_{0-120}}} \tag{4.10}$$

where $\bar{G}_{0-120}$, and $\bar{I}_{0-120}$ are the mean level of glucose and insulin calculated as the AUC_{0-120} divided by the time period.

In spite of the fact that the Matsuda index is widely applied, it does not use all samples from the OGTT to gather information for estimation of insulin sensitivity and it is also sensitive to extreme values. Thus, besides using the Matsuda Composite Index together with HOMA-IR (Paper C) to derive insulin sensitivity in the Japanese and Caucasian cohort, also the OMM for glucose was applied (See Paper D) which uses all samples from the glucose and insulin profiles. The equations for the oral glucose minimal model for estimation of insulin sensitivity is presented below:

$$\begin{aligned} \frac{dG}{dt} &= -(S_g + X)G + S_g G_b V + RA, \qquad G_0 = G_b V \\ \frac{dX}{dt} &= -p_2 X + p_2 S_I (INS - I_b), \qquad X_0 = 0 \end{aligned}$$

here G presents the amount of glucose present in the central compartment (blood), and X the composite insulin action on muscle, adipose tissue, and liver. The parameters S_I, and S_g present the insulin dependent, and the non-insulin dependent clearance of glucose, respectively. G_b is the basal glucose concentration measured in mg/dL. The distribution volume is expressed by V. The parameter p_2 expresses the decay rate for the insulin effect on glucose. INS is a linear interpolation of the insulin curve, and RA the rate of glucose absorption presented by a simplification of the equation presented in [Hansen, 2004][2]

$$RA = MS_1 \exp(-\alpha t)(1 - exp(-\alpha t)) \tag{4.11}$$

This way of presenting the glucose absorption profile is chosen based on the few number of parameters and a prior analysis showing no significant improvements with more complex profiles [Klim, 2009]. Prediction of glucose concentrations following the OGTT can be described by [3]

$$Y = (\frac{G}{V})(1 + \epsilon_2) + \epsilon_1 \tag{4.12}$$

[2]In paper D, the piecewise absorption profile has been applied instead [Man et al., 2004]. Based on studies not presented here it was observed that in a population setting the presented formula had the best performance for estimation of insulin sensitivity

[3]This is the observation equation used in the population approach and is slightly different from the one based on single-subject estimation

Due to the fact that the insulin sensitivity index (S_I) applies full glucose and insulin profiles it can be understood as a composite index in the sense that the both the sensitivity in liver and peripheral tissue contributes to the value. In case one wants separate estimates of the insulin sensitivity originating from liver and peripheral tissues, generally it is needed to add a tracer to the glucose/meal as proposed by Dalla Man et. al [Man et al., 2008].

4.4 Indices for GLP-1 secretion (Paper B)

As stated in the previous chapter, the presence of GLP-1 in combination of glucose is of main importance in the process of insulin secretion. A subject with severely low secretion of GLP-1 following an OGTT will thus typically have a low beta-cell function. In paper B, a model for secretion of GLP-1 providing indices for secretion ability was developed. The model is built using glucose, insulin, and GLP-1 data from an OGTT [Hansen et al., 2007]. First approach was to let the production of GLP-1 be driven solely by the glucose absorption rate, but this was not sufficient to describe the pattern of the data. Instead in the final model GLP-1 secretion is stimulated both by a fast component peaking around 25 min. and a slower component peaking at around 100 min. The fast component is suggested to be caused by a neuro-endocrine loop, whereas the slower phase, by a direct interaction between ingested nutrients and the L-cells placed in the distal gut [Lim and Brubaker, 2006], [Lim et al., 2009]. The model for GLP-1 was developed in close collaboration with professor Bill Jusko, University at Buffalo, NY and the structural setup is presented in Figure 4.2 whereas the parameter values are presented in Figure 4.3.

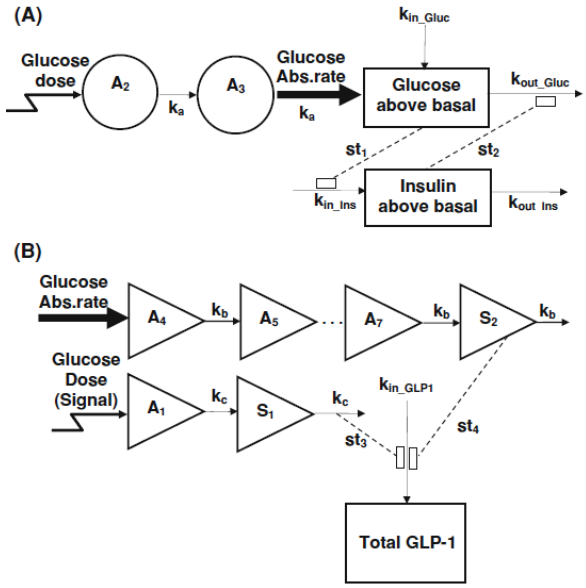

Figure 4.2: A: Diagram of glucose/insulin model for estimation of the glucose absorption rate, B: GLP-1 secretion model. Absorption rate for glucose is identical to that estimated in the glucose/insulin model. The model is an indirect-response (IDR) model with two components stimulating the synthesis of GLP-1. The two components originate from ingestion of glucose with a magnitude presented by st_3, and absorption of glucose with a magnitude equal to st_4. Symbols are as defined in Figure 4.3. See also Paper B

Parameter	Interpretation	Value	SEM (%)	IIV (CV%)	Shr (%)
f (−)	*Absorption fraction*	*0.722*	–	–	–
k_a (min^{-1})	Abs. rate constant	0.0359	2	0.0581(24)	5
k_b (min^{-1})	Transit rate constant	0.0962	8	0.0357(12)	20
k_c (min^{-1})	Neural signal rate constant	0.0566	11	0.270(52)	20
k_{out_GLP1} (min^{-1})	First-order elimination rate constant of GLP-1	0.0644	18	0 FIXED	–
κ [−]	Proportionality between IIV on st_3 and st_4	0.775	10	0 FIXED	–
st_3 [mg^{-1}]	Stimulation factor of GLP-1 production by early signal	$8.64 \cdot 10^{-5}$	10	0.939(97)	6
st_4 [mg^{-1}]	Stimulation factor of GLP-1 production by late signal	$26.2 \cdot 10^{-5}$	3	–	–
SD_{glp} [pmol l^{-1}]	Additive error	0.998	5	–	–
CV_{glp} (%) [pmol l^{-1}]	Proportional error	9	–	–	–

Figure 4.3: The f is obtained from Ref. [Silber et al., 2009] and k_a is estimated from glucose/insulin data
. No IIV on parameters are indicated by: 0 FIXED. See Paper B for further description.

Chapter 5

Ethnic differences in progression of Type 2 diabetes

This chapter deals with ethnic differences related to the development of T2D. First section will have a focus on ethnic differences in general, and the following section on the specific differences between Caucasians and Japanese.

5.1 General ethnic differences

Figure 5.1 shows the prevalence of T2D among different ethnicities in the US, from a survey performed in adults between 2007 and 2009 [Lutsey et al., 2010]. From the figure it can be seen that the prevalence is almost the double in Non-hispanic blacks compared to Non-hispanic whites (Caucasians). Large differences can also be found within ethnicities [Chiang et al., 2011]. Figure 5.2 presents data obtained in Singapore from Chinese, Malay, and Indian subjects indicating that the prevalence in Indian is almost the double as in Chinese placed in Singapore. It is thus clear that T2D is an example of a disease in which differences between ethnic groups exists.

Besides various articles discussing ethnic difference in prevalence among specific races, some reveiws deals also with the area from a more broad perspective.

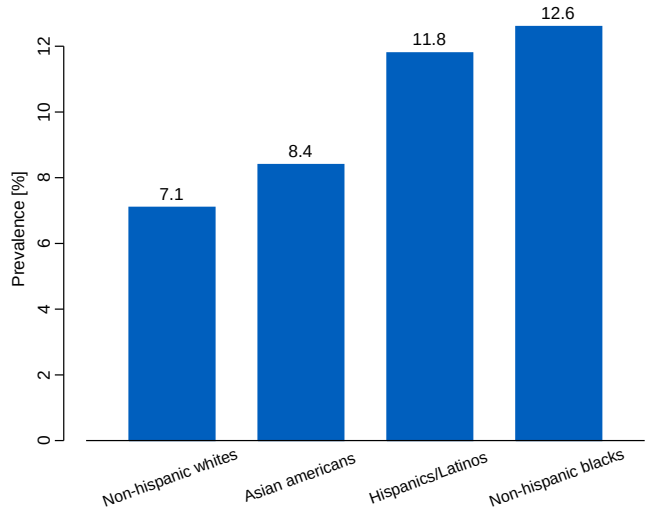

Figure 5.1: Prevalence of diabetes in US in population 20 years or older, adjusted for population age differences. Data obtained from [Lutsey et al., 2010]

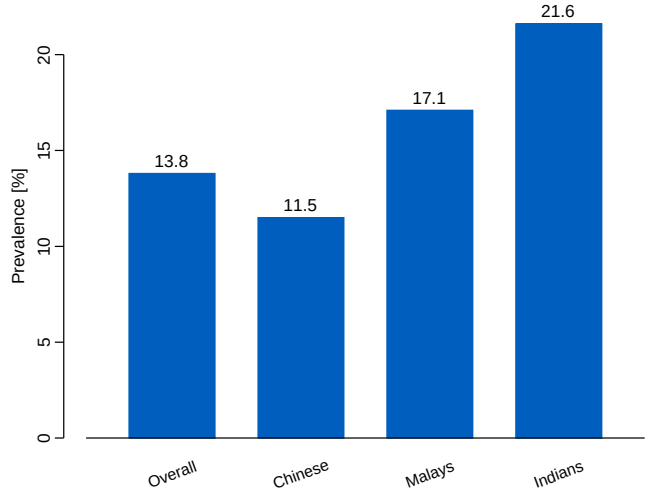

Figure 5.2: Prevalence of diabetes in Asian ethnicities aged 40-95, adjusted for population age differences. Data obtained from [Chiang et al., 2011]

Abate et al. focuses both on epidemiological and pathophysiological aspects [Abate and Chandalia, 2001] and mentions the adoption of the western lifestyle

to have a huge impact on the prevalence of diabetes in ethnicities different from Caucasian. In support of this, the Native African-americans (non-Hispanic blacks) have a prevalence of 1% (not shown), whereas African americans have a prevalence of 12.6% (See Figure 5.1). Another example is intake of fat, which in mean is 32.4g in Japanese-American versus 16.7g of fat in Japanese men living in Japan. The traditional diet of the Japanese was fish and vegetables until the end of the nineteenth century, and in general dietary differences among ethnic groups and level of physical activitity may contribute to the interethnic differences in prevalence of T2D.

So the question is how does lifestyle factors and obesity mechanistically affect the pathogenesis of diabetes in various ethnic groups ? When subjects approach IGT status, they can either be dominated by decreased insulin sensitivity or decreased beta-cell function or a similar contribution of both. One example is the disease development of Pima Indians from NGT to IGT which are characterised both by severe insulin resistance and beta-cell dysfunction [Weyer et al., 1999]. In contrast, African-americans approaching diabetic state have been reported to frequently have relatively low rate of insulin resistance, whereas in European-Americans with mild diabetes, severe insulin resistance is reported. Conclusively the predominant mechanism leading to T2D seems to be different in various ethnic groups.

Abate et al. 1995 shows relation between increasing body fat and insulin resistance, and further states that insulin resistance is mainly dominant in people with BMI above 30 kg/m^2. Obese human subjects have lower tyrosine kinase activity and that activitity is restored post weight loss. Thus, in general it can be concluded that development of obesity has a significant impact on the development of both insulin resistance and beta-cell dysfunction and has been shown to explain about 30% of the variability in insulin sensitivity in a Caucasian cohort [Clausen et al., 1996] although numbers closer to 50% have also been reported [F.Fletcher et al., 1999][1].

One special case is the Asian Indians which are of particular interest as they have significantly more insulin resistance than Europeans despite the low occurrence of obesity. In order to specifically define the role of adiposity and fat distribution on the larger occurence of insulin resistance observed in Asian Indians, Chandalia et al., 2007, performed euglycemic-hyperinsulinemic clamp for calculation of insulin sensitivity in European-Americans and Asian-Indians matched for BMI [Chandalia et al., 2007]. They showed that the percentage of total body fat and adipocyte size was significantly higher and that the insulin sensitivity was significantly lower in the Asian cohort, compared to the

[1]This number is strongly dependent on the methods use to assess obesity and insulin sensitivity

Caucasian. After adjustment for percentage of body fat, Asian Indians still had higher insulin resistance and larger adipocyte size, suggesting the size of the fat cells to explain the lower insulin sensitivity. One could also speculate whether this could be due to lower level of physical activity (PA), but Liew et. al., 2003 found that differences between Caucasians and Asian Indians in insulin sensitivity obtained from clamp, could not be explained by differences in PA [Liew et al., 2003]. In general these arguments suggests that a primary metabolic defect related to genetics could be present in this group, however more studies are needed to draw such conclusions.

The presence of some ethnic groups being predisposed to insulin resistance may have developed as a genetic advantage in populations such as the Hispanics and Asians. The so-called thrifty genotype hypothesis proposed by Neel et al. [Neel, 1962] suggests that predisposition to insulin resistance may have protected individuals during periods of food deprivation by reducing muscle utilization of glucose. The fast change to excessive food availability and less PA has caused a pathological condition characterised by a too low glucose utilization. What was a genetic advantage in the past is now a disadvantage. In spite of the fact that the hypothesis seems reasonable, it is important to note that present analysis did not find any evidence for the thrifty hypothesis [Southam et al., 2009].

It has also been found that the relation between body fat and BMI is ethnic specific. As stated above people refer to the fact that Asians have higher body fat percentage compared to Caucasians with same BMI [Chandalia et al., 2007], [Nair et al., 2008], [Gallagher et al., 2000] although some have reported the opposite [Cruz et al., 2001]. In the following chapter it will be adressed whether such differences can explain differences between Caucasians and Japanese. Based on the understanding of different body fat percentages, the BMI cut-off for obesity is 25 kg/m^2 in Japanese, 26 kg/m^2 in Indians, 27.5 kg/m^2 in Chinese, as compared to 30 kg/m^2 in Caucasians [Kanazawa et al., 2002], [Deurenberg-Yap et al., 2001]. In spite of these differences there seems to be a clear relation between BMI and the prevalence of T2D across different ethnicities (See Figure 5.3[2]).

[2]This source is the most updated data source, but shows statistics for diabetes in general, which is a good indication for T2D as approximately 90% of diabetics has T2D [WHO, 1999]

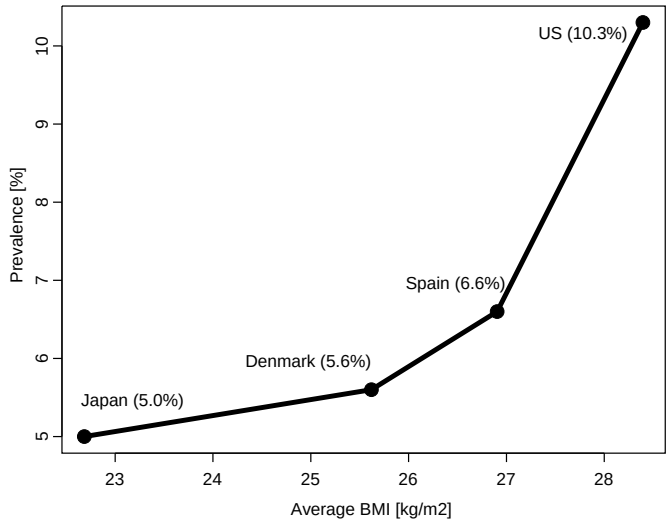

Figure 5.3: Prevalence of diabetes vs BMI. The prevalences are adjusted for population age differences.. Data obtained from [diabetes foundation, 2011] and [of Metabolic Risk Factors of Chronic Diseases Collaborating Group, 2011]

5.2 Differences between Japanese and Caucasian

The present increase in T2D prevalence is different in Asia than it is in other parts of the world. It has developed much faster, in a younger age group, and in people with lower BMI [He et al., 1994]. Compared to people of European origin, some studies indicate a larger proportion of body fat and abdominal obesity as one of the arguments for this difference [Park et al., 2001], [He et al., 2002]. Another suggestion is the presence of dysfunction in early (first-phase) insulin secretion, which have been proposed in a variety of papers, mainly based on findings in Japanese subjects [Chen et al., 1995], [Matsumoto et al., 1997], and [Fukushima et al., 2004]. All these studies calculated the first-phase beta-cell function based on the Insulinogenic index as presented in Chapter 4.

From Figure 5.3 it can be seen that the age-adjusted prevalence of diabetes in Japan is 5.0%, whereas it is 5.6% in Denmark in spite of the fact that the average BMI in Denmark is much higher than in Japanese. Based on the current literature it is generally agreed that the pathogenesis of T2D in Japanese individuals differs from that in Caucasian. More specifically, it is suggested that Japanese can not compensate insulin resistance with increased insulin secretion to the same extent as Caucasians [Fukushima et al., 2004]. This understanding

will be challenged below by a review of disease progression in the two ethnicities. A short review is further presented in the introduction of paper C.

Kuroe et al. studied 453 healthy Japanese NGT spanning a wide range of BMI values. Insulin resistance was calculated using HOMA-IR and Matsuda Composite Index, and beta-cell function using the Insulinogenic Index. They found that HOMA-IR generally increased with BMI whereas a decrease in Matsuda Composite was only observed at larger BMI values (>25). Furthermore they found a decrease in Insulinogenic index towards a larger BMI (up to 27.5 kg/m^2). As all subjects in the study were NGT, they suggest that this finding supports the presence of impaired early phase insulin secretion in Japanese, despite NGT [Kuroe et al., 2003].

In another study including 379 Japanese men, an increase in HOMA-IR and a decrease in Insulinogenic from NGT to IGT and also from IGT to isolated post-challenge hyperglycemia (IPH) was reported [Suzuki et al., 2003]. Another large study with 684 Japanese males/females stratified in NGT, IGT, and T2D groups with mean BMI about 24 kg/m^2, reported a small increase in HOMA-IR from NGT to IGT and again a small decrease from IGT to T2D [Fukushima et al., 2004]. Also HOMA-B and Insulinogenic Index were reported to be significantly decreased from NGT to T2D. These findings were supported by another study, also performed in NGT, IGTs, and T2Ds with OGTT profiles showing a decreased early insulin response (up to 60 min.) in IGTs compared to NGTs. In these subjects, also the insulin sensitivity was decreased from NGT to IGT and from IGT to T2D [Nishi et al., 2005].

Abdul-Ghani et al. also studied Japanese subjects and this paper is one of the few papers that report the calculation of the disposition index, using the product of Matsuda Composite Index and Insulinogenic. The Japanese subjects in both the NGT and the IGT group had BMI≈24 and was shown to have a significant decrease in disposition index from NGT to IGT. This decrease was reported to be caused both by a significant decrease in insulin sensitivity and in insulin secretion [Abdul-Ghani et al., 2007].

The Botnia study is one of the large studies investigating T2D disease progression in Caucasians. The study involved 5396 subjects of mainly finnish inheritance and reports increased OGTT insulin response from NGT to IGT, but a decrease from IGT to T2D. HOMA-IR was further found to have a significant decrease from IGT to T2D [Tripathy et al., 2000]. In 2003 Hanefeld et al. performed another study in 307 Caucasian subjects with a mean BMI about 27 kg/m^2, showing decreased early (Insulinogenic) and late-phase (ratio of full insulin AUC to glucose AUC) beta-cell function, despite increased insulin AUC from NGT to IGT.

Torrens et al., 2004 performed a study in premenopausal women including subjects both from non-hispanic (Caucasian) and Japanese inheritance. Using stepwise multivariable regression analysis of covariance models (ANCOVA), the impact of ethnicity was found to be significant on HOMA-B after correcting for confounders. This suggests the presence of difference also in beta-cell function at fasting state between Caucasian and Japanese.

Fukushima et al., 2004 also compared Caucasian and Japanese, by combining the Botnia study with own findings. Japanese subjects had a less pronounced increase in HOMA-IR from NGT to T2D, but in general had lower early insulin secretion at all stages of glucose tolerance. Based on this it was suggested, that main determinant for decreased glucose tolerance from NGT to IGT in Japanese, is a decreased early-phased insulin response [Fukushima et al., 2004].

In summary, the literature seem to agree upon the fact, that Caucasian and Japanese decline in both beta-cell function (either measured by HOMA-B or Insulinogenic index) and insulin sensitivity (either measured by HOMA-IR or Matsuda index) when progressing from NGT to T2D. Contrary some controversy exist whether the early - and late phase insulin response is increased from NGT to IGT in both ethnicities. Furthermore it is not clear whether Japanese can compensate increasing insulin resistance with increasing insulin secretion to the same extent as Caucasians.

Despite the fact, that the above review has outlined a variety of papers, stating possible differences in T2D disease progression in Caucasian and Japanese, no parallel matched studies performed in each region has been conducted. This makes it difficult to compare the two ethnicities. A parallel study performed in Japan in collaboration with a site in a country with mainly Caucasians can help eliminate the effect of environment and inclusion bias in general. The analysis and modelling of data from such a study was completed throughout the PhD period and will be presented in the following chapter.

Chapter 6

Study of the ethnic difference between Caucasian and Japanese subjects

6.1 Introduction

As described in the last chapter, T2D is an example of a disease in which differences between ethnic groups have been reported. As was further stated, a major part of scientific litterature, reporting ethnic differences is built on the hypothesis that Japanese and Caucasians differ with respect to development of T2D. In spite of this no extensive comparison has been performed using both simple measures and modeling techniques for identifying underlying pathophysiology combined with a thorough assessment of both demographic, biochemical, and genetic information. Information obtained from such a study can potentially support future decisions regarding whether or not to use bridging studies when developing anti-diabetic medicines for the two regions in place. The main objectives of the study investigating ethnic differences between Japanese and Caucasians performed in this thesis were

1. Quantify differences in beta-cell function and insulin sensitivity in Caucasian and Japanese in subjects with:

- Normal glucose tolerance (NGT)[1]
- Impaired glucose tolerance (IGT)
- Type 2 diabetes (T2D)

2. Estimate fraction of differences between Caucasian and Japanese that can be explained by covariates for measures of:
   - Beta-cell function
   - Insulin sensitivity

6.2 Materials and methods

6.2.1 Study design and participants

The study investigated 150 Caucasian subjects (Northern European background for at least three generations) enrolled at Copenhagen University Hospital, Denmark, and 120 Japanese (Japanese background for at least three generations) enrolled at Tokyo University Hospital, Japan. Potential participants (males and females aged 40 to 65 years) were carefully screened to exclude individuals with metabolic conditions other than T2D known to influence body composition. Other key exclusion criteria were: treatment with insulin, recent or ongoing infection, history of malignant disease, and use of thiazolidinedione (TZD) based medications within last three months. Participation also required normal results from the physical examination, blood screening, electrocardiogram, urinalysis, and a stable bodyweight ($\pm 10\%$) for the past year. Participants were stratified into two groups of low and high BMI, respectively. A high BMI was defined for Japanese as $>25\ kg/m^2$ and for Caucasians as BMI$>30\ kg/m^2$ in accordance with regional obesity definitions [Kanazawa et al., 2002], [Organization., 2000]. The participants were classified as having either NGT, IGT or T2D [WHO, 1999], on the basis of blood glucose levels while fasting and at 2 hours during the OGTT (See Chapter 3 for classification). The study protocol was approved by The Regional Committee on Biomedical Research Ethics in Denmark (Journal no H-C-2008-101) and by the Research Ethics Committee of Graduate School of Medicine, University of Tokyo, Japan. Informed consent was obtained from all participants. Each subject was characterised according to the following measurements:

- Lifestyle factors

[1] See classification criteria in section 3

Dual-emission X-ray absorptiometry (DXA) scan for body fat

VO_2max for physical activity

International Physical Activity Questionnaire (IPAQ)

- Biochemical assessments

  Cytokines and hormones (IL-6, TNF-α, leptin, and adiponectin)

  Lipids (HDL, LDL, triglycerides, cholesterol, and FFA)

- Genetics

  Single-nucleotide-polymorphisms (SNPs) validated for T2D

For assessment of beta-cell function and insulin sensitivity, each subject underwent an extended 75g frequently sampled oral glucose tolerance test (OGTT). After an approximately 12-h overnight fast, venous blood samples were drawn at -30, and 0 minutes before the glucose intake and at 10, 20, 30, 60, 90, 120, 150, 180, 240, 300 minutes after. Serum insulin, plasma glucose, and C-peptide were measured at all timepoints. At timepoints 0, 20, 30, 60, 120, and 240 samples were measured for GLP-1 whereas at 0, 20, 60, 120, 180, and 300 min samples were measured for free-fatty-acids (FFA). Data analysis of GLP-1 and FFA data will not be considered in this thesis.

6.2.2 Hardware and software

All data, including OGTT measurements, were delivered by the two sites in Excel files. For handling and analysis of data the commercial software S-plus was applied. The statistical analysis of the data was carried out by developing specific S-plus scripts. Data files for the model-based approach was also prepared using S-plus scripts. Single-subject estimation was carried out using Matlab v.12 and population estimation using NONMEM VII, both installed on a linux cluster at DTU and NN, respectively.

6.2.3 Covariate selection

Strategies for selection of covariates in regression models has been studied in various publications in both the classical statistics area [Hooking, 1976], [Gupta and Huang, 1988], [Cheng et al., 2009] and the population PK/PD area [Kowalski and Hutmacher, 2001], [Denti et al., 2010], [Ribbing and Jonsson, 2004]. In summary, the proposed methods are all based on some or one of the following procedures outlined below [Madsen and Thyregod, 2011]:

- Forward selection: Inclusion of one variable at a time in order of increasing p-value
- Backward elimination: Starting with a full model and reduction of model, one variable at a time
- Stepwise regression: A modified version of the forward selection where variables are added step-by-step and other variables are tested for significance at each step
- For a number $k = 1, 2, 3, ...$ an algorithm identifies a given number of best regression models using k variables

In the present study a large pool of covariates (>30) were included in the analysis and many of these coviariates were highly correlated (BMI, height, waist, fat-mass etc.). Furthermore, as mentioned above, the study was designed with stratification into different groups based on both BMI, race and glucose tolerance. Based on this, a classical covariate procedure was not found appropriate for selection. Instead the following procedure was used:

1. Define full covariate model with pre-specified covariates and possible additional covariates
   - Type[NGT,IGT,T2D], race[Caucasian,Japanese], age[40;65], sex[male,female], BMI[≥18], and additional covariates
2. Inclusion of additional covariates in case they are significant and explain a large fraction of the variability remaining after inclusion of pre-specified covariates
   - Significant (p<0.01)
   - Explained variability > 10%[2]
   - Not highly correlated with other covariates (r<0.5)
3. Backwards elimination[3]
   - Significance (p>0.001) or explained variability < 10 %[4]
4. Testing order of elimination

[2]Explained variability is calculated using the variance of residuals from model with and without the additional covariate

[3]The full model without the use of backward elimination is used to generate Forest plot as will be shown below

[4]In the NONMEM analysis presented in this chapter the p-value is used and corresponds to a change in OFV of 10.84

- First: Covariates explaining least variability
- Last: Predefined covariates such age, sex, and BMI

By applying this procedure a limited amount of covariates was included, and those covariates that were kept in the final model are not only significant, but also have a high probability of being clinically relevant as descibed by Tunblad et al. [Tunblad et al., 2008].

In order to estimate fraction of differences in beta-cell function and insulin sensitivity that could be explained by each covariate in final model, a measure for how much each covariate explains of the difference was further derived according to the following formula:

$$\frac{\sum_{i=NGT,IGT,T2D} |\bar{E}_{C,i} - \bar{E}_{JPN,i}| \quad - \sum_{i=NGT,IGT,T2D} |\bar{E}_{C.Adj,i} - \bar{E}_{JPN.Adj,i}|}{\sum_{i=NGT,IGT,T2D} |\bar{E}_{C,i} - \bar{E}_{JPN,i}|} \cdot 100\%$$

where $\bar{E}_{C,i}$ represents mean of endpoint for Caucasians calculated for the disease group i which is either NGT, IGT or T2D. $\bar{E}_{C.Adj,i}$ represents mean of endpoint adjusted for covariates. Assuming a linear relationship between the covariates and the logarithm of the endpoint, the calculation of the adjusted endpoint would be

$$E_{Adj} = exp(log(E) - \beta_1 \cdot x_1 - \beta_2 \cdot x_2 - ...) \tag{6.1}$$

where β_i is the slope obtained from a linear model having the given endpoint (E) as the dependent variable and the covariate subtracted for median (x_i) as independent variable together with race and disease type such that the intercept can be different in each group. The median is subtracted from the covariate such that the intercept is calculated at that point.

6.3 Results

In this section, the results from the analysis of the development of T2D in Caucasians and Japanese will be presented. The main analysis has been performed by calculating different measures for beta-cell function and insulin sensitivity using data from the OGTT, for subjects in each disease group (NGT, IGT, or T2D). Measures for insulin sensitivity and beta-cell function have been calculated based on 1) simple measures derived from glucose and insulin profiles such as the HOMA-IR [Matthews et al., 1985] and Insulinogenic index [Phillips et al., 1994], and 2) using a model-based approach that builds on knowledge

about the PK/PD relations between glucose, insulin, and C-peptide following an OGTT [Breda et al., 2001], [Man et al., 2002]. These models have been implemented in a) single-subject approach where parameters for each subject is estimated using the profile of the given subject as originally proposed by Breda et al., 2001, and Dalla Man et al., 2002, and b) a population-approach where all data are used simultaneously to estimate population parameters and individual parameters as done for the C-peptide model for estimation of beta-cell function in [Møller et al., 2010].

Paper C included in this thesis describes in detail the part of the analysis performed using simple measures, whereas paper D deals with the single-subject analysis of the model-based approach. The results presented in this chapter will thus mainly be based on the population analysis.

6.3.1 Beta-cell function and insulin sensitivity

In this subsection, the estimates for insulin sensitivity and beta-cell function will be presented. The results originate from population implementations of the minimal models for glucose and C-peptide as presented in Chapter 4. Each barplot (Figure 6.1 - Figure 6.3) represent mean values and standard error of the mean (SEM) in each disease group (NGT, IGT, or T2D) for Caucasians and Japanese. These values are calculated based on the individual estimates of insulin sensitivity (S_I) and beta-cell function (ϕ_d,ϕ_s) obtained from the population analysis. Test for significant differences between Japanese and Caucasian is performed for each disease group on an α=0.05 level (one star) and on α=0.01 level (two stars)[5]. Significance was obtained from ANOVA model. The results of the analysis is shown later in this subsection.

The dynamic secretion index (Figure 6.1) influence model predictions of C-peptide until the point were the slope of the glucose curve becomes less than or equal to 0. It can thus be understood as a first-phase index and has a significant relation to the first-phase index derived from an IVGTT (See paper A). The results regarding differences in model-based beta-cell function and insulin sensitivity obtained from the population estimation is generally in line with the results obtained using the single-subject approach presented in paper D. One difference is for the dynamic index (Φ_d) in IGTs, which is not significant based on the population analysis. The fact, that the Caucasian NGTs seem to have numerically lower values than Japanese NGTs is in contrast to what is observed for the non-model based estimates of first-phase (Insulinogenic) presented in

[5]Multiple statistical tests was performed without Bonferroni correction indicating that significance for some tests will occur from time to time. Bonferroni correction was not performed due to insufficient number of subjects in this trial

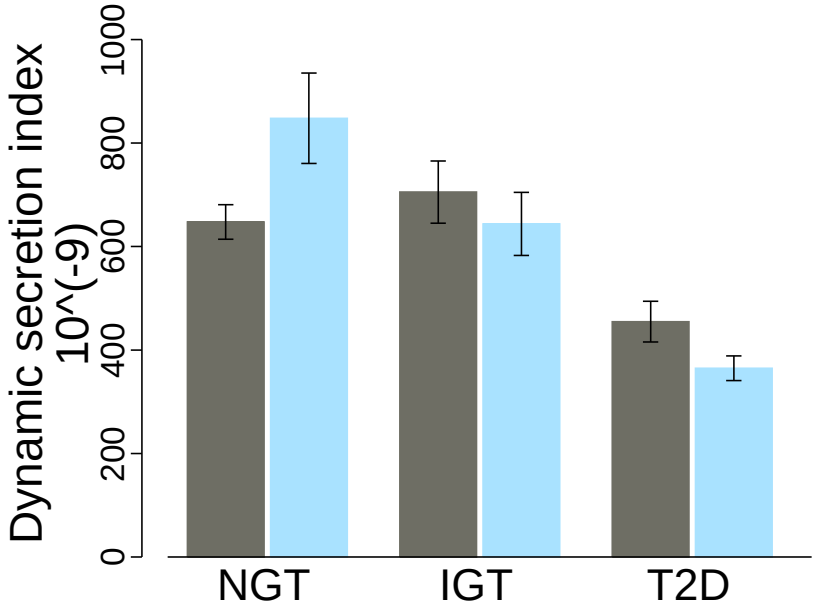

Figure 6.1: Dynamic secretion index (Φ_d) for NGT, IGT, and T2D in Caucasian and Japanese

Paper C. To investigate this further, Figure 6.4 presents impact from the dynamic (SR_d) and the static (SR_s) part of secretion. Based on calculation of AUC from 0-30 min from these curves (Figure 6.4), the dynamic part represents 23%, and 33% of the full effect in Caucasian and Japanese, respectively. Thus, only a fraction of the response (which is reflected in the total C-peptide/insulin profile) originates from the dynamic part, whereas Insulinogenic uses the raw samples (100% of the insulin response). This could partly explain the reason for the difference observed for Insulinogenic and dynamic index. In fact, it is rather expected to see the same trend for the static index, which plays a bigger role for the shape of the C-peptide and thus also the insulin curve.

The estimates for the static secretion index estimated by the NLME approach is presented in Figure 6.2. As for the single-subject results presented in paper D, it indicates a similar trend in the transition from NGT to T2D between Japanese and Caucasians with decreasing static secretion index. It is also observed that the static secretion index is numerically lower in the Japanese compared to the corresponding Caucasians in all disease groups which was also seen for the Insulinogenic index and the insulin secretion ratio (See paper C).

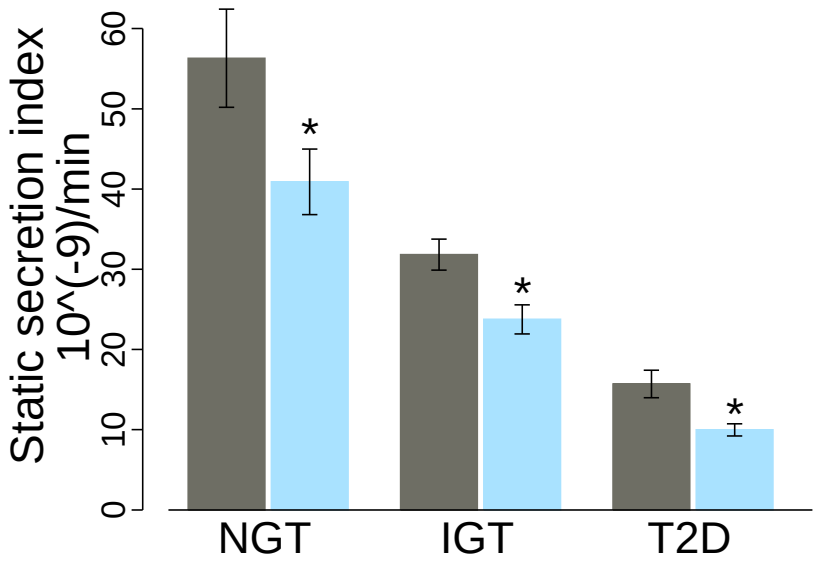

Figure 6.2: Static secretion index (Φ_s) for NGT, IGT, and T2D in Caucasian and Japanese

In Figure 6.3, the estimates for population model based insulin sensitivity (S_I) are presented. Again the estimates seem to be in accordance with what is presented from the single subject analysis in Paper D, although there seems to be a tendency towards a higher sensitivity in Caucasian NGTs estimated using the single-subject model. The population estimates thus agree better with the Matsuda Composite Index which is presented in paper C. By inspecting the values obtained from the single-subject analysis, some more extreme values were found, although the correlation between population and single-subject estimates were r$\approx$ 0.90 both for log-transformed and non-transformed variables.

In order to better analyse possible differences in beta-cell function as a function of continuous disease state, the dynamic and static indices are plotted against glucose at 2 hours (G2H) (Figure 6.5). In order to analyse the difference in slopes and intercepts, the following statistical model was applied (here shown for Φ_d)

$$log(\Phi_d) \sim Race + log(G2H_{adj}) + Race : log(G2H_{adj}) \tag{6.2}$$

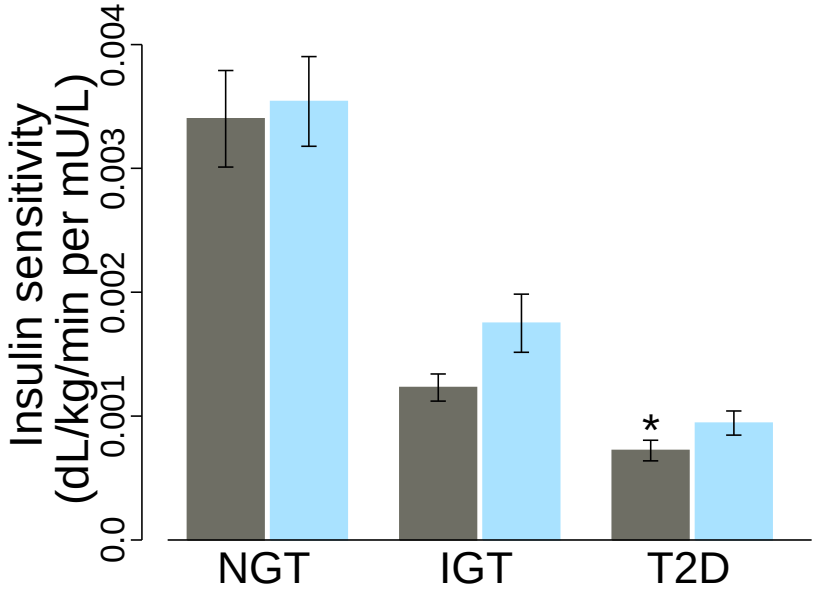

Figure 6.3: Insulin sensitivity (S_I) for NGT, IGT, and T2D in Caucasian and Japanese

where $log(G2H_{adj})$ is equal to log(G2H) - median(log(G2H)). The median in log-domain has been subtracted from G2H as it does not give sense to investigate difference in intercept at a glucose level equal to 0.

For the dynamic index, it seems that there might be a difference in the slopes, although the impact of ethnicity on slope was borderline significant (p≈0.06). For the static secretion index the slopes are nearly identical, which is also reported from the statistical model, indicating a p-value on the effect of ethnicity on the slope to be p≈0.8.

In summary, the estimates of beta-cell function in general were higher in the Caucasian cohort than in the Japanese, and a trend towards lower insulin sensitivity in Caucasians were found. Furthermore, for the dynamic secretion index, it seems the Caucasians do not decrease from NGT to IGT, which is the case for Japanese. This seems mainly to be driven by the high values of ϕ_d in the

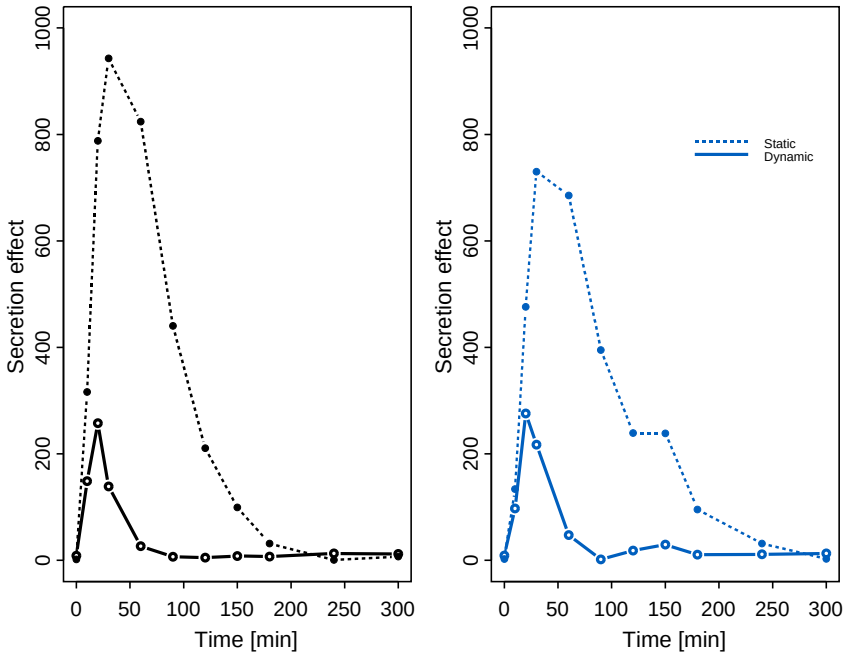

Figure 6.4: Contribution of dynamic and static part on secretion. Left: Caucasians, Right: Japanese.

Japanese NGTs[6], although neither in the NGT or in the IGT group there is a significant difference between the two cohorts. The borderline significant different slopes obtained from the continous analysis, having G2H at the abscissa also seem to be driven by these differences. Based on the analysis of ϕ_s (Fig.6.5,right) with G2H on the abscissa, it seems that Caucasians and Japanese have similar decline in static secretion index for worsening disease state (G2H).

In the following section, covariate analysis on the insulin sensitivity (S_I) and beta-cell function (Φ_d, Φ_s) indices will be performed. From this analysis it will be clear whether the difference observed between Japanese and Caucasian seem to inherit from unknown ethnic factors or can be attributed to difference in lifestyle/demographic factors.

[6] A further investigation showed that the high mean value in the Japanese NGT group was driven by 4 values that were around 3 fold higher than the rest of the values

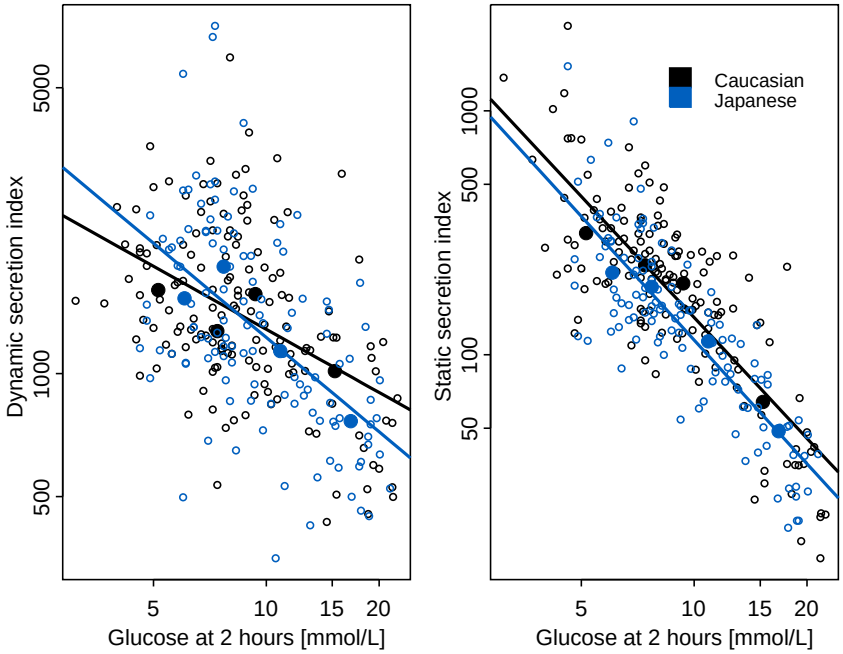

Figure 6.5: Left: Dynamic secretion index (Φ_d) vs. G2H. Right: Static secretion index (Φ_S). Large dots represent median in quartiles and lines are obtained from linear regression on log-transformed data.

6.3.2 Covariate analysis on model based insulin sensitivity

The results from covariate analysis on S_I obtained from the NLME version of the oral minimal glucose model will be presented first. In order to present an exploratory analysis of the predictability of each covariate relating to insulin sensitivity, Table 6.1 shows how much of the variability in S_I each covariate explains. The values in the table have been obtained by using a regression model with post-hoc estimates of S_I as dependent variable, and type, race, and the additional covariate as independent variable. Explained variability have been calculated using the variance of residuals of the model with and without the additional covariate, and the difference explained according to the procedure outlined above. From the table it is clear that android fat is a good predictor for insulin sensitivity.

For covariate selection, the procedure outlined above was followed. First the explained variability was calculated for each covariate, having the pre-specified

covariates already in model. The covariates that explained more than 10 % of the remaining variability is presented in Table 6.2[7]. Thus, as a preliminary

Covariate	Exp.var.(%)	Diff.exp.(%)
Android fat [%]	27	11.4
Trunk fat [%]	23.3	55.7
Waist [cm]	17.5	-17.2
VO2Max per kg [mL/min/kg]	16.2	30.4
Leptin [pg/ml]	14.1	31.2
Whole body fat [%]	13.0	56.7
Hip [%]	12.6	-80.6
Triglycerides [mmol/L]	12.3	20.8
Whole body fat free [%]	12.3	57.8
IL6 [pg/ml]	10	25.4
BMI [kg/m^2]	10	20.4

Table 6.1: Results from adding each covariate one at a time having type, race, and interaction between the two already in model. Exp.var(%) is calculated based on variance of residuals in regression model with and without the given covariate and Diff.exp(%) is calculated according to the formula presented above.

analysis, each covariate was added to a linear model having having type, race, age, sex, BMI, and the interaction between type and race already in model. Based on the variance of residuals from the linear model with - and without the additional covariate, a measure was obtained for each covariate indicating how many percent the given covariate could explain of the remaining variability (after inclusion of type, race, age, sex, and BMI). Output from this analysis is shown in Table 6.2 below (diff. exp. not shown as the table is solely used for selection of additional covariates).

In order to check for correlated covariates, the correlation between the variables already in the model (Age, BMI etc.) and the ones presented in Table 6.2 was calculated. All covariates in Table 6.2 was found to be highly correlated with BMI ($r > 0.5$). Based on the fact, that the percentage of android fat clearly explained much more of the variability in S_I than BMI (27 % vs. 10 %), it was chosen to add android fat to the model and remove BMI. The full model thus consisted of the following covariates: Type, race, age, sex, and android fat. The correlation coefficient between age and android fat was $r < 0.1$ and the implementation of S_I was performed in the following way:

$$S_I \approx \theta_{1,i}\theta_2^{Race}\theta_3^{Gender}\left(\frac{Age}{mAge}\right)^{\theta_4}\left(\frac{Android\ fat}{mAndroid}\right)^{\theta_5} exp(\eta_1) \qquad (6.3)$$

[7]For all presented covariates, $p < 0.001$, and thus the p-value is not presented

Covariate	Exp.var.(%)
Android fat [%]	20.5
Trunk fat [%]	19.8
Whole body fat [%]	14.5
Leptin [pg/ml]	14.1
Upper limb fat [%]	10.5

Table 6.2: Results from one-on analysis having type, race, age, BMI and sex in model. Only covariates explaining more than 10% residual variability is presented. Exp.var(%) is calculated based on variance of residuals in regression model with and without the given covariate

where i is either NGT, IGT, or T2D assuming different mean values of S_I in each group. The medians of Age and Android fat is identified as mAge and mAndroid, and $\theta_1 - \theta_5$ present covariate relations between the individual values of insulin sensitivity and the given covariate. NONMEM VII provides estimates and uncertainty (standard error) for each covariate parameter. The implementation above thus enables graphical representation of covariate effects for clarification of the effect on the dependent variable. For discrete variables such as race, the parameter θ_2 will be around 1 if race does not have a significant impact. For continuous variables, the visualisation of effect is obtained using the rounded values of the highest and lowest observations for the given covariate and the corresponding value of the estimates of the dependent variable with a 90% confidence interval. By applying this method, the covariate effects can be visualised in a so-called Forest plot (or blobbogram) [Lalkhen, 2009]. This way of visualizing covariate effects was recently proposed by FDA as an easy way to present the effect of covariates on PK responses [Menon-Andersen et al., 2011].

Figure 6.6 shows the effect of covariates on S_I. The plot is interpreted in the way, that if the lines intersect the value of 1, the effect is not statistically significant. The interval 0.8-1.25 reflects the bioequivalence for when the differences are considered relevant.

From the Forest plot of S_I and corresponding covariates it is observed that a person with an age around 40 has a significantly higher expected insulin sensitivity than a corresponding person with an age around 65. Similarly a person with an android fat mass around 1 % is predicted to have a 3 times higher sensitivity than a person with an android fat mass around 3.25 %. As the interval on the effect of race in all three groups (NGT, IGT, and T2D) include 1, race does not seem to have en impact on the level of S_I. This is also seen for gender.

For the dynamic and static indices of beta-cell function, no additional covariates explained more than 10% of the residual variability having the pre-specified

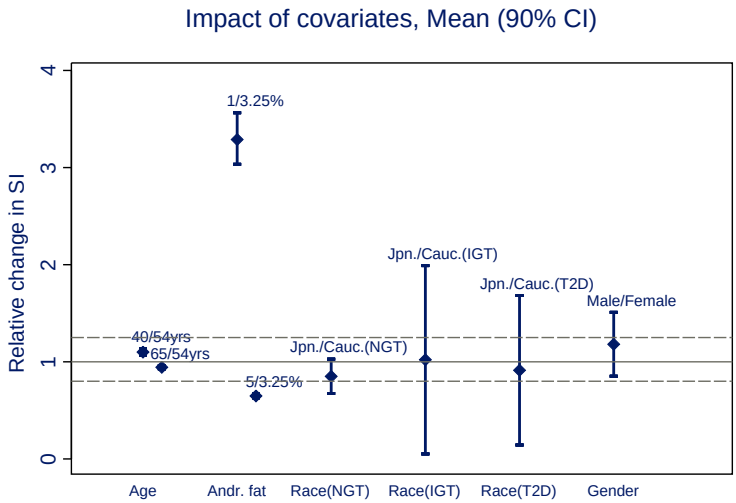

Figure 6.6: Impact of covariates on insulin sensitivity (S_I)

covariates in regression model. The Forest plots presented in Figure 6.7, and 6.8 is thus visualised using the pre-specified covariates defined by disease type (NGT, IGT, T2D), race, age, sex, and BMI. It is clear that BMI has a significant impact on both beta-cell function indices. It is also observed that race does not have a relevant impact on either of the two indices, except for ϕ_d in NGT[8]. In order to obtain a reduced model, a backwards elimination procedure was also performed for S_I, ϕ_d, and ϕ_s using a LRT and a significance criteria of 0.001 corresponding to a change in OFV of 10.83 with a reduction in one degree of freedom. The following reduced models were obtained

$$S_I \sim Type + Android\ fat\ mass \tag{6.4}$$
$$\phi_d \sim Type + BMI \tag{6.5}$$
$$\phi_s \sim Type + BMI \tag{6.6}$$
$$\tag{6.7}$$

[8] As stated earlier it was found that this difference was driven by 4 Japanese subject having 3 fold higher values than the rest of the group. The C-peptide fits of two of those subjects is questionnable, but the values was not considered as outliers. In a following analysis where the 4 subjects were removed, the Forest plot did not indicate any significant differences between the two races

indicating that race did not have any significant impact. In contrast, the Forest plot in Figure 6.7 indicate that race might have an impact on ϕ_d in the NGT group. In order to check this, a statistical analysis of post-hoc estimates was performed both on the ODE and SDE model without covariates. For both cases race was significant for ϕ_d and it is thus concluded that race might have an impact on the dynamic secretion index. In spite of this it is worth mentioning that, 1) the difference seems mainly to be driven by a difference in the NGT group, 2) Post-hoc values analysis in the classical statistics approach can suffer from shrinkage [Savic and Karlsson, 2009], 3) the dynamic secretion index seems to have limited impact on the C-peptide (and thus also insulin) curve, which is the important factor for controlling a subjects glucose level.

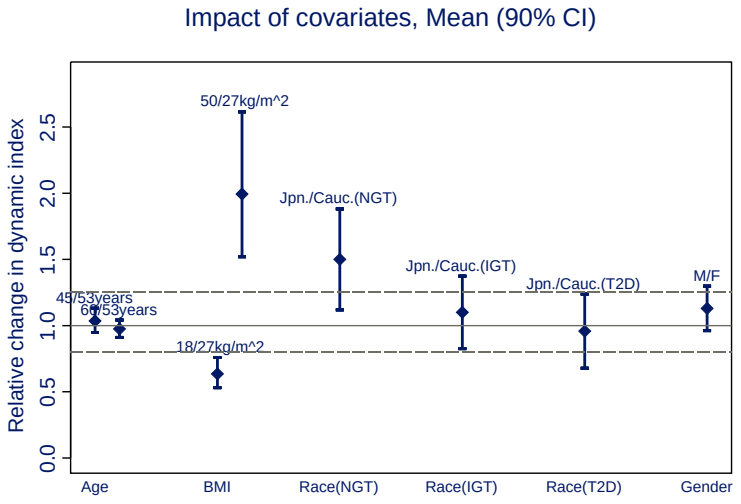

Figure 6.7: Impact of covariates on dynamic secretion index (ϕ_d)

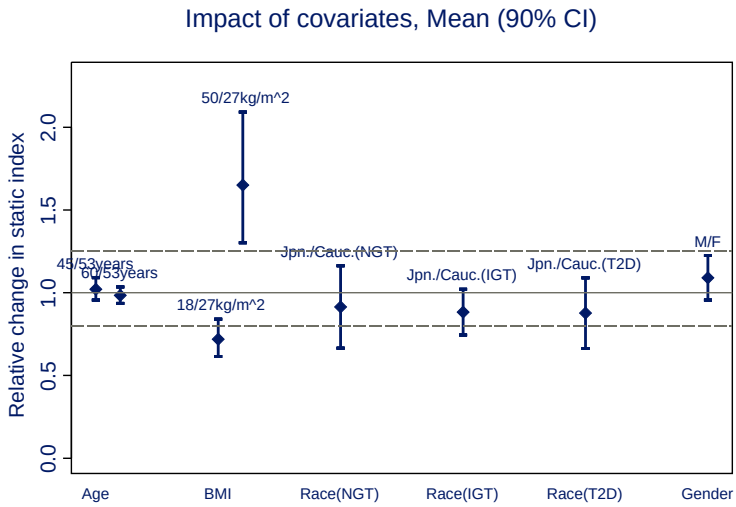

Figure 6.8: Impact of covariates on static secretion index (ϕ_s)

Chapter 7

Discussion and Perspectives

7.1 SDEs in PK/PD modelling

In general the use of SDEs have gained more and more attention in the PK/PD community. In spite of this, it is very few companies that applies these techniques and to my knowledge none of the regulatory authorities. One major issue is the lack of validated software tools that can handle SDEs in a population setting. At present only one software package exists, which constitutes an R library [Klim et al., 2009] that requires in-depth understanding of the R language, although specific subroutines have been implemented in more validated software packages such as NONMEM 7 [Bauer, 2011]. One unfortunate property in both these tools is the restriction, that the system noise is additive, and can not be dependent on the state without transformations of the state space. Future studies are needed to investigate the errors introduced by assuming system noise as being additive, in situations where this is not necessarily the case.

Another issue is the fairly few practical case studies which has proven SDEs to be beneficial in PK/PD modelling [Kristensen et al., 2005], [Overgaard, 2006], [Tornøe, 2005]. Paper A in this thesis adds to the pile by showing improved prediction performance with SDEs in modelling of the glucose/insulin system.

7.2 Modelling to improve understanding of T2D

In early phases of drug development, PK/PD models models can be applied to understand disease progression and drug action, and in later phases to extrapolate treatment regimens to other populations, races etc. In Paper B, included in this thesis, a model for GLP-1 secretion was developed. It suggests the presence of a neuro-endocrine loop, that can cause a fast secretion of GLP-1. In this way the model might help to improve the understanding of disease progression of T2D. As it provides indices describing secretion on an individual level, it would be of interest to study the impact of demographic factors such as age, ethnicity etc. on these indices.

7.3 Study of ethnic differences in T2D

From the study performed on data from the Caucasian and Japanese cohorte, it was found that the paradigm, that Japanese have a different pathogenesis of T2D than Caucasians, seem to be mainly driven by the general difference in BMI. As BMI has a strong relation to development of insulin resistance, the studied Japanese cohort was found to develop a form of T2D less driven by insulin resistance and more by low beta-cell function. Due to the fact, that BMI seems to explain the majority of the difference, similar results would thus be expected in Caucasians having low BMI as that observed for Japanese. In spite of this, it seems that even when adjusted for lifestyle factors etc., it is suggested that Asian Indians develop T2D much easier than eg. non-Hispanic whites [Lee et al., 2011]. This suggests that a study as the one analysed in this thesis including Asian Indians would be of value for further understanding of T2D and for future anti-diabetic drug development.

In this thesis, the OGTT from the two cohorts was analysed with simple indices (Paper C), and single-subject model-based indices (Paper D), and population based modelling approaches (Chapter 6). As outlined, the different methods have provided slightly different results. In spite of this, the application of these different methods helped to improve the understanding of the data and results from each method was used to challenge the results obtained using the other methods.

7.4 Application of study in drug development

The process of obtaining regulatory approval for pharmaceuticals in new geographic regions has usually required extensive clinical development programs in the new regions despite the fact the pharmaceutical is already tested in another region. However, with the implementation of the ICH Guideline "Ethnic factors in the Acceptability of Foreign Clinical Data" [184, 1998] it became possible to obtain approval for a new drug based on efficacy data from a foreign region under the assumption that the PK and PD properties could be bridged between the two ethnic groups based on a limited clinical development program. Using a bridging strategy can in many cases lead to shorter development time and inclusion of fewer patients than is the case with a full clinical development program [Uyama et al., 2005].

In case a bridging approach is considered, it is of main importance whether a drug can be characterised as sensitive or insensitive to ethnic differences. In the latter case, the probability of a successful bridging study is much higher. As drugs generally interact with the physiology of the human body, also possible ethnic differences in development of diseases is of main importance.

The results presented in this thesis outlines the ethnic differences in T2D disease development between Japanese and Caucasians and shows that such differences seem to mainly be explained by differences in phenotype characterised by factors such as BMI or other measures describing the degree of obesity. These results thus support the use of global clinical trials and bridging studies in future antidiabetic drug development between Japanese and Caucasians, although other studies supporting the presented results will substantiate the conclusion.

Chapter 8

Conclusion

Pharmacokinetic/Pharmacodynamic (PK/PD) modelling is gaining more and more importance in the phases of clinical drug development. Especially in phase 3, where patients are recruited from different regions, population PK/PD modelling has shown to be an invaluable tool in identifying covariate effects such as race, age etc.

In application of PK/PD models it is of main importance that such models are flexible so they can be used to predict dose-response relationships in different treatment regimens etc. This points to the fact, that such models must be based not only on clinical data, but on prior knowledge of underlying pathophysiology. By using mechanism-based modelling, models build in early phases for exploratory purposes can be used in confimatory settings at later phases. This can help to decrease the probability of Type 1 and Type 2 errors in test procedures.

In the present PhD thesis, various models dealing with the pathophysiology of type 2 diabets have been studied, and more specifically models for estimation of GLP-1 secretion, beta-cell function, and insulin sensitivity have been implemented in a non-linear mixed effects population setting. The models for beta-cell function and insulin sensitivity have been applied for characterising type 2 diabetes disease progression in Japanese and Caucasians.

The results from the analysis show that Japanese generally have lower beta-cell function and higher insulin sensitivity compared to Caucasians and that the major part of these differences can be explained by a difference in body-size (BMI) in contrast to race in itself. Furthermore the results showed that the disposition index is similar in the two cohorts at all levels of glucose tolerance (NGT, IGT, and T2D).

More specifically, the achievements from this project have been summarized in the present report and a major part of these are described in further details in the papers attached in the appendix. Some of these achievements include:

1. Paper A: The application of SDEs for improving predictive performance for glucose/insulin models implemented using a NLME population approach was investigated. It was observed that the SDE implementation could account for correlated residuals and thus caused improved predictive performance of the oral minimal C-peptide model for determination of beta-cell function.
2. Paper B: A mechanism-based population model of the GLP-1 secretion following an OGTT was developed, which succesfully could describe the systematic behaviour of GLP-1 data. The model included an early - and a late phase stimulation of GLP-1 production originating from ingestion and absorption of glucose.
3. Paper C: A complete analysis of insulin sensitivity and beta-cell function was performed on data from an OGTT obtained in Japanese and Caucasian subject. Paper C summarises the findings obtained from analysis using simple indices, and showed that lower beta-cell function combined with higher insulin sensitivity observed in Japanese compared to Caucasian mainly can be explained by differences in BMI.
4. Paper D: In this paper, results from a corresponding model-based single-subject approach is presented. Based on these, it became clear that besides having lower beta-cell function, the Japanese subjects furthermore had a higher hepatic extraction ratio. Also, the net-effect of insulin sensitivity and beta-cell function (disposition index) was assessed, and was found to be similar across all disease states in the Japanese and Caucasian cohort.
5. Chapter 6: This section mainly involve results obtained from NLME population models applied on the data in Japanese and Caucasians. The model setup enabled simultaneous estimation of measures for beta-cell function and insulin sensitivity and the effects of covariates on these parameters. Based on obtained estimates and their corresponding confidence intervals, a visual representation clarified that android fat seem to be an important predictor for insulin sensitivity and BMI for beta-cell function.

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Part II

Papers

Paper A

Predictive performance for population models using stochastic differential equations applied on data from an oral glucose tolerance test

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Abstract Several articles have investigated stochastic differential equations (SDEs) in PK/PD models, but few have quantitatively investigated the benefits to predictive performance of models based on real data. Estimation of first phase insulin secretion which reflects beta-cell function using models of the OGTT is a difficult problem in need of further investigation. The present work aimed at investigating the power of SDEs to predict the first phase insulin secretion (AIR_{0-8}) in the IVGTT based on parameters obtained from the minimal model of the OGTT, published by Breda et al. (Diabetes 50(1):150–158, 2001). In total 174 subjects underwent both an OGTT and a tolbutamide modified IVGTT. Estimation of parameters in the oral minimal model (OMM) was performed using the *FOCE*-method in NONMEM VI on insulin and C-peptide measurements. The suggested SDE models were based on a continuous AR(1) process, i.e. the Ornstein-Uhlenbeck process, and the extended Kalman filter was implemented in order to estimate the parameters of the models. Inclusion of the Ornstein-Uhlenbeck (OU) process caused improved description of the variation in the data as measured by the auto-correlation function (ACF) of one-step prediction errors. A main result was that application of SDE models improved the correlation between the individual first phase indexes obtained from OGTT and AIR_{0-8} ($r = 0.36$ to $r = 0.49$ and $r = 0.32$ to $r = 0.47$ with C-peptide and insulin measurements, respectively). In addition to the increased correlation also the properties of the indexes obtained using the SDE

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models more correctly assessed the properties of the first phase indexes obtained from the IVGTT. In general it is concluded that the presented SDE approach not only caused autocorrelation of errors to decrease but also improved estimation of clinical measures obtained from the glucose tolerance tests. Since, the estimation time of extended models was not heavily increased compared to basic models, the applied method is concluded to have high relevance not only in theory but also in practice.

Keywords Pharmacokinetic (PK) · Oral glucose tolerance test (OGTT) · Intravenous glucose tolerance test (IVGTT) · Acute insulin response (AIR) · Oral minimal model (OMM) · Autocorrelation function (ACF) · Stochastic differential equations (SDEs) · Ornstein-Uhlenbeck (OU) · Extended Kalman filter (EKF)

Introduction

The present article deals with the application of mathematical models for the description of the dynamics of insulin response following an oral glucose tolerance test (OGTT) [1]. Traditionally the parameters in these models are estimated using single subject estimation as performed in [2]. In this paper the data was instead modelled using non-linear mixed-effects (NLME) population models. This approach handles the data from various patients as a population which enables simultaneous estimation of inter- and intra-subject variability, influence of measured concomitant effects, and covariates on the fixed effects parameters. This way of estimating parameters is the preferred method in population PK/PD modeling because it provides reliable predictions of variability and is the only practical method for analyzing data from multiple patients in a single data analysis. As a result of this, the models become cornerstones in the simulation of future trials and thus have high value for the pharmaceutical companies. Compared to a single-subject estimation, the population method is also less time consuming and has been shown in [3–5] to provide more correct and robust estimates of metabolic indices.

In the development of PK/PD models a correct determination of the magnitude of unexplained variability is of great importance. Clearly the efficacy and safety of the drug might decrease as unexplained variability increases [6]. Mathematical models applied to time-dependent data are in general said to be falsified if the prediction errors have a systematic trend across the time-scale [7]. In line with this it was found in [8] that misspecification of the residual error impacts the type I error rate in inclusion of covariates. In population analysis this is not an uncommon phenomenon and various examples can be found in [9]. One way to account for time-correlated prediction errors is to use a more complex error structure or introduce stochastic differential equations in the model building as performed in [9, 10], respectively.

In the application of SDEs the differences between individual predictions and observations are explained not by one, but by two fundamentally different types of noise [11].

- The measurement noise, which represents the serial uncorrelated part of the residual variability that may be due to assay error or if the sample concentration is not representative for the true concentration in plasma.
- The dynamic noise, which enters through the dynamics of the system and may originate from model deficiencies, true random fluctuations within the system, or simply unknown system inputs.

Quantification of the dynamic component is thus a unique tool to tell if the proposed PK/PD model is precise enough to satisfactorily describe the underlying system. An estimate of large dynamic noise could be an indication that a model is too simple or simply describing the system badly.

In summary, the inclusion of a diffusion term, representing dynamic noise, allows the SDE model to explain a larger portion of the variation in a given data set compared to a basic ODE approach. Additionally, estimation of this component provides information on model uncertainties and can be used in general model building as performed in [10]. In agreement it was suggested in [12] that the variability between occasions in PK/PD modelling may be more appropriately modelled using SDEs rather than ODEs.

The application of SDEs in describing the glucose/insulin system has been successfully performed in only few studies. These include modelling data from an euglycaemic clamp study, first in [13] and later in [14], data from an IVGTT [3], and finally also data from a 24-h profile in [15, 16]. In spite of the different applications of SDEs in PK/PD model development it generally holds, that the direct benefits seen on practical applications are scarce and evaluation of these e.g. to predictive performance of parameters on real data has not been performed.

In this paper we investigated the predictive performance of population PK/PD models based on ODEs and SDEs built on data obtained from an OGTT. Various models have been built with the purpose of describing the observed dynamics and a major part of them is presented in [1, 2, 17–24]. The purpose of using these models is to create a metabolic portrait of the subjects under investigation. A complete assessment of all models is beyond the scope and here we used the oral minimal model (OMM) described by Breda et al. [1] which is widely used.

We focused solely on the part of the metabolic portrait concerned as the beta-cell function index. Estimation of this measure was used to evaluate the performance of the studied models through a comparison with the standard beta-cell function index obtained from an IVGTT (AIR_{0-8}). Besides measuring the correlation between the index obtained from the OGTT and AIR_{0-8}, we also investigated their respective relation to different covariates. It was thus not the goal to obtain maximal correlation with AIR_{0-8} as in [24], but rather to investigate how well the beta-cell function index obtained from the applied OGTT model relates to AIR_{0-8} and how application of SDEs can effect these relations.

In the selection of a beta-cell function index from the OGTT models, as stated above, a reasonable goal could be to obtain a measure that reflects the properties of the widely used index obtained from the IVGTT, AIR_{0-8}. This measure can be interpreted as the response to an impulse of glucose. Strictly speaking it is a measure

describing the magnitude of the direct insulin response to a rapid increase in glucose. In [2] a correlation of 0.28 was obtained between a dynamic beta-cell function index (Φ_d) and AIR_{0-10}, using single-subject modelling applied to 17 nondiabetic subjects. An extended OGTT model was presented in [22] which also takes incretin effects into account. The obtained correlation between the different indices was 0.67 and estimation was performed using a population approach on 40 healthy subjects. In [24] a regression based approach using measurements from the OGTT and subject specific covariates as independent variables and AIR_{0-8} as dependent variable was used. A correlation equal to 0.74 from the estimation dataset and a value of 0.67 on an internal validation set was obtained. Based on these results it seems reasonable to assume that some information on the first phase index on IVGTT can be derived from models of the OGTT.

Methods

Research design

In this study we applied the dataset originally described in [24] where each subject underwent an oral glucose tolerance test (OGTT) (18 samples during 240 min) and a tolbutamide-modified intravenous glucose tolerance test (IVGTT) (33 samples during 180 min). The cleaned dataset applied here consisted of samples taken from 174 individuals. The condition of subjects was categorized according to the level of fasting plasma glucose (FPG) and level 2 h after glucose ingestion ($OGTT_{120}$) measured in mmol/l. The classification criteria applied, agreed with the ones described in [25] and resulted in a distribution of subjects as presented in Table 1. The study was approved by Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki.

IVGTT

All subjects underwent a 33-point tolbutamide-modified, frequently sampled IVGTT within 1 week after the OGTT examination except for a few individuals who underwent an IVGTT within 4 weeks. The trials were carried out after 12 h of

Table 1 Study population

	NGT	IFG-IGT-T2D	Total
Age (years)	42.19 (11.55)	44.45 (12.55)	42.45 (11.65)
BMI (kg $^{-2}$)	25.70 (4.238)	30.77 (4.715)	26.28 (4.577)
FPG (mmol L^{-1})	5.084 (0.436)	6.025 (0.693)	5.192 (0.558)
C_b-Ins (pmol L^{-1})	37.76 (20.98)	78.86 (55.38)	42.48 (29.98)
C_b-Cpep (pmol L^{-1})	497.3 (169.4)	725.0 (250.2)	523.5 (193.8)
Number of subjects	154	20	174

Mean and standard deviation (SD) of demographic characteristics of the studied population

fasting and samples were drawn at −30, −5 and 0 min before the IVGTT, and at 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min after glucose ingestion. Measurements were taken on serum insulin, plasma glucose and serum C-peptide. At $t = 0$, the glucose was injected intravenously. At $t = 20$ min, a bolus of 3 mg tolbutamide/(kg body-weight) was injected in 5 s.

OGTT

All subjects furthermore underwent a standardized and extended 75-g frequently sampled OGTT. After a 12-h overnight fast, venous blood samples were drawn in duplicate at −30, −10, 0 before the glucose intake and at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140, 160, 180, 210, 240 after. As for the IVGTT, serum insulin, plasma glucose, and serum C-peptide were measured.

Generally the glucose tolerance study was modelled using the glucose measured in mmol/l, the insulin in pmol/l, and the C-peptide in pmol/l.

Mathematical and statistical methods

Structural models

The structural oral glucose models applied in this study are closely related to the oral minimal model (OMM) presented in [1]. The hypothesis is that the insulin secretion is controlled by a static and a dynamic glucose component. The static component consist of a delayed version of the glucose level above a given threshold whereas the dynamic component consist of direct change in the glucose level. The static and the dynamic components are presented in the model by sr_s and sr_d respectively. Applying the model on C-peptide measurements thus leads to the following equations

$$\begin{aligned} \frac{dC_1}{dt} &= sr_s + sr_d - k_1 C_1 + k_2 C_2 - k_3 C_1 \\ \frac{dC_2}{dt} &= k_1 C_1 - k_2 C_2 \end{aligned} \tag{1}$$

in which k_1, k_2, and k_3 are the standard C-peptide kinetic parameters as calculated by Cauter et al. [26]. C_1 and C_2 are the C-peptide concentration above baseline in the central and peripheral compartments. The dynamics of the static secretion component sr_s are described by

$$\frac{dsr_s}{dt} = -\tau^{-1}(sr_s - \Phi_s[G - G_{0'}]^+) \tag{2}$$

where $[G - G_{0'}]^+$ equals $G - G_{0'}$ if $G > G_{0'}$ and 0 otherwise. After a stepwise increase of glucose, sr_s approaches a steady-state value linearly related through the parameter Φ_s, with a rate constant equal to τ^{-1} which corresponds to a delay equal

to τ (min). The dynamic secretion sr_d corresponds to the secretion of promptly releasable insulin stored in the beta-cells, and is proportional to the rate of increase of glucose through a parameter Φ_d

$$sr_d = \begin{cases} \Phi_d \frac{dG}{dt} & \text{if} \quad \frac{dG}{dt} > 0 \\ 0 & \text{Otherwise} \end{cases} \tag{3}$$

For simplicity, parameters and units are summarised in Table 2.

Compared to the minimal model presented in [1] the presented model differs in the following ways

1. The glucose threshold is not estimated but instead fixed to $G_{0'}$
2. The glucose signal ($G - G_{0'}$) is forced to be non-negative
3. The baseline secretion (sr_b) is not estimated as a parameter. Instead baseline concentration is added in the measurement equation.

All these assumptions are applied in order to gain robust population estimation for all parameters in the model.

The corresponding model applied to the insulin data is

$$\frac{dsr_s}{dt} = -\tau^{-1}(sr_s - \Phi_s[G - G_{0'}]^+) \tag{4}$$

$$\frac{dC_2}{dt} = sr_s + sr_d - k_e C_2 \tag{5}$$

where $k_e = 0.161$ [24] is the elimination constant of insulin and corresponds to $t_{1/2} \approx 4.3$ (min). Insulin concentration above baseline is presented by the state of the central compartment (C_2). The other parameters are interpreted as in the model for C-peptide as explained above. Compared to the C-peptide model which was described using three differential equations (ODEs) the insulin model can be described using only two differential equations.

SDE extension

One argument for using the SDEs compared to ODEs is that correlation between the prediction errors at time s and t can be taken into account. In this paper we have applied an Ornstein-Uhlenbeck (OU) process which can improve model performance and to a certain extent remove the correlation and "push" the model in the

Table 2 Parameter units

Symbols	Unit
sr_s, sr_d, sr_b	(pmol l^{-1} min^{-1})
Φ_s	(10^{-9} min^{-1})
Φ_d	(10^{-9})
τ	(min)
C_1, C_2	(pmol l^{-1})
k_1, k_2, k_3	(min^{-1})

Units of applied parameters and variables

direction of less correlated prediction errors. This is obtained as the OU process itself has correlation between points at time s and t and—by obtaining optimal parameter estimates—the correlation in the process will counterbalance that of the original errors thus cancelling some of the correlation already present.

The OU-process is a so-called continuous version of the AR(1)-process, as previously introduced in PK/PD modelling by Karlsson et al. [9] (See [7] for mathematical details) and is described by an SDE which evolves according to

$$dU = -\gamma U dt + \sigma_{w_1} dw_1 \tag{6}$$

where γ is the drift coefficient, σ_{w1} the diffusion coefficient, and w_1 a standard Wiener process. In general the process has the following covariance structure

$$Cov[U_s, U_t] = Var(U_t) exp(-\gamma |s - t|) \tag{7}$$

which corresponds to an exponential decaying correlation with rate constant γ, decreasing with the distance between timepoint s and t. In general correlation between prediction errors for models based on ODEs can be interpreted as either a symptom of inadequate structural model or true fluctuations in parameters. This is one of the reasons why we find it important to use SDEs in PKPD models.

Model implementation

All models were implemented as non-linear mixed-effects models in NONMEM VI using *FOCE* with interaction. Fixed effects parameters τ, Φ_s, Φ_d, and estimates for inter-subject variabilities $\eta_\tau, \eta_{\Phi_s}, \eta_{\Phi_d}$ were estimated according to the formula $\theta_i = \theta \exp(\eta_i)$ where θ represent the fixed effect parameter. In the SDE models parameter values for correlation decay rate γ and magnitude of Wiener process σ_{w1} were estimated according to the method presented by Tornøe et al. [27], and were assumed equal across subjects. A general interpretation of parameters is presented in Table 3.

Data from the OGTT and the IVGTT was treated individually such that no false correlation was introduced for the first-phase and the indices in the OGTT models. Due to skewness, the dependent variables were log-transformed thus causing an error model of the form

$$log(OBS) = log(C_{pred} + C_b) + \epsilon \tag{8}$$

Table 3 Description of parameters; interpretation of parameters

Symbols	Description
Φ_s	Static secretion index
Φ_d	Dynamic secretion index
τ	Delay between glucose input and insulin/C-peptide feedback
$\eta_{\Phi_s}, \eta_{\Phi_d}, \eta_\tau$	Inter-subject variability of Φ_s, Φ_d and τ
γ	Correlation decay rate
σ_{w_1}	Magnitude of Wiener process

where C_{pred} is the individual predicted concentration of either C-peptide or insulin above baseline, C_b is the corresponding individual baseline level, and OBS, the individual observation.

In the extended SDE models the OU-process is added, thus causing the prediction

$$log(OBS) = log(C_{pred} + C_b) + U + \epsilon \quad (9)$$

where U is the OU-process as presented in Eq. 6. Estimation in these extended models was performed in NONMEM using the extended Kalman filter with equations for mean and covariance as described in [27].

Autocorrelation function

Graphical presentations of prediction errors and their corresponding autocorrelation function (ACF) are used in validation of model assumptions. In general the ACF describes the serial correlation present in a time series calculated for different time lags and is a useful tool to identify insufficient error structure of mathematical models (See also [7]). In this paper the ACF was calculated from the vector of the prediction errors obtained by appending the errors at different timepoints from each individual thus causing a vector with $16 \cdot 174 = 2{,}784$ elements. Estimating the ACF in this way can lead to slightly underestimated correlations as it does not take into account the changes present in the transition from one subject to the other where points from the different subjects usually not are correlated. In spite of this it still provides valuable information about where the model is consistently over and under estimating at the various time points.

Covariate effects for Φ_d

In order to estimate the effects of different covariates such as BMI, Age, FPG etc. on the first-phase index, the parameter Φ_d is presented in NONMEM by the equation

$$log(\Phi_d) = log(\theta_1) + \theta_2\left(\frac{X - \mu_X}{\sigma_X}\right) + \eta \quad (10)$$

which means that θ_2 can be interpreted as the slope between a normalised covariate X and $\log(\Phi_d)$. In the results part, this correlation is compared to the correlation between X and $\log(AIR_{0\text{–}8})$ which can be used to compare the two measures of the beta-cell function.

For clarity, the implementation of different models can be read from Table 4 indicating that each model built on C-peptide and insulin measurements were implemented in five different ways, including a basic model and the four different covariate inclusions. All models were further implemented with a more complex error structure by using the OU-process as described earlier in this section. In total results were thus obtained from 20 different models.

Table 4 Correlation estimates

Model	C-peptide (ODE)	(SDE)	Insulin (ODE)	(SDE)
No covariates	0.36	0.49	0.32	0.48
BMI	0.39	0.49	0.33	0.46
AGE	0.34	0.49	0.36	0.46
FPG	0.35	0.50	0.24	0.50
C_b	0.38	0.49	0.34	0.46
Mean	(0.36)	(0.49)	(0.32)	(0.47)

Correlation (r) between $log(\Phi_d)$ and $log(AIR_{0-8})$. Last row indicates mean for the given model presented in column header

Results

Model diagnostics

Individual model fits from the C-peptide and the insulin model using ODEs and SDEs are presented for a single subject in Fig. 1. Left plots present obtained predictions from the models based on ODEs using C-peptide and insulin respectively whereas plots to the right show fits obtained from the models including the OU error term. As seen the predictions obtained using SDEs more correctly reflect the dynamics of the system compared to those obtained using the ODEs. Note that the presence of cracks in the ODE predictions is caused by the linear interpolations of predictions.

In order to further diagnose the different models, evaluation has been based also on the ACF of one step prediction errors (PE) which equals the observation subtracted by the one step prediction obtained from the Kalman filter at the given time-point. ACF values are presented in Fig. 2 for the different models not including covariates. For models based on C-peptide and insulin, it is observed that the autocorrelation between residuals is significantly smaller for SDEs than for ODEs. Notice that for dynamic models small (or zero) values of the ACF indicate that the model provides a sufficient description of the data.

Correlation between Φ_d and AIR_{0-8}

This section evaluates the ability of the OGTT models to predict the first phase response obtained from the IVGTT. The evaluation was performed through an analysis of the correlation between $log(\Phi_d)$ derived from the implemented OGTT models and $log(AIR_{0-8})$ obtained from the IVGTT. The correlations are presented in Table 4. The value 0.36 in the first row thus indicates that the correlation between the individual parameters for $log(\Phi_d)$ obtained using the C-peptide model with no covariates included, correlates with the individual estimates of $log(AIR_{0-8})$ with a coefficient of 0.36. In all cases the correlation is increased going from the ODE to the SDE model. Furthermore the difference between correlations obtained from the models using ODEs and SDEs (Δr) and corresponding SD obtained from 1,000 bootstraps were 0.130(0.059), and 0.166(0.056) for the C-peptide and insulin

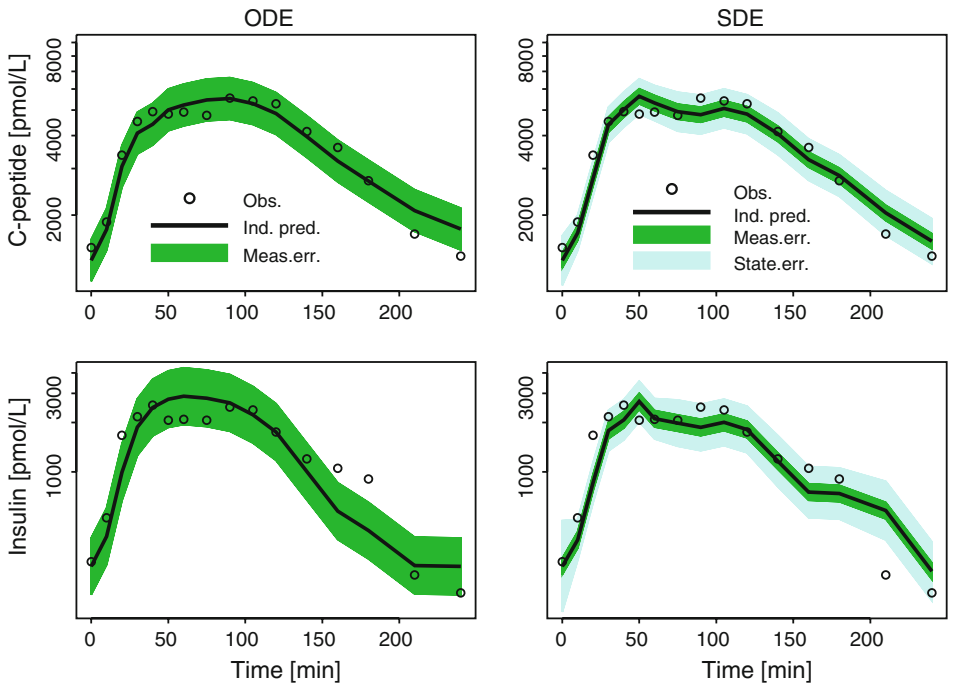

Fig. 1 Individual predictions for implemented OGTT models of C-peptide and insulin using ODEs and SDEs. *Dark* band identifies measurement error whereas *light* band identifies the state error obtained from the extended Kalman filter

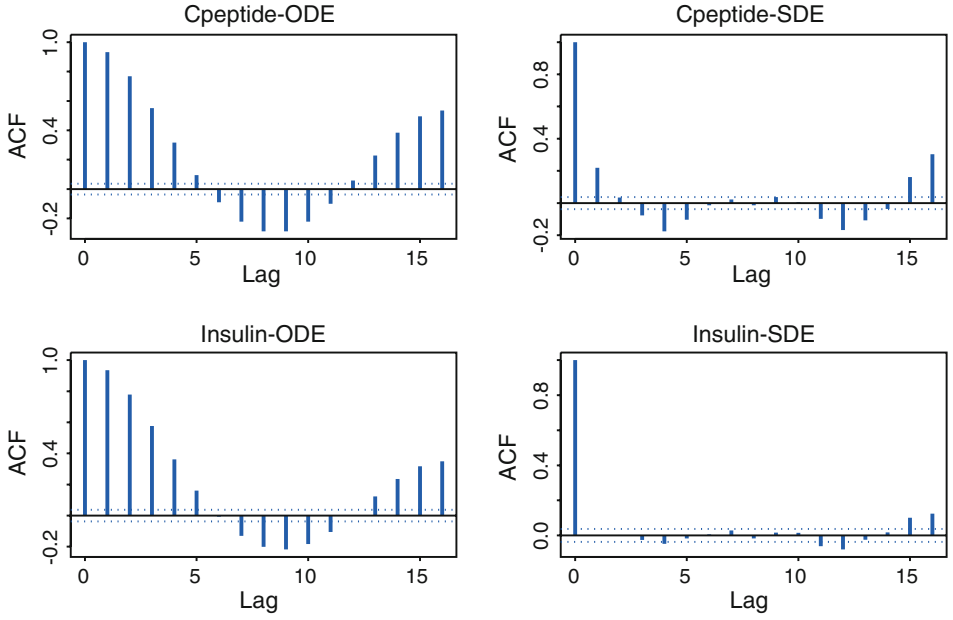

Fig. 2 Autocorrelation functions (ACFs) of one step prediction errors (PEs)

models without covariates and was concluded to be significant as $\Delta r > 2SD$. The bootstrap was performed by picking 174 random parameter estimates 1,000 times from each model. Afterwards the distributions of correlation coefficients obtained from each model were compared.

Covariate relationships for Φ_d and AIR_{0-8}

In addition to estimating the correlation between $log(\Phi_d)$ and $log(AIR_{0-8})$, the predictive performance of the models was also analysed through the ability of Φ_d to describe properties in the AIR_{0-8} as presented by relations to given covariates. This was measured through covariate relationships as presented in Table 5. Values in the second column were calculated as the slope between $log(AIR_{0-8})$ and the corresponding covariate indicated in the first column normalized according to Eq. 10. Last four columns present parameter values obtained for θ_2 (See also Eq. 10) indicating the relationship between $log(\Phi_d)$ and the given covariate. The value 0.273 in first row thus indicates that the slope between normalized BMI and $log(\Phi_d)$ in the basic C-peptide model equals 0.273. Equality between slopes obtained for the AIR_{0-8} and Φ_d indicates that the index obtained using the OGTT model has same relation as AIR_{0-8} to the given covariate. These slopes are thus applied as a measure for the similarity between model derived index and AIR_{0-8}. We believe this gives a clearer picture of the similarities between the two beta-cell function indexes compared to using only the correlation. In all cases the extended error structure based on the OU process caused the slopes for the covariates to better reflect the ones observed for the $log(AIR_{0-8})$. It shall be noted that we did not expect to get exactly equal values for $log(\Phi_d)$ and $log(AIR_{0-8})$ as the physiological interpretations of these parameters are different. Note also that a full covariate analysis on first phase indexes was determined to be out of the scope of this paper but is subject for future research.

Table 5 Covariate estimates

Index	$log(AIR_{0-8})$	$log(\Phi_d)$			
Data		C-peptide		Insulin	
Model		ODE	SDE	ODE	SDE
BMI	0.176(0.052)	0.273	0.106	0.490	0.196
AGE	−0.017(0.053)	0.030	−0.017	0.207	0.033
FPG	−0.199(0.052)	0.030	−0.105	0.177	−0.050
$I_{0'}$	0.159(0.052)	0.287	0.147	0.543	0.271

Relationship between beta-cell indices and selected covariates for C-peptide and insulin models built using ODEs and SDEs

(.) Indicate standard error (SE)

Discussion

In this paper we applied population PK/PD modelling with an error description which can take correlation of model errors between different time points into account. During early model development our approach was to use SDEs with additive system noise as done in [11]. We experienced that an inadequate description of insulin concentrations at high levels was obtained which we speculate was due to the non-state dependent inclusion of the system noise. As there is no standard solution to the problem of implementing SDEs with state-dependent system noise we instead chose to add an extra state with dynamics described by an SDE of the Ornstein-Uhlenbeck type. By adding this state to the measurement equation we obtained an error model following a continuous AR-process as presented in Karlsson et al. [9]. As shown, the solution help describe the correlation between prediction errors also at high insulin concentrations. The drawback using this approach compared to a setup with state-dependent noise entering the original system equations is that it can not be directly linked to a physiological description. However, it is a mathematical technique to better explain the correlation in the measurements compared to a standard ODE approach. The method was applied to a real data set obtained from an OGTT which enabled a study of model performance based on various measures other than those obtained from basic residual plots. These measures all relate to the predictive performance of the first-phase index Φ_d compared to AIR_{0-8}.

Today various experimental methods and modelling approaches have been developed to assess beta-cell function. Some of these include hyperglycaemic clamp [28], minimal model of C-peptide secretion during the IVGTT test [29], and homeostasis model assessment [30]. In this paper we have focused on Φ_d obtained from the oral minimal model and AIR obtained from the IV test as these measures are widely used in various applications both in research and in the pharmaceutical industry. Correlation between beta-cell function indexes calculated from IVGTT and OGTT have been performed recently in [22, 24]. In both articles a correlation around 0.67 is obtained.

In this paper we applied a fairly simple OGTT model and correlation was estimated to be around 0.4 using ODEs compared to 0.5 using SDEs. In general we believe that models using the whole time scale (as the ones presented here) seem most promising as they contain more information than the regression based models. We further believe that the models concerned here serve as a good basis, but should be extended also to include incretins as done in [24]. For a systematic improvement of the models the SDEs provide an attractive tool as shown in [10].

In the study we analysed the oral minimal model applied to either C-peptide or insulin measurements. The half-life of C-peptide is much larger than that of insulin causing a less marked first pass effect through liver. In the application of the insulin model the obtained secretion rates thus reflects post-hepatic secretion. In spite of this we found it interesting to apply a model based on insulin measurements as the higher elimination rate causes changes in secretion to produce more pronounced changes in plasma concentration compared to C-peptide.

Concerning the model development it was first attempted to implement the OMM exactly as presented in [1]. From experimental research it was observed that allowing the glucose signal to go below baseline in general caused unstable population models which caused NONMEM errors in the integration routine. Furthermore, setting the glucose threshold equal to the threshold minus the glucose baseline caused extremely low estimated values and was thus substituted by the glucose baseline. Finally, it was observed that adding the insulin baseline to the prediction instead of having baseline secretion as a parameter caused more stable models with approximately same prediction performance as for the basic OMM.

In conclusion, we showed that improved model performance can be obtained using a more complex error structure where time-correlated prediction errors are taken into account by the use of an SDE of the Ornstein-Uhlenbeck type as a part of the total state space model. Furthermore we have shown that estimation of covariate effects was highly dependent on how correlated the prediction errors are for the given model. In practice we thus recommend to check the serial correlation e.g. through an inspection of the ACF calculated for the prediction errors before concluding on the estimated covariate effects. As the SDE extension presented here not only provided less serially correlated errors, but also provided better estimation of clinical measures, the method is concluded to have high relevance not only in theory but also in practice.

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Paper B

Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following and oral glucose tolerance test

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Mechanism-based population modelling for assessment of L-cell function based on total GLP-1 response following an oral glucose tolerance test

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Abstract GLP-1 is an insulinotropic hormone that synergistically with glucose gives rise to an increased insulin response. Its secretion is increased following a meal and it is thus of interest to describe the secretion of this hormone following an oral glucose tolerance test (OGTT). The aim of this study was to build a mechanism-based population model that describes the time course of total GLP-1 and provides indices for capability of secretion in each subject. The goal was thus to model the secretion of GLP-1, and not its effect on insulin production. Single 75 g doses of glucose were administered orally to a mixed group of subjects ranging from healthy volunteers to patients with type 2 diabetes (T2D). Glucose, insulin, and total GLP-1 concentrations were measured. Prior population data analysis on measurements of glucose and insulin were performed in order to estimate the glucose absorption rate. The individual estimates of absorption rate constants were used in the model for GLP-1 secretion. Estimation of parameters was performed using the FOCE method with interaction implemented in NONMEM VI. The final transit/

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indirect-response model obtained for GLP-1 production following an OGTT included two stimulation components (fast, slow) for the zero-order production rate. The fast stimulation was estimated to be faster than the glucose absorption rate, supporting the presence of a proximal–distal loop for fast secretion from L-cells. The fast component ($st_3 = 8.64 \cdot 10^{-5}$ [mg^{-1}]) was estimated to peak around 25 min after glucose ingestion, whereas the slower component ($st_4 = 26.2 \cdot 10^{-5}$ [mg^{-1}]) was estimated to peak around 100 min. Elimination of total GLP-1 was characterised by a first-order loss. The individual values of the early phase GLP-1 secretion parameter (st_3) were correlated ($r = 0.52$) with the AUC(0–60 min.) for GLP-1. A mechanistic population model was successfully developed to describe total GLP-1 concentrations over time observed after an OGTT. The model provides indices related to different mechanisms of subject abilities to secrete GLP-1. The model provides a good basis to study influence of different demographic factors on these components, presented mainly by indices of the fast- and slow phases of GLP-1 response.

Keywords GLP-1 · L-cells · Oral glucose tolerance test (OGTT) · Indirect response model · NONMEM

Introduction

Type 2 diabetes (T2D) is a result of decreased insulin sensitivity combined with decreased beta-cell function. The beta-cell function is described by the ability of the beta-cells to provide an insulin response to a given glucose load.

One of the main determinants of beta-cell function is the presence of the insulinotropic hormone glucagon-like-peptide 1 (GLP-1) [1, 2] in combination with glucose. More specifically Brandt et al. [2] demonstrated in vivo glucose dependency of the action of postprandial physiological concentrations of GLP-1 in healthy subjects over the plasma glucose range of 5–10 mM.

GLP-1 is a gut derived peptide secreted from intestinal L-cells [3] and circulating levels increase after a meal or an oral glucose load [4, 5]. It is derived from a transcription product of the proglucagon gene and the active molecule is identified as GLP-1 (7–36). Once in the circulation it has a very short half-life estimated to be around 2–3 min in healthy volunteers [4].

The GLP-1 response in terms of area under the curve from 0 to 240 min. after the start of the meal is significantly decreased in most patients with type 2 diabetes [6]. Combined with the finding that the short half-life of GLP-1 does not seem to differ in healthy volunteers and patients with T2D [1], this suggests that the decreased GLP-1 response observed in patients with T2 diabetes is due to a lower post-prandial secretion. This also seems to be the case comparing patients with impaired glucose tolerance (IGT) and healthy volunteers [5]. In general we believe that analysis of the GLP-1 response observed after an OGTT would be valuable in understanding the mechanisms underlying the post-prandial secretion profile.

The overall aim of this study was to develop a mechanism-based population model providing descriptive indices of the observed GLP-1 secretion following an

OGTT. The goal was thus not to model the GLP-1 effect on insulin secretion, but rather to build a model providing indices for capability of GLP-1 secretion. Based on the mechanisms of action, we propose to model the stimulation of GLP-1, using an indirect response model [7]. Compared to earlier non-compartmental analysis (as in [8]) of the GLP-1 secretion profiles observed after an OGTT, a compartmental population model approach takes into account variability in measurements and time (compartmental) and variability between subjects (population). This kind of model further provides a good basis for future inclusion of covariates (such as demographic factors) on obtained model parameters.

Methods

Study participants

The data applied in this study is a subset of the dataset originally described in [9]. In this study available plasma GLP-1 profiles obtained after an oral glucose load are included. Only full profiles were included and seven profiles were removed because of erratic behaviour inconsistent with basic physiology and the dynamics of the rest of the population. The cleaned dataset applied here thus consisted of samples taken from 135 individuals distributed as presented in Table 1. The classification of individuals was categorized according to concentrations of plasma glucose (FPG) fasting and 2 h after glucose ingestion ($OGTT_{120}$) measured in mmol/L. The classification criteria, agreed with the ones described in [10]. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki.

Study conditions

All participants underwent a standardized and extended 75-g frequently sampled OGTT. After a 12-h overnight fast, venous blood samples were drawn in duplicate at −30, −10, 0 before the glucose intake and then at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140, 160, 180, 210, 240. Plasma glucose and serum insulin were measured. The plasma glucose concentration was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany). Serum insulin was determined by

Table 1 Mean and standard deviation (SD) of demographics of study subjects

Subjects	Normal	IFG-IGT-T2D	Total
Number	117	18	135
Age [yr]	41.8 (11.4)	45.6 (12.7)	42.3 (11.6)
Fasting plasma glucose [mg dl^{-1}]	93.0 (8.1)	109.8 (13)	95.3 (10.5)
Fasting plasma insulin [pmol l^{-1}]	5.43 (3.1)	11.66 (8.4)	6.26 (4.6)
Fasting plasma GLP-1(total) [pmol l^{-1}]	5.35 (3.3)	4.61 (2.6)	5.26 (3.2)

IFG Impaired fasting glucose, *IGT* Impaired glucose tolerance, *T2D* Type 2 diabetics

enzyme-linked immunoadsorbent assay with a narrow specificity excluding des (31, 32)-proinsulin and intact proinsulin (DAKO Diagnostics, Ely, UK) [11].

Fasting plasma GLP-1 were analysed in duplicate and at single measurements post glucose load at time points 10, 20, 30, 40, 60, 90, 120, 180, and 240 min. All blood samples for GLP-1 analysis were kept on ice, and the protease inhibitor aprotinin (Novo Nordisk, Denmark) was added in a concentration of 0.08 mg/ml blood. The GLP-1 concentrations were measured after extraction of plasma with 70% ethanol (vol/vol). The plasma concentrations of GLP-1 were measured [12] using standards of synthetic GLP-1 7–36 amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 derived from the intestine. The results of the assay reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9–36 amide, into which GLP-1 is rapidly converted [13]. The assay sensitivity was below 1 pmol/l, intra-assay coefficient of variation below 0.06 at 20 pmol/l, and recovery of standard added to plasma before extraction was 100% when corrected for losses inherent in the plasma extraction procedure. Very few samples were under the LLOQ, and these were not included in analysis.

Non-compartmental analysis

The individual incremental areas under the curve for GLP-1 were calculated using a linear up/linear down trapezoidal method. Peak AUCs identified in the report as $AUCP_{GLP\text{-}1}$ were calculated as incremental AUCs up to 60 min. The software S-plus was used for this part of the analysis.

Compartmental population modelling

For preliminary analysis, the absorption rate constant (k_a) of glucose was obtained from glucose and insulin data by applying the model presented by Lima et al. [14], using two compartments for description of absorption rate according to Eq. (3) and (4). This was done in order not to bias the estimation of this parameter towards the fitting of GLP-1.

Baseline GLP-1 values were calculated as the average from pre-dose samples for each individual. Considering the fact that the inclusion of these baseline values as either fixed or estimated can influence the bias of other parameters [15], we implemented these values as either fixed, fixed with a variance, or estimated. In general the GLP-1 data was modelled using a population model build in NONMEM VI using the FOCE Inter method. Model selection was based on individual/population predicted profiles, variance and independence of residuals, and obtained objective function value (OFV), and inspection of visual predictive check (VPC).

Structural model

The final structural models for glucose/insulin and secretion of GLP-1 are presented in Fig. 1. The glucose/insulin model was applied in order to obtain estimates of glucose absorption rate. The model for the GLP-1 secretion reflects an indirect

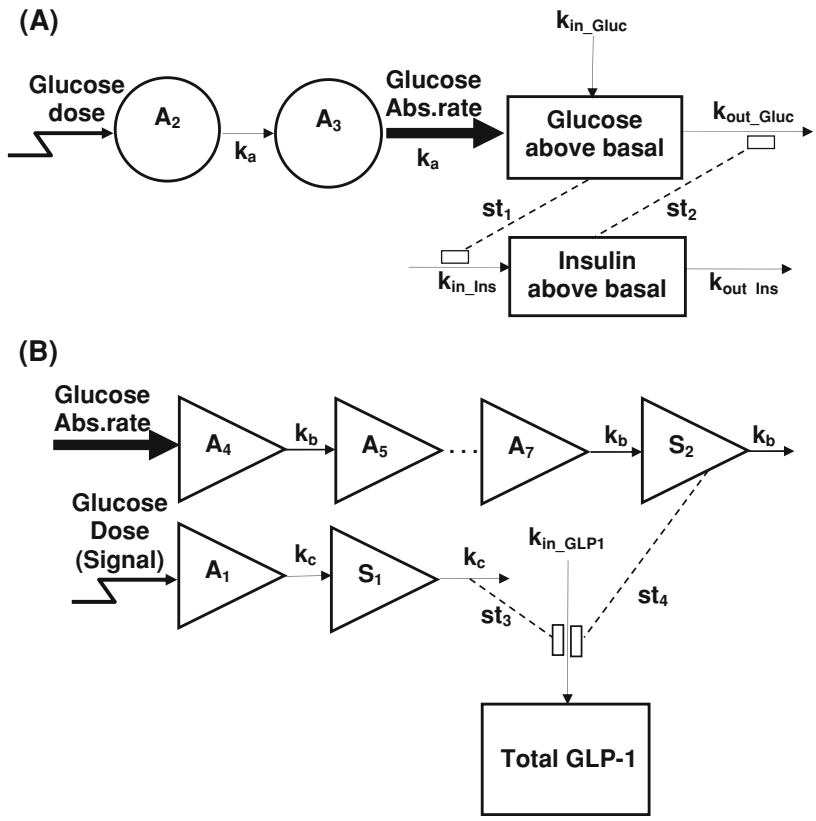

Fig. 1 **a** Diagram of glucose/insulin model for estimation of glucose absorption rate constant, **b** GLP-1 secretion model. Absorption rate for glucose is identical to that estimated in the glucose/insulin model. Symbols are defined in Table 2

response model with zero-order input and first-order loss. The zero-order input was found to be stimulated by two mechanisms differentiated by time of onset. The first part was estimated to be faster than the absorption of glucose and caused a peak in the GLP-1 concentration around 40 min as also identified in [16]. The ingestion signal was included as being proportional to the glucose dose size as:

$$\frac{dA_1}{dt} = -k_c \cdot A_1, \quad A_1(0) = Dose \tag{1}$$

$$\frac{dS_1}{dt} = k_c \cdot A_1 - k_c \cdot S_1, \quad S_1(0) = 0 \tag{2}$$

where $1/k_c$ [min] determines the length of the signal caused by the intake of the amount of glucose, defined by *D*ose. The A_1 and S_1 define the first and second transit compartments in the early response signal originating from ingestion of glucose. The second part was related to a delayed version of the absorption of glucose in gut. The delay was implemented with the use of transit compartments.

The optimal number of transit compartments for description of the delay was determined based on an explicit solution [17] together with the obtained OF Vs.

From the obtained number of compartments and rate constants, this signal was identified to peak around 100 min. The equations below define the glucose absorption rate ($k_a \cdot A_3$) and the stimulus of GLP-1 production related to the absorption rate (S_2).

$$\frac{dA_2}{dt} = -k_a \cdot A_2, \quad A_2(0) = Dose \cdot f \tag{3}$$

$$\frac{dA_3}{dt} = k_a \cdot A_2 - k_a \cdot A_3, \quad A_3(0) = 0 \tag{4}$$

$$\frac{dA_4}{dt} = k_a \cdot A_3 - k_b \cdot A_4, \quad A_4(0) = 0 \tag{5}$$

$$\frac{dA_5}{dt} = k_b \cdot A_4 - k_b \cdot A_5, \quad A_5(0) = 0 \tag{6}$$

$$\vdots$$

$$\frac{dS_2}{dt} = k_b \cdot A_6 - k_b \cdot S_2, \quad S_2(0) = 0 \tag{7}$$

The value of f was fixed to 0.722 based on the bioavailability of glucose observed from an OGTT in healthy subjects [18].

Specifically A_2 presents the glucose at absorption site, and $k_a \cdot A_3$ the glucose absorption rate as stated above. The absorption rate constant k_a was estimated using the compartment absorption structure of glucose (A_2 and A_3) connected to an indirect response model for the interaction between glucose and insulin [14], see Fig. 1. The rate constant k_b defines the delay between glucose absorption rate and stimulation of late-phase GLP-1 secretion. The S_2 thus defines the signal related to stimulation of GLP-1 production by glucose absorption.

The elimination of GLP-1 was implemented as a first-order process. In total, the concentration of total GLP-1 following the OGTT is described by

$$\begin{aligned} &\frac{dC_{GLP1}}{dt} = k_{in\_GLP1} \cdot [1 + st_3 \cdot S_1 + st_4 \cdot S_2] - k_{out\_GLP1} \cdot C_{GLP1}, \\ &C_{GLP1}(0) = B_{GLP1} \end{aligned} \tag{8}$$

where k_{in_GLP1} (pmol $l^{-1} \cdot min^{-1}$) is the endogenous production rate of GLP-1 and k_{out_GLP1} (min^{-1}) the first-order rate constant of GLP-1 elimination with the steady-state condition defined by

$$k_{in\_GLP1} = B_{GLP1} \cdot k_{out\_GLP1} \tag{9}$$

where B_{GLP1} is the baseline level of GLP-1. The parameters st_3 and st_4 present first- and second-phase stimulation factors related to the first- and second phase stimulation signals (S_1 and S_2).

Individual model

Inclusion of Inter-individual variability (IIV) was done according to a log-normal distribution of individual parameters. The IIV was included for all estimated

parameters except k_{out_GLP1} which is experimentally found not to vary significantly between subjects [1]. Due to a high correlation, the same random effect was used for st_3 and st_4, and these were estimated according to

$$st_3 = \theta_1 \cdot \exp(\eta_1) \tag{10}$$

$$st_4 = \theta_2 \cdot \exp(\kappa \cdot \eta_1) \tag{11}$$

where θ_1 is the typical value of st_3 and η_1 the random effects parameter related to the inter-subject variability of st_3 and similar for st_4. Note that the inter-variability between the individual estimates of st_4 is proportional to the inter-variability of the st_3 estimates using the constant κ.

Residual error model

Additive, proportional, and combined error models were tested. The combined error model appeared superior.

Results

Four individual GLP-1 concentration versus time profiles together with model predictions are shown in Fig. 2. High variability in the profiles is present both for the baseline and in the dynamics of the GLP-1 hormone.

Figure 3 presents population predictions together with individual observations and their mean. Figure 4 presents the autocorrelation function (ACF) of residuals [19]. A visual predictive check (VPC) of the model is presented in Fig. 5. These figures indicate that the model seems to adequately capture the main GLP-1 dynamics measured in the studied population. There is no need to implement the presented model using stochastic differential equations (SDEs). This is augmented by the fact that for all lags >0 there is small correlation and only the correlation at lag $= 2$ (corresponding to the correlation between residuals shifted two time-points) is significant (See Fig. 4).

Interpretation, estimated values, and inter-individual variability (IIV) of each parameter is presented in Table 2. For each parameter estimated with IIV we have also reported the η-shrinkages (*shr*) as these measures are of importance e.g. for a study incorporating covariate effects [20]. The shrinkage on the residual error was found to be very small.

In order to check that our model was consistent with NCA-analysis, we plotted the fast stimulation index st_3 versus $\text{AUCP}_{\text{GLP-1}}$ which has been used previously [21] to measure the size of the fast response (see Fig. 6). A significant correlation between the two measures ($r = 0.52$) was obtained.

Figure 7 presents the time course of mean signals related to the fast and the slow GLP-1 responses (simulation of compartment S_1 and S_2 above using the estimated typical values of k_a, k_b and k_c) together with the mean of simulated A_3, the presenting compartment related to glucose absorption rate. For the fast response a

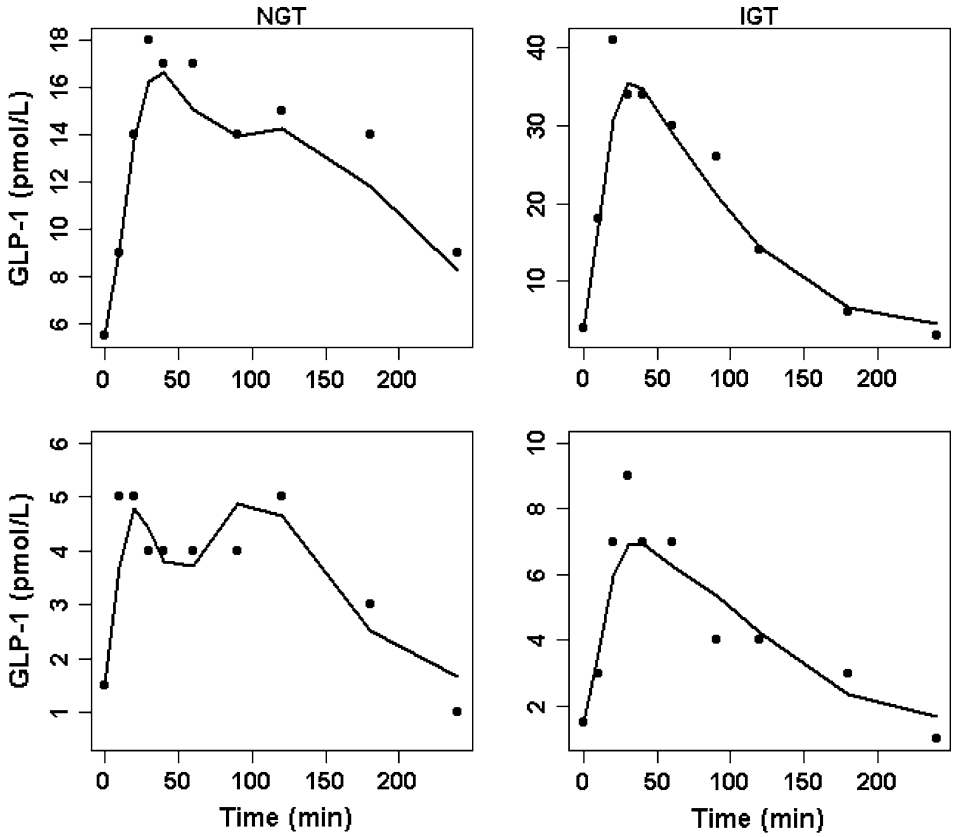

Fig. 2 Measurements and individual predictions of total GLP-1 versus time after the oral dose of glucose. *Left*: Normal glucose tolerant subjects (NGT). *Right*: Impaired glucose tolerant subjects (IGT)

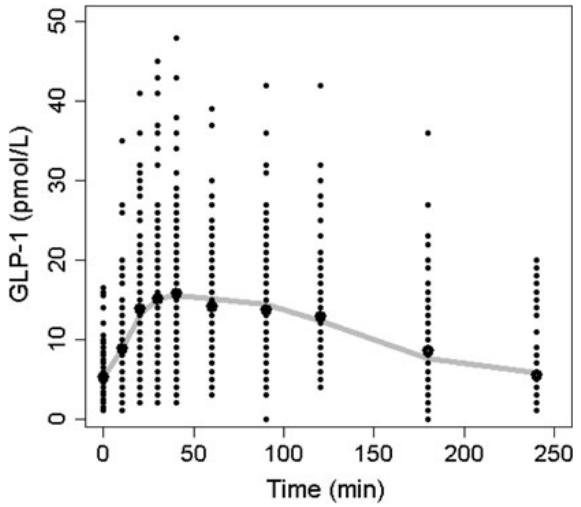

Fig. 3 Comparison between mean DV and population prediction. *Black small dots*: plasma concentrations of GLP-1 versus time for all subjects. *Large dots*: mean observed GLP-1 concentrations. *Gray curve*: population prediction

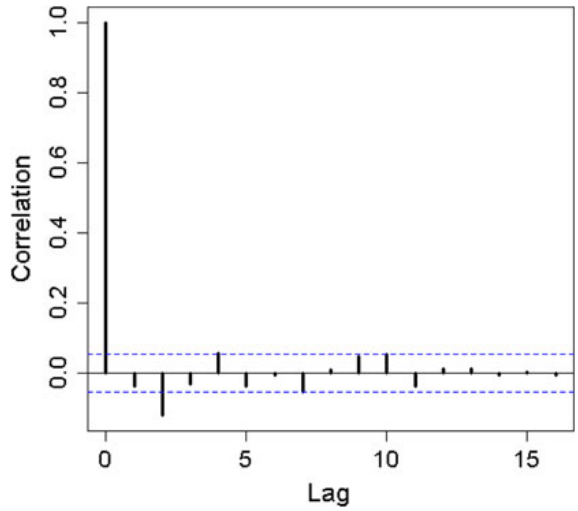

Fig. 4 Autocorrelation function (ACF) calculated based on appended residuals from each subject

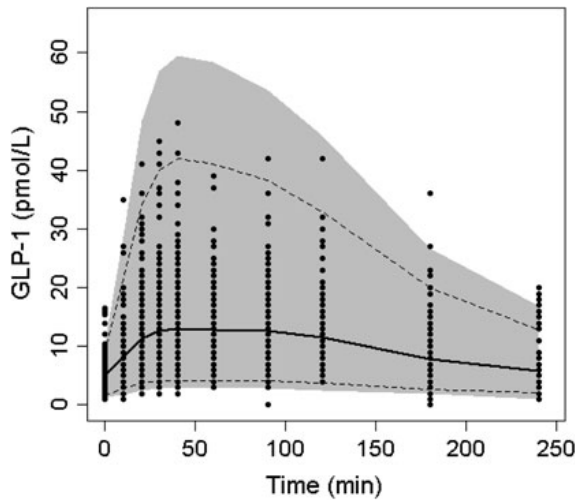

Fig. 5 Visual predictive check (VPC) of GLP-1 measurements versus time. Shaded area defines the 5–95th percentiles of predictions and *dotted line*, the 10–90th percentiles for predictions. *Full line* presents median of prediction, whereas *dots* represent data points

peak around 25 min is observed, whereas the slow response peaked around 100 min.

Discussion

In this study we modelled the sum of intact GLP-1 and the primary metabolite, GLP-1 (9–36), into which GLP-1 is rapidly converted. This sum therefore reflects the rate of secretion of GLP-1. The obtained total GLP-1 concentrations following the OGTT could be described by an indirect response model with zero-order production rate and first-order loss. Stimulation of GLP-1 production by glucose was characterized with a fast stimulation signal and a signal related to a delayed version of the absorption rate of glucose. Elimination was characterized by a non-saturable

Table 2 Obtained parameter estimates for GLP-1 dynamics

Parameter	Interpretation	Value	SEM (%)	IIV (CV%)	Shr (%)
f (−)	*Absorption fraction*	*0.722*	–	–	–
k_a (min^{-1})	Abs. rate constant	0.0359	2	0.0581(24)	5
k_b (min^{-1})	Transit rate constant	0.0962	8	0.0357(12)	20
k_c (min^{-1})	Neural signal rate constant	0.0566	11	0.270(52)	20
k_{out_GLP1} (min^{-1})	First-order elimination rate constant of GLP-1	0.0644	18	0 FIXED	–
κ [−]	Proportionality between IIV on st_3 and st_4	0.775	10	0 FIXED	–
st_3 [mg^{-1}]	Stimulation factor of GLP-1 production by early signal	$8.64 \cdot 10^{-5}$	10	0.939(97)	6
st_4 [mg^{-1}]	Stimulation factor of GLP-1 production by late signal	$26.2 \cdot 10^{-5}$	3	–	–
SD_{glp} [pmol l^{-1}]	Additive error	0.998	5	–	–
CV_{glp} (%) [pmol l^{-1}]	Proportional error	9	–	–	–

The f is obtained from Ref. [18] and k_a is estimated from glucose/insulin data

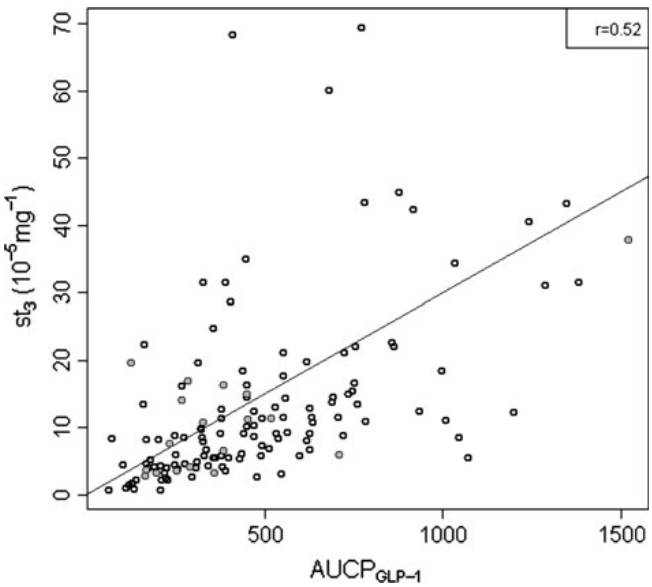

Fig. 6 Individual predictions of parameter st_3 versus $AUCP_{GLP\text{-}1}$ calculated as AUC from 0–60 min. above baseline values. *Open circles*: NGTs, *Gray filled circles*: IFG-IGT-T2Ds. *Line* presents relation: $st_3 = 0.03\ AUCP_{GLP\text{-}1}$, obtained using perpendicular least squares

elimination pathway. The model for glucose/insulin was estimated separately from the GLP-1 secretion model. This was done in order not to bias the estimation of glucose absorption towards the prediction of GLP-1 concentrations. Besides, the

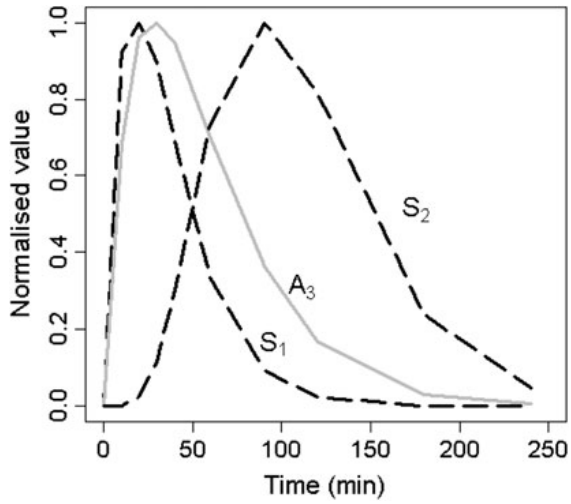

Fig. 7 Normalized mean of simulations of compartments S_1, A_3 and S_2 versus time

simultaneous estimation was very time-consuming causing separate estimation to be preferred.

The GLP-1 secretion model was successfully applied to a mixed-effects model setting using NONMEM VI, thus providing both information about intra-variability and inter-variability in the studied population.

To our knowledge compartmental modelling of GLP-1 secretion following an OGTT has not been performed previously. As observed from our individual profiles there is very high variability between subjects and the response is considered complex which relates to the fact that determinants of the secretion are not fully understood. Based on this we initially started out using a simple indirect response model using one stimulus related solely to the glucose absorption rate. This stimulus was not adequate to describe the GLP-1 secretion and we observed that two phases of secretion could be identified. Based on the estimation of the rate constants k_b and k_c, the peaks of these stimuli were observed to be around 25 and 100 min. This seems to be consistent with the GLP-1 profiles following a mixed meal [21] indicating maximum GLP-1 concentrations shortly after the peak stimulation times.

The fast response (peak around 25 min.) is hypothesized to be caused mainly by nutrients in the duodenum activating a proximal—distal neuroendocrine loop stimulating GLP-1 secretion from L-cells and colon (3). In our study we estimated the rate constant (k_c) related to the first-phase to be significantly faster than the rate constant (k_a) related to glucose absorption. This provides evidence for the possibility of the neuroendocrine regulation of L-cell secretion (3), although more insights could be gained from further experiments.

In this study we chose not to perform covariate analysis on the individual parameters for secretion, and did thus also not analyse the effect of disease state on the obtained estimates. Such an analysis belongs to another study, and must be performed with data that has more subjects identified with T2D.

The developed model should be seen as a tool that in future can be applied to investigate factors such as disease state, drug effect, or ethnicity on the parameters characterizing GLP-1 secretion.

Estimating the rate of absorption of glucose without the use of tracer has been a subject in various publications [18, 22]. We applied a simple approach using only one parameter without information from tracer kinetics.

In order to investigate the dependence of our approach on different glucose absorption models, we implemented two alternative models [18, 22]. The objective function values, population fittings and correlation obtained between st_3 and $AUCP_{GLP\text{-}1}$ appeared similar to the present results. A clear drawback of our study is that the absorption rate for glucose is not necessarily captured with high accuracy. It will be of future interest to see how the model performs knowing the rate of absorption obtained with a tracer [22, 23].

Another limitation of this study is that only one dose level of the OGTT was administered. Possible non-linearities in the GLP-1 response are thus unidentifiable. For further model development it would be informative to repeat the experiments performed in this analysis with different glucose doses.

Regarding the number of transit compartments one could argue that the possibility of having different individual numbers would be reasonable. This was tested using an explicit solution [17], but was found to cause the model not to be uniquely identifiable thus causing unstable estimation of parameters. Instead we chose to have IIV on k_b, thus enabling individual differences in time of onset of S_2. In spite of the fact that IIV was only 12% in k_b values, we observed significantly higher OFV and a worse model fit. That was the reason for having k_b not fixed to 0.

The value of k_{out_GLP1} indicates a half-life of total GLP-1 of around 10 min. This agrees with values in the range of 3–11 min obtained experimentally in vivo [13], although it seems to be slightly higher than values obtained for active GLP-1 (7–36), specifically measured in humans [4, 24, 25]. As Holst et al. [16] describe, there are different types of GLP-1 and in this study the measured concentration reflects the sum of the active GLP-1 (7–36) and the inactive form, GLP-1 (9–37). The inactive form has a much longer half-life [16] which will be the main determinant for the half-life. In general it is important to note that the degradation of GLP-1 (7–36) is known to be fairly complex and involves both an inactivation in the gut and degradation in liver which is not taken into account here. It would thus be of future interest to build a more complex model based on data obtained in different tissues and from the different metabolites.

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Paper C

Pathophysiology of Type 2 diabetes in Japanese versus Caucasians: A Direct Comparative Study

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Submitted

Pathophysiology of Type 2 Diabetes in Japanese versus Caucasians: A Direct Comparative Study

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BACKGROUND Lifestyle and ethnicity are known determinants for the occurrence of type 2 diabetes (T2D). Previous studies have indicated differences in the pathogenesis between Japanese and Caucasian individuals.

METHODS We conducted a cross-sectional, clinical study in Denmark and Japan. 150 Caucasian and 120 Japanese males and females aged 40 to 65 years were enrolled to obtain a comparable distribution of high/low body mass index (BMI) values across glucose tolerance

states (normal [NGT], impaired [IGT], or T2D). Participants underwent oral glucose tolerance tests according to WHO guidelines, and were classified as having low/high BMI according to regional definitions of obesity.

RESULTS Mean glucose profiles were similar in the two ethnic cohorts, whereas in Japanese the insulin response was smaller, with substantially lower insulin profiles in subjects with IGT and T2D compared to Caucasians. Insulin sensitivity was higher in Japanese as indicated by the HOMA-IR and Matsuda indices, whereas beta-cell function appeared lower in Japanese as measured by the HOMA-B and insulinogenic indices, as well as the insulin secretion ratio. The major part of these differences in insulin sensitivity and beta-cell function were explained by differences in BMI. The measure of beta-cell function relative to insulin resistance – the disposition index – was similar for the two ethnic cohorts at all glucose tolerance states.

CONCLUSIONS We confirm the existence of differences between Japanese and Caucasians in beta-cell function and insulin sensitivity, and further demonstrate that the major part of these differences can be explained by differences in body size (BMI).

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INTRODUCTION

Type 2 diabetes (T2D) poses a global health problem approaching epidemic proportions. The total number of diabetes patients, estimated at 366 million in 2011, is expected to rise to an alarming level of 552 million by 2030[1]; this is mainly caused by increasing incidence in countries with large shifts in lifestyle due to urbanization, as is currently the case in many Asian countries.[2, 3]

Lifestyle factors as well as ethnicity are known determinants for the development of T2D.[4] The importance of lifestyle factors has been shown by a higher incidence of T2D for Japanese-Americans compared to native Japanese, mainly caused by higher fat intake and less physical activity following adaptation to a Western lifestyle.[5]

T2D is characterized by insulin resistance and beta-cell failure.[6] It is thought to be triggered by insulin resistance, which is compensated initially by increased beta-cell function, leading eventually to T2D due to exhaustion of the pancreas.[6-8]

According to current understanding, the pathophysiology of T2D is different in Japanese compared to Caucasians, in the sense that Japanese are unable to compensate insulin resistance with increased insulin secretion to the same extent as Caucasians. Pre-diabetes and early-stage diabetes in Japanese are characterized by a reduced beta-cell function combined with a lower degree of insulin resistance compared to Caucasians.[9-12] In a prospective, cross-sectional study of individuals with normal and impaired glucose tolerance (NGT and IGT), it was demonstrated that Japanese people living in Japan were more insulin-sensitive than Mexican-Americans living in the United States and Arabs living in Israel.[13] The three ethnic groups also differed with regard to beta-cell function, but the disposition index – a measure of insulin secretion relative to insulin resistance – was similar across ethnicities for NGT and IGT participants. In summary, these studies suggest that profound ethnic differences exist

with regard to the pathophysiology of T2D. However, few attempts have been made to establish to what extent these differences are caused by factors other than ethnicity.

These perceived ethnic differences in the pathophysiology of T2D may influence treatment in clinical practice. For instance, T2D patients in Japan have traditionally been considered more insulin-sensitive compared to their counterparts in Western countries, and sulphonylureas have been the most widely used antidiabetic drug in Japan.[14] This is in contrast to general diabetes treatment practices in other countries, where primarily insulin sensitizers such as metformin are being used as first-line medication.

The present study represents the first prospective, cross-sectional study in well-characterized Japanese and Caucasian individuals with the following aims: 1) to characterize insulin sensitivity and beta-cell function at various glucose tolerance states, and 2) to investigate the role of factors such as body size as underlying predictors for possible ethnic differences in insulin sensitivity and beta-cell function. Individuals with NGT, IGT and T2D were enrolled in the study and a comparable distribution of individuals with high and low BMI values was secured in each subgroup of glucose tolerance state. This allowed us to identify the relative influence of ethnicity and body size on insulin sensitivity and beta-cell function. Here we present the key results of the study, focusing on the role of BMI, a known risk factor for the occurrence of T2D.[15]

METHODS

Study Design

The study investigated 150 Caucasian participants (Northern European background for at least three generations) enrolled at Copenhagen University Hospital, Denmark, and 120 Japanese participants (Japanese background for at least three generations) enrolled at Tokyo University Hospital, Japan. Potential participants (males and females aged 40 to 65

years) were screened to exclude individuals with metabolic conditions other than T2D. Other key exclusion criteria were: treatment with insulin, recent or ongoing infection, history of malignant disease, or use of thiazolidinedione-based medications within three months. Participation also required normal results from the physical examination, blood screening, electrocardiogram, urinalysis, and a stable body weight (±10%) for the past year. Participants were assigned into groups of either low or high BMI, respectively. A low BMI was defined for Japanese as <25 kg/m^2 and for Caucasians <30 kg/m^2, in accordance with regional obesity definitions.[16, 17] The participants were classified as having either NGT, IGT, or T2D[18] on the basis of blood glucose levels while fasting and at 2 hours during the oral glucose tolerance test (OGTT). The study was powered at 80% to detect a 50% difference between Japanese and Caucasians in insulin response 30 minutes after oral glucose intake.

The study protocol was approved by the Regional Committee on Biomedical Research Ethics in Denmark (Journal no. H-C-2008-101) and by the Research Ethics Committee, Graduate School of Medicine, University of Tokyo, Japan. Informed consent was obtained from all participants, and the handling of data was approved by the Danish Data Protection Agency.

Procedures

At screening (Visit 1), participants reported to the clinic in the morning after an overnight fast. They were evaluated according to the exclusion and inclusion criteria, and demographic characteristics were assessed. Female participants were scheduled to visit within the 14th day ±4 days of their menstruation cycle. Concomitant medication, physical conditions including body measurements, and vital signs were recorded, and blood samples were collected for hematological and biochemical assessment. For the Japanese cohort, an indicatory OGTT was conducted to assist with the recruitment of participants into glucose tolerance subgroups.

For all participants, an OGTT was performed at Visit 2 with an oral bolus corresponding to 75 g dissolved glucose. Plasma samples for measurement of glucose and insulin concentrations were collected at times -30, 0, 10, 20, 30, 60, 90, 120, 150, 180, 240, and 300 minutes relative to the time of glucose ingestion. All samples were stored frozen pending analysis at a central laboratory in Denmark.

Assessment of Insulin Sensitivity and Beta-cell Function

In order to assess insulin action both while fasting and following glucose challenge, we calculated the homeostatic model assessment insulin resistance (HOMA-IR) index[19] and the Matsuda index.[20] The HOMA-IR is based on fasting-state glucose and insulin concentrations, whereas the Matsuda index employs glucose and insulin concentrations up to 120 minutes during the OGTT. Beta-cell function was evaluated using three indices: HOMA-B,[19] which is based on fasting-state glucose and insulin concentrations; the insulinogenic index,[21] which is based on insulin and glucose concentrations at fasting and 30 minutes after glucose challenge; and the insulin secretion ratio, which is based on insulin and glucose concentrations between 0 and 120 minutes following glucose intake.[22] The disposition index was calculated as the product of the insulinogenic index and the Matsuda index,[23, 24] as well as the product of the insulin secretion ratio and the Matsuda index.[22]

Statistical Analysis

For each measure of insulin sensitivity and beta-cell function, an ANOVA was conducted on log-transformed endpoints with glucose tolerance state, ethnicity, the interaction between the two, with and without BMI, all as fixed effects. No correction for multiple testing was made. Spearman's rank correlation coefficients between BMI and metabolic measures were calculated using log-transformed values of insulin sensitivity and beta-cell function. For the plots, endpoints were adjusted to correspond to the median BMI value for the entire

population in order to illustrate the predictive value of BMI for ethnic differences in insulin resistance and beta-cell function. Adjustments of the endpoints were based on coefficients estimated under the ANOVA model with BMI included.

RESULTS

The baseline characteristics of the study participants (Table 1) showed a similar demographic distribution across the two ethnic cohorts, except for measures of body size (height, weight, and BMI), which, in accordance with the study design, showed lower values in Japanese compared to Caucasians. Consequently, mean BMI values for Caucasians were substantially higher than for Japanese participants.

Similar mean glucose profiles in NGTs, IGTs, and T2Ds were observed for the two ethnic cohorts, with regards to both magnitudes and profile shapes (Fig. 1). In contrast, the insulin responses appeared lower in Japanese compared to Caucasians at all glucose tolerance states.

We calculated measures of insulin resistance (HOMA-IR and Matsuda index) and beta-cell function (HOMA-B, insulinogenic index, and insulin secretion ratio) at each glucose tolerance state (Fig. 2A–2E). The pattern of change in insulin resistance (HOMA-IR) and Matsuda index from NGT to IGT and further to T2D was similar in the two ethnic cohorts (Fig. 2A–2B). A large increase in insulin resistance was apparent in Caucasians in the transition from IGT to T2D, whereas the corresponding increase in Japanese was less pronounced (Fig. 2A). Marked decreases in insulin sensitivity (Matsuda index) were observed from NGT to T2D for both ethnic cohorts. The mean insulin sensitivity was significantly higher in Japanese individuals at all glucose tolerance states (Fig. 2A–2B).

Measures of beta-cell function at fasting state (HOMA-B) also showed a similar pattern of change from NGT to T2D for Japanese and Caucasians, but with overall lower mean values in Japanese individuals. The HOMA-B appeared similar in NGT and IGT, and to

decrease from IGT to T2D in both Japanese and Caucasians. The insulinogenic index and the insulin secretion ratio appeared to decline progressively from NGT to T2D in both ethnicities. Although mean values of the insulinogenic index were numerically higher in Caucasians at all glucose tolerance states, statistical significance between the two ethnicities was present in the T2D group only (Fig. 2D). Likewise, the insulin secretion ratio appeared higher in Caucasians reaching statistical significance in the IGT and T2D groups (Fig. 2E).

In order to establish the importance of BMI for ethnic differences, we further investigated the relationship between measures of BMI on one hand, and insulin sensitivity and beta-cell function on the other. Significant correlations were observed between BMI and insulin sensitivity as well as beta-cell function (Fig. 3). The inherent difference in BMI in the two cohorts (Table 1) related to the study design led us to calculate BMI-adjusted endpoints (Fig. 2F–2J). For all five indices, the differences between Caucasians and Japanese were no longer statistically significant after accounting for BMI in either the NGT or the IGT group. Caucasians with T2D were still found to have significantly higher beta-cell function (HOMA-B, $P<0.05$, Fig 2H)) and insulin resistance in the fasting state (HOMA-IR, $P<0.01$, Fig 2F) compared to the Japanese with T2D. The Matsuda index remained lower in Caucasians compared to the Japanese counterparts after accounting for BMI ($P<0.01$).

To obtain a measure of the beta-cell function relative to insulin resistance, the disposition index was calculated for the NGT, IGT, and T2D groups using the insulinogenic index as well as the insulin secretion ratio for beta-cell function and the Matsuda index for insulin sensitivity (Fig. 4A–4B). No statistically significant differences between Japanese and Caucasians were found for any of the glucose tolerance states.

DISCUSSION

This study was conducted to investigate 1) whether Caucasians and Japanese have similar beta-cell function and insulin sensitivity across glucose tolerance states, and 2) whether differences found can be explained by lifestyle-related or demographic factors, with an emphasis on the role of BMI. Other risk factors were also evaluated in the study (please refer to the supplemental material), but the measures associated with the degree of obesity turned out to be the most important. Of these, we focused on the role of BMI, which was a design parameter in the study and correlated highly with other measures of body size such as body weight and waist circumference.

In contrast to previous studies investigating pathophysiological differences in T2D between Caucasians and Japanese, this study assigned participants into two groups of low and high BMI, for each state of glucose tolerance (NGT, IGT, or T2D). This assignment enabled us to assess the importance of body size for beta-cell function and insulin sensitivity in each glucose tolerance subgroup.

The results obtained from the OGTTs showed similar mean glucose profiles in Japanese and Caucasians, whereas insulin responses were substantially lower in Japanese participants compared to Caucasians. In line with these observations, measurements of beta-cell function were generally lower in Japanese, who simultaneously had higher insulin sensitivity. The major part of differences in insulin sensitivity and beta-cell function between the two ethnic cohorts could be explained by the different BMI distributions, with higher mean BMI in Caucasians. After accounting for BMI, the differences were no longer statistically significant, with the exception of the T2D group for which values of HOMA-IR and HOMA-B remained significantly higher in Caucasians, whereas the Matsuda index remained higher in Japanese. This could be caused by a difference in disease state within the T2D group, being more advanced in the Caucasian participants as reflected by the higher

mean fasting plasma glucose, or by differences not explained by BMI, such as their genetic disposition.

Assessments of insulin sensitivity and beta-cell function at various glucose tolerance states have previously been performed in both Caucasians and Japanese. One of the largest studies performed in Caucasians is the Botnia study published in 2000, which included 5,396 Caucasians ranging from NGT to T2D.[11] In that study, the mean insulin response following an OGTT was observed to increase from NGT to IGT and to decrease from IGT to T2D. However, when the insulin response was evaluated relative to the glucose concentration, there was a significant decline both from NGT to IGT and from IGT to T2D. In addition, the HOMA-IR increased from NGT to T2D, which is in agreement with the results in the present study.

The results from the Botnia study have been compared with a similar study performed in Japanese.[9] This comparison indicated differential insulin profiles, with Japanese IGTs having a lower mean response compared to Japanese NGTs. This finding was not supported by Tanaka et al. or Kanauchi, who reported an increased mean insulin response in Japanese in the transition from NGT to IGT.[25, 26] The present study provides further support for an increase in insulin response in Caucasians as well as in Japanese, in the transition from NGT to IGT.

With regard to the magnitude of insulin responses, our results support the presence of differences between Caucasians and Japanese, as the responses appeared lower in Japanese compared to Caucasians at all glucose tolerance states. Our study thus confirms the previous finding that Japanese are characterized by a lower degree of insulin resistance and a more pronounced beta-cell dysfunction compared to Caucasians, regardless of glucose tolerance state.[27]

Despite a lower insulin response in Japanese individuals, Japanese and Caucasians had similar disposition indices, i.e. lower insulin secretion for a given degree of insulin resistance, throughout the range of glucose tolerance. This finding indicates a similar capacity for beta-cell compensation, which is also reflected in the similar glucose profiles for NGT, IGT and T2D participants.

The observed relationship between insulin sensitivity and BMI is well established in both Japanese and Caucasians,[28-30] confirming that obesity is a risk factor of T2D.[31, 32] The negative correlation between the Matsuda index and BMI in Caucasians reported by Clausen and collaborators[33] is in line with this relationship. In a large Japanese cohort of NGT participants, the HOMA-IR increased with BMI, whereas the Matsuda index decreased with increasing BMI,[30] which is in agreement with our results.

The strong correlation between BMI and beta-cell function in the present study is most likely driven by decreasing insulin sensitivity with increasing BMI, and therefore a compensatory need for more insulin. The inverse relationship between insulin sensitivity and beta-cell function reflects the compensatory nature of the beta-cell function in response to insulin resistance.[34]

In summary, our study confirms the existence of differences in insulin sensitivity and beta-cell function between Japanese and Caucasians, and shows for the first time that a major part of these differences can be explained by differences in BMI. Our results provide evidence for a common pathogenesis of T2D in Japanese and Caucasians and may potentially have an impact on future treatment recommendations for the two populations.

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Table 1. Baseline Characteristics of the Participants.

	Caucasians			Japanese		
	NGT	IGT	T2D	NGT	IGT	T2D
Total	63	39	48	46	26	48
Low BMI[a]	32 (51%)	14 (36%)	24 (50%)	25 (54%)	12 (46%)	27 (56%)
Age — yr	53 (7)	54 (8)	57 (7)	49 (7)	54 (8)	57 (7)
Sex — male	29 (46%)	15 (38%)	29 (60%)	21 (46%)	12 (46%)	33 (69%)
Height — m	1.7 (0.1)	1.7 (0.1)	1.8 (0.1)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)
BMI — kg/m^2	29.8 (5.9)	33.0 (6.3)	30.4 (5.7)	24.0 (3.2)	26.3 (5.0)	25.3 (4.4)
Waist-to-hip ratio	0.91 (0.09)	0.94 (0.08)	0.97 (0.08)	0.93 (0.05)	0.94 (0.05)	0.95 (0.05)
FPG — mmol/liter	5.5 (0.5)	5.9 (0.4)	8.3 (2.0)	5.5 (0.5)	6.0 (0.6)	7.7 (1.3)
FSI — pmol/liter	46 (36)	56 (34)	77 (47)	30 (19)	36 (24)	36 (23)

[a]Cut-offs for the low-BMI group were <25 and <30 kg/m^2 for Japanese and Caucasians, respectively.

Data are presented as number of participants (%) or mean (SD). To convert the values for glucose to mg/dL, divide by 0.05551. To convert the values for insulin to mU/L, divide by 6.945. BMI denotes body mass index, FPG fasting plasma glucose, and FSI fasting serum insulin.

FIGURE LEGENDS

Figure 1. Plasma glucose (Panels A and B) and serum insulin (Panels C and D) profiles in Caucasians and Japanese NGT, IGT and T2D participants following standard oral glucose tolerance tests. Data are presented as mean±SEM.

Figure 2. Left panels: Mean ±SEM of insulin sensitivity (Panels A and B) and beta-cell function (Panels C, D and E). Statistical tests were obtained from ANOVA with ethnicity, glucose tolerance state, and the interaction between the two in the model. Right panels: BMI-adjusted measures. Statistical tests were obtained by including BMI in the ANOVA model. *P<0.05, **P<0.01. Gray (dark): Caucasians, blue (light): Japanese.

Figure 3. Insulin sensitivity (Panels A and B) or beta-cell function (Panels C, D and E) versus BMI. The ordinate is presented on a logarithmic scale. Correlation coefficients are calculated on the basis of log-transformed values for all individuals. All correlations were statistically significant (p<0.01). Black (open): Caucasians, blue (filled): Japanese.

Figure 4. Disposition indices (DI) (mean±SEM) calculated as products of the insulinogenic index (Panel A) and the insulin secretion ratio (Panel B) and the Matsuda index. Statistical tests were obtained from ANOVA with ethnicity, glucose tolerance state, and the interaction between the two in the model.

FIGURES

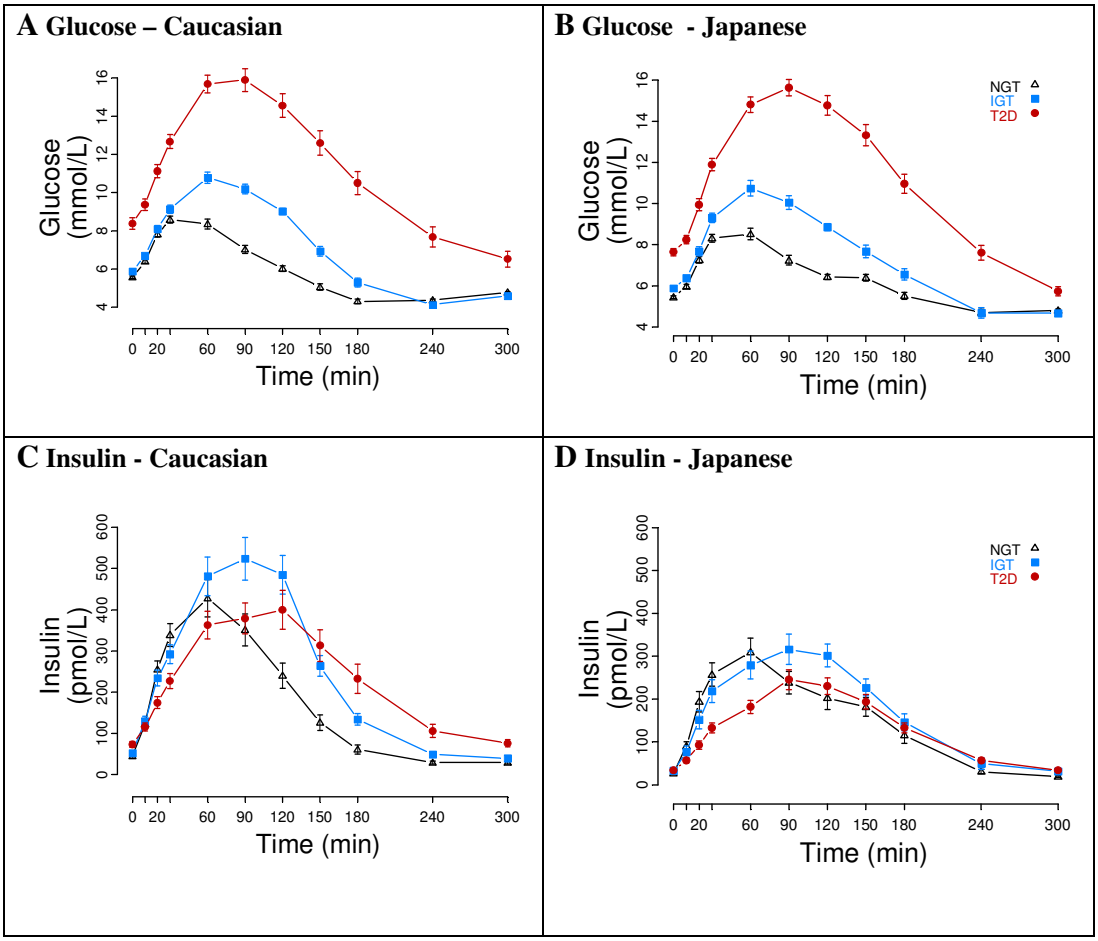
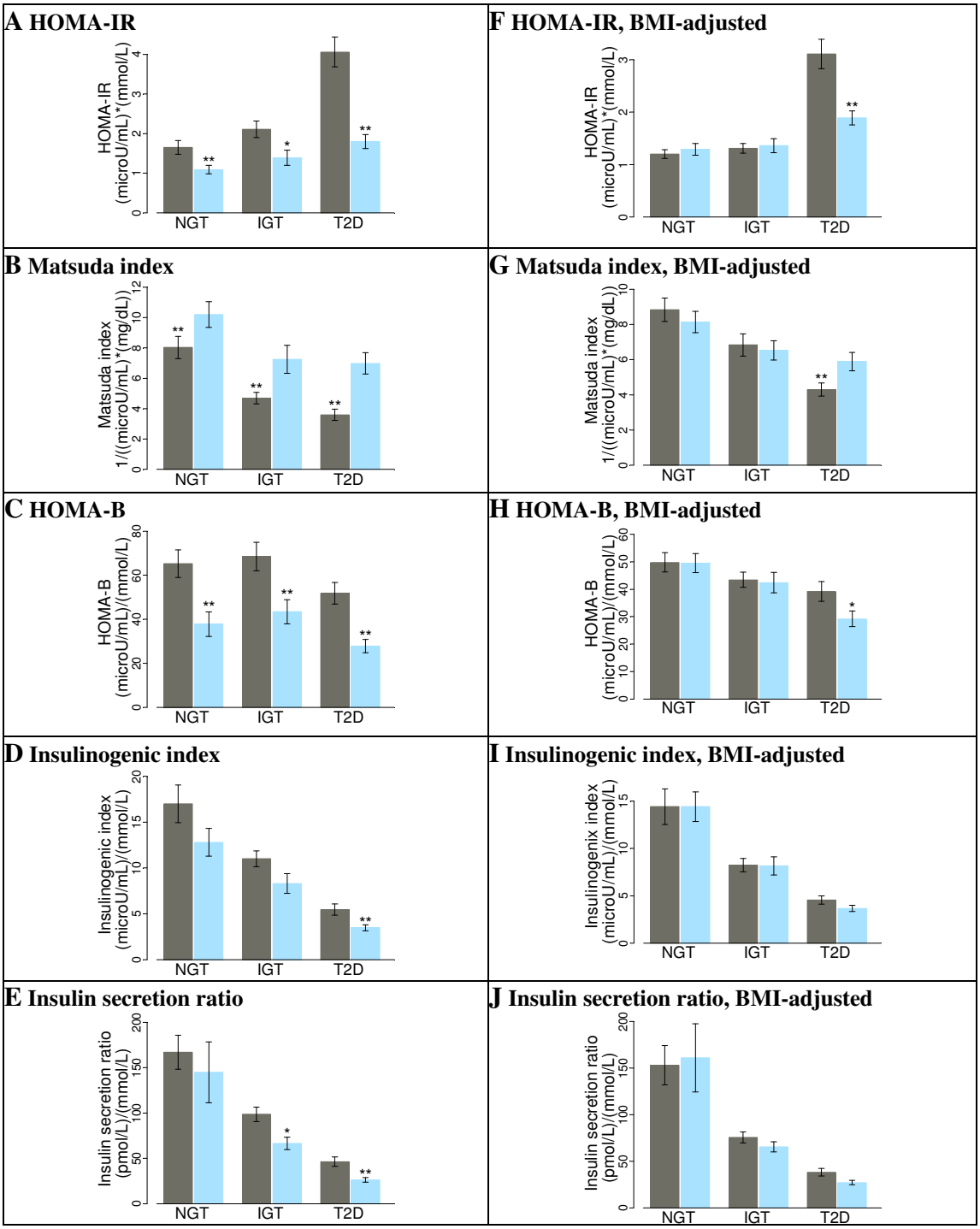


Figure 1


A HOMA-IR
HOMA-IR (microU/mL)*(mmol/L)
NGT
IGT
T2D
F HOMA-IR, BMI-adjusted
HOMA-IR (microU/mL)*(mmol/L)
NGT
IGT
T2D
B Matsuda index
Matsuda index 1/((microU/mL)*(mg/dL))
NGT
IGT
T2D
G Matsuda index, BMI-adjusted
Matsuda index 1/((microU/mL)*(mg/dL))
NGT
IGT
T2D
C HOMA-B
HOMA-B (microU/mL)/(mmol/L)
NGT
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T2D
H HOMA-B, BMI-adjusted
HOMA-B (microU/mL)/(mmol/L)
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D Insulinogenic index
Insulinogenic index (microU/mL)/(mmol/L)
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I Insulinogenic index, BMI-adjusted
Insulinogenix index (microU/mL)/(mmol/L)
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E Insulin secretion ratio
Insulin secretion ratio (pmol/L)/(mmol/L)
NGT
IGT
T2D
J Insulin secretion ratio, BMI-adjusted
Insulin secretion ratio (pmol/L)/(mmol/L)
NGT
IGT
T2D

Figure 2

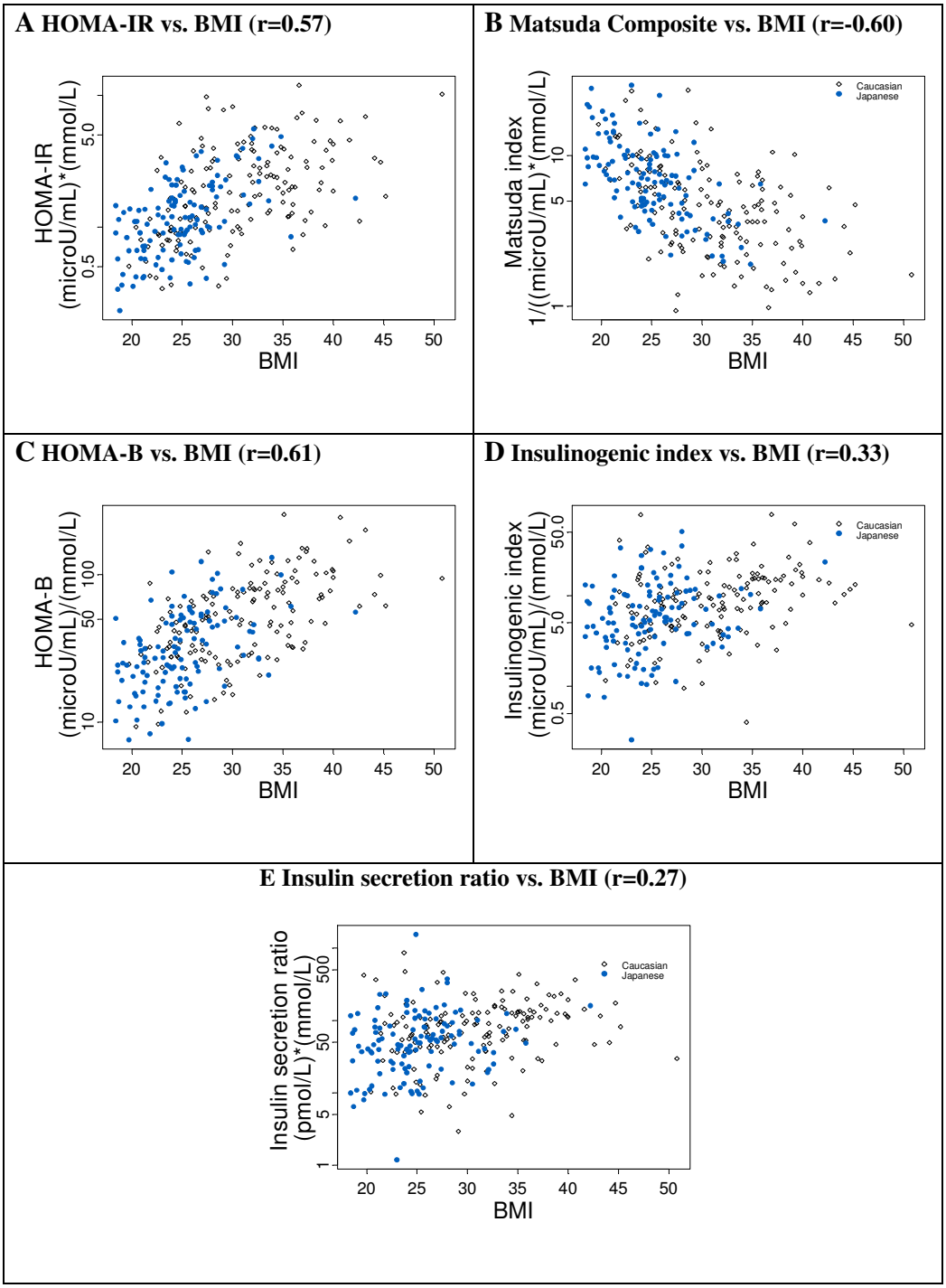
A HOMA-IR vs. BMI (r=0.57)
HOMA-IR (microU/mL)*(mmol/L)
0.5
5.0
20 25 30 35 40 45 50
BMI
B Matsuda Composite vs. BMI (r=-0.60)
Matsuda index 1/((microU/mL)*(mmol/L)
1
5
10
Caucasian
Japanese
20 25 30 35 40 45 50
BMI
C HOMA-B vs. BMI (r=0.61)
HOMA-B (microU/mL)/(mmol/L)
10
50
100
20 25 30 35 40 45 50
BMI
D Insulinogenic index vs. BMI (r=0.33)
Insulinogenic index (microU/mL)/(mmol/L)
0.5
5.0
50.0
Caucasian
Japanese
20 25 30 35 40 45 50
BMI
E Insulin secretion ratio vs. BMI (r=0.27)
Insulin secretion ratio (pmol/L)*(mmol/L)
1
5
50
500
Caucasian
Japanese
20 25 30 35 40 45 50
BMI

Figure 3

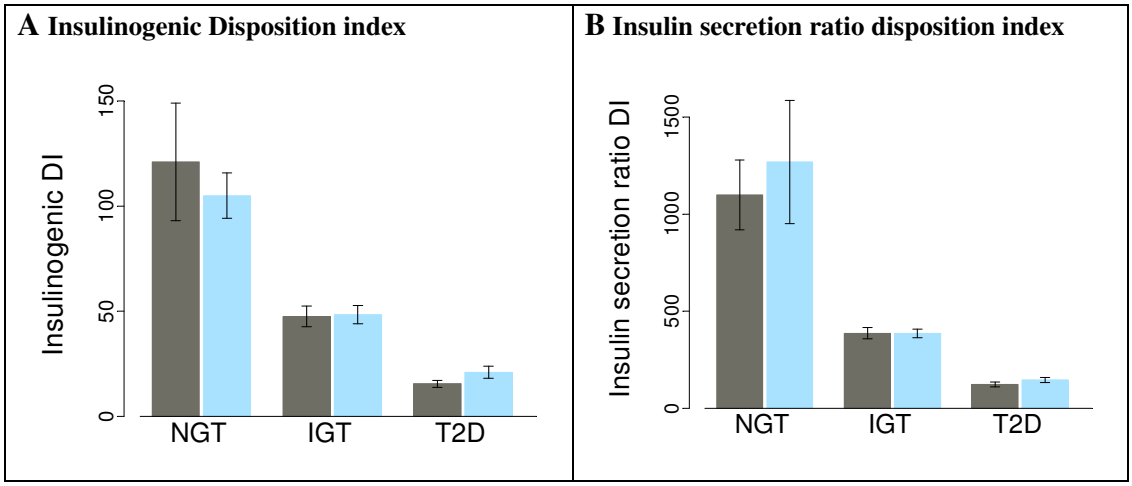
A Insulinogenic Disposition index
Insulinogenic DI
0
50
100
150
NGT
IGT
T2D
B Insulin secretion ratio disposition index
Insulin secretion ratio DI
0
500
1000
1500
NGT
IGT
T2D

Figure 4

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Paper D

Disease progression to type 2 diabetes in Japanese and Caucasians: An oral minimal model analysis

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Manuscript

Title: Disease Progression to Type 2 Diabetes in Japanese and Caucasians: An Oral Minimal Model Analysis

Authors: Jonas B. Møller, Chiara Dalla-man, Rune V. Overgaard, Steen H. Ingwersen, Maria Pedersen, Haruhiko Tanaka, Mitsuru Ohsugi, Bente K. Pedersen, Jan Lynge, Katrine Almind, Nina-Maria Vasconcelos,Charlotte Keller, Claudio Cobelli

Summary

Objective: Type 2 diabetes (T2D) a disease in which differences between ethnic groups have been reported. However, an extensive comparison of the pathophysiology in different ethnicities using an oral minimal model based approach has not yet been published. In this paper we aimed at assessing the potential differences in beta-cell function, insulin sensitivity and hepatic extraction between Caucasian and Japanese subjects ranging from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and to type 2 diabetics (T2D).

Research design and methods: Beta-cell function and insulin sensitivity was calculated for 150 Caucasians and 120 Japanese subjects using glucose, insulin, and C-peptide measurements obtained from an oral glucose tolerance test (OGTT). Subjects were stratified according to glucose tolerance (NGT, IGT, and T2D) and into high and low BMI groups. Caucasian subjects were stratified to the high group, when BMI≥30 kg/m^2, and Japanese when BMI≥25 kg/m^2. Basal, dynamic, and static beta-cell responsivity (Φ_b, Φ_d, Φ_s) and S_I were assessed by oral C-peptide and glucose minimal models, and hepatic extraction were estimated using the C-peptide minimal model in combination with a newly developed insulin model. Furthermore, the two disposition indices (DI_d, DI_S), which adjust beta-cell secretion for insulin action were calculated in order to describe the ability of the beta-cell to compensate for insulin resistance.

Results: Despite similar OGTT glucose profiles, Japanese in general had lower insulin and C-peptide secretion as compared to Caucasians both at basal conditions and following the OGTT. At all disease states, the basal beta-cell function (Φ_b) was lower in Japanese ($P<0.01$). Estimates of the dynamic (Φ_d) and static (Φ_s) beta-cell responsivity indices indicated significantly lower values in the Japanese IGT group compared to Caucasians ($P<0.05$). In contrast, values of insulin action (S_I) showed higher sensitivity in the Japanese IGT subjects. Hepatic extraction at basal (HE_b) and during the OGTT (HE_{post}) was similar in the NGT and IGT groups, but significantly higher in the Japanese T2D subjects compared to the Caucasians ($P<0.01$). The disposition indices (DI_d, DI_S) were similar in Japanese and Caucasians at all disease states.

Conclusions: Our findings show the existence of differences in beta-cell function and insulin sensitivity between Caucasians and Japanese and a similar ability to compensate for increased insulin resistance at all states of glucose tolerance. Furthermore we have shown that significant differences in hepatic insulin extraction ratios exist at the state of T2D.

Introduction

The existence of ethnic difference in the pathogenesis of type 2 diabetes (T2D) has been shown in a variety of papers (Fukushima et al. 831-35;Kuroe et al. 71-77;Takeuchi et al. 370-76;Jensen et al. 2170-78;Torréns et al. 354-61;Fukushima, Suzuki, and Seino S37-S43;Hanefeld et al. 868-74;Chandalia et al. e812;Chandler-Laney et al. 2086-92;Ferrannini et al. 3251-57;Liew et al. 784-89;Nakanishi et al. 571-77;Weiss et al. 571-79;Izuka et al. 41,45;Mitsui et al. 53-58;Tripathy et al. 975-80). Many of these papers rely on data obtained in Caucasians and Japanese, and the general hypothesis outlined is that Japanese can not compensate with increased insulin secretion when progressing from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to the same degree as Caucasians. Some of these studies refer specifically to the difference in first-phase insulin response (Hanefeld et al. 868-74;Fukushima, Suzuki, and Seino S37-S43), although other studies have reported contrasting results (Torréns et al. 354-61).

In general comparisons between Japanese and Caucasians have been performed using data from different studies with different inclusion criteria etc. This could potentially lead to biased and misleading conclusions. To our knowledge no study has reported results from a study where inclusion and enrolment of patients has been matched for the purpose of studying the potential difference in development of T2D between the two ethnicities.

It is well-known that pathogenesis of T2D involves decreased insulin sensitivity in combination with impaired beta-cell function (Lyssenko et al. 166-74) . One approach to investigate ethnic differences in development of the disease is to assess beta-cell function and insulin sensitivity in subjects with different glucose tolerance using data from an oral glucose tolerance test (OGTT).

Classical measures for beta-cell function and insulin sensitivity such as the homeostatic indices (Matthews et al. 412-19) or the Insulinogenic index (Phillips et al. 286-92) have been widely applied in previous analyses of ethnic differences (Fukushima et al. 831-35;Suzuki et al. 1211-15,Ref. article 1). One drawback with these methods of calculating beta-cell function is that they only take into account a fraction of the available data obtained from the OGTT and are typically calculated using glucose and insulin samples. As approximately 50% of newly secreted insulin is degraded in liver (Vølund, Polonsky, and Bergman 1195-202), and the hepatic extraction ratio can be different from one individual to another, such measures can potentially lead to misleading conclusions.

Alternatively beta-cell function may be estimated by a mathematical model which applies C-peptide concentrations to obtain measures for insulin secretion rate. For such an assessment, the oral minimal model of C-peptide (Breda et al. 150-58) has been applied to provide indices for both first- and second phase insulin secretion estimated as the dynamic and static beta-cell responsivity index (Basu et al. 866-72;Basu et al. 2001-14;Sunehag et al. 233-39). When combined with a model for insulin kinetics, information of hepatic extraction during an OGTT can be obtained, thus providing a more complete metabolic description compared to classical methods (Campioni et al. E941-E948).

As stated previously, it is well-established that T2D is characterised by insulin resistance accompanied by beta-cell defects (Bergman et al. S212-S220). In non-diabetic subjects a close relationship between insulin resistance and beta-cell function is observed (Kahn et al. 1663-72).

Progression to T2D is hypothesised to happen when beta-cells can not compensate increasing insulin resistance (Stumvoll, Goldstein, and van Haeften 1333-46). The disposition index reflects beta-cell function interpreted in light of prevailing insulin sensitivity, and be used as a surrogate marker for diabetes state from a pathophysiological point of view (Cobelli et al. E1-E15).

To our knowledge, no study has extensively assessed the progression to T2D in a matched cohort of Caucasian and Japanese subjects applying state of the art methods for estimation of beta-cell function, insulin sensitivity, and hepatic extraction. Thus, whether a similar pathophysiology characterises the transition from NGT to IGT to T2D in Japanese and Caucasians still seems to be unresolved.

In our study, we assessed the metabolic sequence of events that takes place in the transition from NGT to IGT to T2D in Caucasians and Japanese by using a frequently-sampled OGTT in each cohort. By applying the oral minimal models (Breda et al. 150-58), (Dalla Man, Caumo, and Cobelli 419-29), (Campioni et al. E941-E948) we determined indices for beta-cell function, insulin sensitivity, hepatic extraction, and disposition indices at each glucose tolerance state.

Methods

Study design and participants

This study included 150 subjects enrolled at Rigshospitalet, Denmark, and 120 subjects enrolled at Tokyo University Hospital in Japan. The 270 subjects in total, were stratified according to BMI and glucose tolerance state as outlined in WHO criteria (normal glucose tolerant (NGT), impaired glucose tolerant (IGT), or type 2 diabetes (T2D)) (WHO). Subjects were aged 40 to 65 years, and each cohort had a similar distribution of males and females. Baseline characteristics and distribution of subjects is presented in Table 1. The study protocol was approved by The Regional Committee on Biomedical Research Ethics in Denmark (Journal no H-C-2008-101) and by the Research Ethics Committee of Graduate School of Medicine, the University of Tokyo in Japan. Informed consent was obtained from all participants and the handling of data was approved by the Danish Data Protection Agency.

Procedures

All subjects received an oral bolus corresponding to 75g of glucose. Plasma samples were collected at times -30, 0, 10,20,30,60, 90, 120, 150, 180, 240, and 300 min for determination of glucose, insulin and C-peptide plasma concentrations, and were assayed at the same lab using validated methods. In

order to assess measures for beta-cell function and insulin sensitivity, we respectively applied the oral minimal model (OMM) for C-peptide (Breda et al. 150-58), and glucose (Dalla Man, Caumo, and Cobelli 419-29). The OMM for C-peptide provides measures of basal, dynamic, and static (Φ_b, Φ_d,Φ_s) beta-cell responsivity, whereas the OMM for glucose provides measures of insulin sensitivity (S_I). The combination of the C-peptide model with a model for insulin delivery rate proposed by Campioni et al. was applied for estimation of hepatic extraction at basal and under the glucose challenge (Campioni et al. E941-E948). Disposition indices DI_d and DI_s were calculated using the product of indices for beta-cell responsivity and insulin sensitivity. All estimation procedures were carried out using a single-subject approach and commercial software package Matlab v. 14.

Statistical analysis

Data are presented as means ± SE. For each measure of insulin sensitivity and beta-cell function, an ANOVA was used to test for effects of glucose tolerance state and race. In addition, comparisons between Japanese and Caucasians at each glucose tolerance state were performed using the unpaired t-test. A p-value<0.05 was considered significant, and a p-value<0.01 was indicated with two significance stars.

Results

Plasma glucose, insulin, and C-peptide profiles

Concentration vs. time profiles together with standard-errors of the mean (SEM) for NGTs, IGTs, and T2Ds are shown in Fig. 1. For the glucose profiles, both baseline and maximal concentrations appeared similar between Caucasian and Japanese, but had apparent difference in return to baseline for NGTs and IGTs.

In contrast, basal and maximum concentrations of insulin were significantly higher in Caucasians in all glucose tolerance groups (P<0.05). For all groups it was further observed, that the insulin response in Caucasians mainly seems to be higher following time points after 30 min. Similar trends were observed for C-peptide concentrations being higher in Caucasians both at baseline and at maximum concentrations in all groups of glucose tolerance states.

Insulin action

Figure 2 presents mean values and SEM of indices for insulin action (S_I), beta-cell function (Φ_b, Φ_d,Φ_s), and hepatic extraction ratios (HE) at each disease state (NGT, IGT, and T2D) for Caucasian and Japanese. Generally a decrease in insulin sensitivity from NGT to T2D was observed for both groups. Insulin sensitivity was found to be significantly lower in Caucasian IGTs compared to Japanese (P<0.05), but no difference was found for NGT and T2D subjects. Results from ANOVA performed including all glucose tolerance states on the insulin sensitivity index (S_I) indicated no overall significant effect of ethnicity (P>0.05).

Beta-cell function

No clear trend for beta-cell function at fasting was observed from NGT to T2D, although beta-cell responsivity at basal was significantly lower in Japanese compared to Caucasians at all levels of glucose tolerance ($P<0.01$). In contrast, for both cohorts, the dynamic and static beta-cell function indices decreased around 50% from NGT to T2D. For the dynamic response, a significantly lower mean value was found for the Japanese IGT group compared to the Caucasian IGT group ($P<0.05$), whereas the static index appeared lower in Japanese at all disease states and reached statistical significance both in the IGT and the T2D group ($P<0.01$,$P<0.05$). The ANOVA analysis revealed that for the dynamic index, the interaction term between type and ethnicity was significant ($P<0.05$), whereas for the static index, ethnicity in itself was found to be significant ($P<0.01$).

Hepatic extraction

Caucasians and Japanese had statistically similar hepatic extraction in both the NGT and the IGT group, although numerically the Japanese had slightly higher mean values (Fig. 2). For the T2D group, the difference was significant ($P<0.01$) indicating higher hepatic extraction ratios both at basal (HE_b) and post-glucose load (HE_{post}) in Japanese compared to the Caucasians. Testing the overall effect on the hepatic extraction ratios revealed that ethnicity in itself was significant both for basal – and post-glucose hepatic extraction ratios ($P<0.01$, $P<0.01$).

Disposition indices

Values of disposition indices, calculated to adjust insulin secretion with insulin action, are presented in Figure 3. No statistical significant difference at any of the disease states were observed for any of the disposition indices (DI_d, DI_s) between Caucasians and Japanese, which was supported by ANOVA indicating no significant effect of ethnicity.

Discussion

The present study evaluates and provides new insights regarding the pathogenesis of T2D in Japanese and Caucasians. Beta-cell function, insulin sensitivity, hepatic extraction ratios, and disposition indices were estimated using mathematical methods applied on data from a frequently-sampled OGTT. Indices were calculated for 3 subgroups in each of the Caucasian and Japanese cohorts, stratified according to glucose tolerance into NGT, IGT, or T2D groups.

Compared to NGT subjects, the IGT subjects had lower insulin sensitivity both in the Japanese and Caucasian subjects, although the decrease in insulin sensitivity is much more pronounced in Caucasians, resulting in higher insulin sensitivity in Japanese than in the Caucasians at the IGT state.

Higher maximum insulin and C-peptide concentrations were observed at all stages in the Caucasian subjects compared to Japanese, and the difference seems more pronounced for insulin than for C-peptide. We speculate that this could be due to a higher hepatic insulin extraction ratio and thus applied the newly developed model by Campioni et al. for assessment of hepatic extraction from

an oral test (Campioni et al. E941-E948). As seen in Fig. 2, the hepatic insulin extraction ratio was quantitatively higher in Japanese both before and during the OGTT, and the difference was significant for the T2D group.

The beta-cell function relative to insulin resistance was assessed using dynamic as well as static disposition indices. Both indices were found to be similar at all disease states, thus showing that Japanese and Caucasian have the same ability to compensate for increased insulin resistance during transition from NGT to T2D.

In an earlier publication, we applied simple indices for calculation of insulin sensitivity (HOMA-IR (Matthews et al. 412-19) and Matsuda Composite (Matsuda and Defronzo 1462-70)) and beta-cell function (HOMA-B and Insulinogenic (Phillips et al. 286-92)) which all apply glucose and insulin data. This was supplemented with a minimal model-based approach for several reasons. First of all, this enables us to accurately assess the hepatic extraction ratios before and after the OGTT. Secondly, the model-based approach takes all samples from the OGTT into account, and can handle uncertainty in measurements. Thirdly, the length and timing of the first- and second phase insulin secretion can be different from one individual to another, which is taken into account only in the model-based approach.

In general, the findings presented here are in line with our earlier findings, although some specific differences exist. These differences originate mainly from the before-mentioned difference in the amount of samples included in the calculation of insulin sensitivity and beta-cell function. One such difference is the insulin sensitivity in NGTs measured by the Matsuda Composite or using the glucose minimal model. The Matsuda Composite index uses data up to 120 minutes, whereas the minimal model uses the full profiles, which in our case is up to 300 min. The undershoot observed in the glucose curve for Caucasians with NGT does not contribute to the Matsuda Composite index value, and was reported to be quantitatively higher in Japanese subjects than in Caucasians (Ref. article 1). This is in contrast to the findings presented here for the minimal model insulin sensitivity, indicating quantitatively higher sensitivity in the Caucasian NGTs.

Continuous deterioration of insulin sensitivity in the progression to T2D have been reported both for Caucasians and Japanese (Bock et al. 3536-49;Jensen et al. 2170-78) (Fukushima et al. 831-35). In line with our findings, Nishi et al. also observed decreased insulin sensitivity from NGT to IGT to T2D in Japanese (Nishi et al. 46-52). This provides evidence, that pathogenesis to T2D is characterised by continuous decreasing whole-body insulin sensitivity both in Caucasians and Japanese.

In our previous paper (ref. Article 1), we reported first-phase insulin secretion using the insulinogenic index, and we found no statistical difference between Caucasian and Japanese IGTs. In contrast, in the present analysis, we found a statistical difference in the dynamic index (Φ_d) which is also a surrogate marker for the first-phase insulin secretion. This controversy originates from the fact, that the insulinogenic index uses insulin data, instead of C-peptide, and only uses measurements up to 30 min. For analysis of beta-cell function, it is generally accepted that use of C-peptide data provides the most accurate measure, due to possible differences in hepatic extraction ratio. In spite of

this, the insulinogenic index is widely applied and generally accepted in the diabetes literature as a marker of first-phase insulin secretion ability.

Differences between Japanese and Caucasians in beta-cell function have been suggested in a number of studies (Fukushima et al. 831-35;Fukushima, Suzuki, and Seino S37-S43;Tripathy et al. 975-80). These studies suggest that Japanese cannot compensate insulin resistance with increased beta-cell function to the same extent as Caucasians. In our study, we found lower beta-cell function in Japanese IGTs, and T2Ds calculated using the dynamic and static beta-cell index.

Due to the inherent relation between beta-cell function and insulin sensitivity (Kahn et al. 1663-72), we also calculated the dynamic and static disposition indices which takes into account the degree of insulin sensitivity. The disposition indices can thus be used as markers for the ability of the beta-cell to compensate for prevailing insulin sensitivity. As expected, both indices declined with worsening of glucose tolerance. Furthermore, at all glucose tolerance states, similar values were obtained for Japanese and Caucasians. This provides evidence for a similar ability to compensate for insulin resistance in the two cohorts, which supports our previous findings based on non-model based indices for beta-cell function and insulin sensitivity (Ref. Article 1).

In order for the two cohorts to be representative for their corresponding population, each of the cohorts was stratified in high and low BMI groups according to regional obesity definitions. This lead to a cut-off of 25 kg/m^2 for Japanese and 30 kg/m^2 for Caucasians. As we strived to obtain a well-balanced design with similar number of subjects in the high and low BMI groups, the Caucasian subjects on average had higher BMI compared to the Japanese. In the previous publication (ref. Article 1), we showed that the BMI difference could explain the majority of the difference in insulin sensitivity and beta-cell function at each disease state. Here we did not take BMI into account, as the key point was to compare the findings presented in the previous publication with results from the minimal models and to present the findings for hepatic insulin extraction ratios, which clarifies the underlying physiology for the differences observed between the two cohorts. It is worth mentioning that BMI may also explain a major part of the differences in minimal model insulin sensitivity at each disease state as this was observed also for the Matsuda index (Ref. Article 1).

In our study, we applied a cross-sectional in contrast to a longitudinal study approach. Thus, the results cannot provide evidence about whether Japanese and Caucasians can tolerate the same period of insulin resistance before progression to T2D. In other words, whether Japanese can cope with the stress on the beta-cells in the same period of time as is the case for Caucasians. This is a suggestion for future research.

In summary, our study showed that Caucasian and Japanese NGT, IGT, and T2D had similar glucose profiles following the OGTT. Despite these similarities, beta-cell function calculated using the oral minimal model for C-peptide, in general was lower in Japanese compared to Caucasians in IGT and T2D states. Finally, the combined effect of insulin sensitivity and beta-cell function as estimated by the disposition index showed that Japanese and Caucasian NGTs, IGTs, and T2Ds have similar beta-cell function relative to insulin resistance. This provides evidence that the ability to compensate for increasing insulin resistance is similar in Caucasian and Japanese.

Tables

Table 1: Baseline demographics of participants

	Caucasians			Japanese		
	NGT	**IGT**	**T2D**	**NGT**	**IGT**	**T2D**
Total	63	39	48	46	26	48
Low BMI[a]	32 (51%)	14 (36%)	24 (50%)	25 (54%)	12 (46%)	27 (56%)
Age (years)	53 (7)	54 (8)	57 (7)	49 (7)	54 (8)	57 (7)
Sex (male)	29 (46%)	15 (38%)	29 (60%)	21 (46%)	12 (46%)	33 (69%)
Height (m)	1.7 (0.1)	1.7 (0.1)	1.8 (0.1)	1.6 (0.1)	1.6 (0.1)	1.6 (0.1)
BMI	29.8 (5.9)	33.0 (6.3)	30.4 (5.7)	24.0 (3.2)	26.3 (5.0)	25.3 (4.4)
Waist-to-hip	0.91 (0.09)	0.94 (0.08)	0.97 (0.08)	0.93 (0.05)	0.94 (0.05)	0.95 (0.05)
FPG [mmol/L]	5.5 (0.5)	5.9 (0.4)	8.3 (2.0)	5.5 (0.5)	6.0 (0.6)	7.7 (1.3)
FSI [pmol/L]	46 (36)	56 (34)	77 (47)	30 (19)	36 (24)	36 (23)

[a]Cut-offs for the low-BMI group were <25and <30 kg/m^2 for Japanese and Caucasians, respectively.
Data are presented as number of participants (%) or mean (SD). To convert the values to mg/dL, divide by 0.0555. To convert the values for insulin to mU/L, divide by 6.945. BMI denotes body mass index, FPG denotes fasting plasma glucose, and FSI fasting serum insulin

Figures

Figure 1: Glucose, insulin, and C-peptide profiles for Caucasian (black) and Japanese (blue) at the three different disease stages (NGT, IGT, and T2D). Data are mean ± SEM.

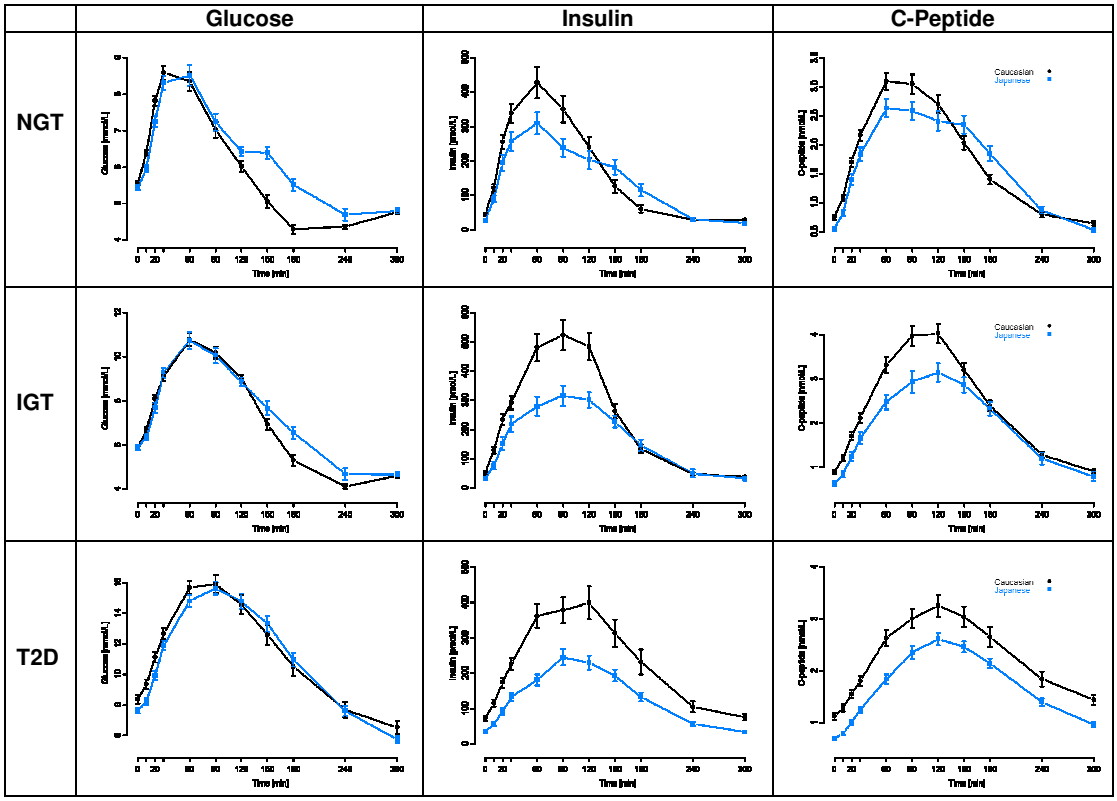


Figure 2 : Measures of insulin sensitivity (S_I), beta-cell function (ϕ_b, ϕ_d, ϕ_s), and hepatic extraction (HE_b, HE_{post})

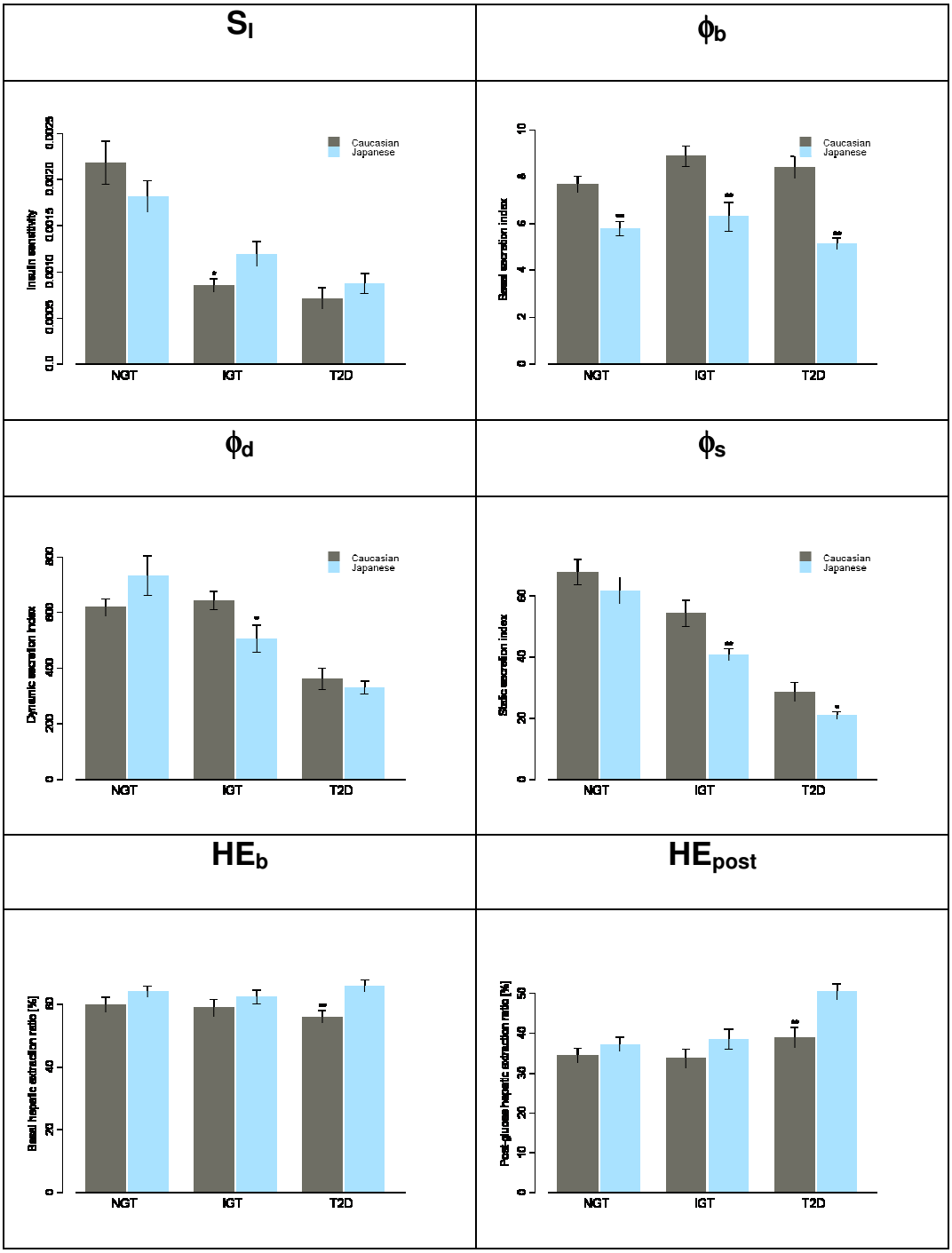
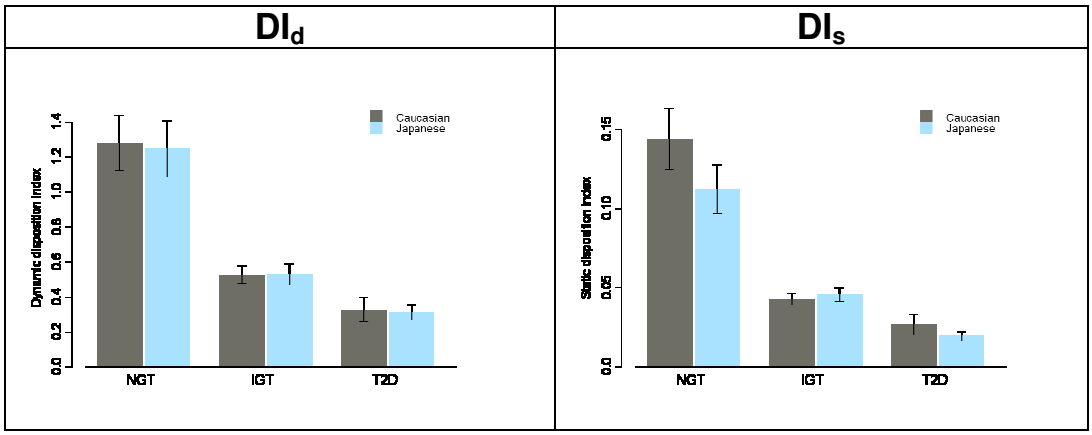


Figure 3 : Disposition indices (DI_d, DI_s), calculated as the product of insulin sensitivity and beta-cell function indices (ϕ_d, ϕ_s).



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