DISSERTATIONS IN
HEALTH SCIENCES

Yuvaraj Mahendran

Identification of Biomarkers for Type 2 Diabetes

Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

YUVARAJ MAHENDRAN

Identification of Biomarkers for Type 2 Diabetes

To be presented by permission of the Faculty of Health Sciences,
University of Eastern Finland for public examination in the Auditorium L3,
Canthia building at the University of Eastern Finland, Kuopio,
on Friday, 14th of March 2014, at 12 noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
214

Department of Medicine, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
Kuopio University Hospital
Kuopio
2014

Juvenes Print - Finnish University Print
Kuopio, 2014

Distributor:
University of Eastern Finland
Kuopio Campus Library
P.O.Box 1627
FI-70211 Kuopio, Finland
http://www.uef.fi/kirjasto

ISBN: 978-952-61-1367-8 (print)
ISBN: 978-952-61-1368-5 (PDF)
ISSN: 1798-5706 (print)
ISSN: 1798-5714 (PDF)
ISSNL: 1798-5706

Author's address: Department of Medicine, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
P.O. Box 1627
FI-70211 Kuopio
Finland
e-mail:yuvaraj.mahendran@uef.fi

Supervisors: Academy Professor Markku Laakso, M.D., Ph.D.
Department of Medicine, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
Kuopio University Hospital

Professor Johanna Kuusisto, M.D., Ph.D.
Department of Medicine, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
Kuopio University Hospital

Reviewers: Professor Risto Kaaja, M.D., Ph.D.
Department of Medicine
Turku University
Turku, Finland

Docent Olavi Ukkola, M.D., Ph.D.
Department of Internal Medicine
University of Oulu
Oulu, Finland

Opponent: Adjunct Professor Heikki Koistinen, M.D., Ph.D.
Department of Medicine, Division of Endocrinology
Helsinki University Central Hospital
Minerva Foundation Institute for Medical Research
Biomedicum 2U, Tukholmankatu 8
Helsinki, Finland

Mahendran, Yuvaraj
Identification of Biomarkers for Type 2 Diabetes
University of Eastern Finland, Faculty of Health Sciences
Publications of the University of Eastern Finland. Dissertations in Health Sciences 214. 2014. 56 p.

ISBN: 978-952-61-1367-8 (print)
ISBN: 978-952-61-1368-5 (PDF)
ISSN: 1798-5706 (print)
ISSN: 1798-5714 (PDF)
ISSNL: 1798-5706

ABSTRACT

Type 2 diabetes (T2D) is a complex disorder characterized by insulin resistance and pancreatic β-cell dysfunction. The incidence and prevalence of T2D have doubled in recent decades, this phenomenon being attributable to obesity, sedentary lifestyle and unhealthy diet. Both genetic and environmental factors are major determinants of this disease. Genome-wide association studies have identified several risk loci for T2D and hyperglycemia, but the biological role of most of these variants remains unknown. The early diagnosis of diabetes is important in order to avoid long-term micro- and macrovascular complications in individuals at high risk of T2D. Therefore, the identification of biomarkers that accurately predict incident T2D is of great interest. The main aim of this study was to identify non-genetic and genetic biomarkers that would predict hyperglycemia and incident T2D in a prospective follow-up of the population-based METSIM (METabolic Syndrome In Men) cohort. We also investigated the significance of insulin sensitivity and insulin secretion as mediators in the associations of metabolites with the deterioration of hyperglycemia and incident T2D. We found that high fasting levels of glycerol, free fatty acids, monounsaturated fatty acids (FAs), and saturated FAs, and omega-7 and -9 FAs associated with increased risk of the development of hyperglycemia and T2D, whereas high levels of omega-6 FAs were associated with reduced risk of hyperglycemia and T2D. Insulin resistance explained these associations at least in part. With respect to erythrocyte membrane FAs, palmitoleic acid, dihomo-gamma-linolenic acid, and the ratios of 16:1n-7/16:0 and 20:3n-6/18:2n-6 associated with the worsening of hyperglycemia, whereas the linoleic acid level and the ratio of 18:1n-7/16:1n-7 were associated with decreases in the hyperglycemia. Palmitoleic acid and the ratio of 16:1n-7/16:0 nominally predicted incident T2D, whereas linoleic acid had an opposite association. These associations were largely independent of insulin sensitivity, insulin secretion and glucose levels. Finally, high levels of acetoacetate and β-hydroxybutyrate predicted the worsening of hyperglycemia, and acetoacetate predicted incident T2D. Impaired insulin secretion, but not insulin resistance, explained these associations. One common variant rs780094 of the glucokinase regulatory protein gene was significantly associated with β-hydroxybutyrate levels. In conclusion, this study identified several novel biomarkers predicting the worsening of hyperglycemia and incident T2D which could be used in clinical practice.

National Library of Medicine Classification: QU 84, QU 90, WK 810, WK 820, WK 880

Medical Subject Headings: Diabetes Mellitus, Type 2; Biological Markers; Genetics; Metabolomics; Hyperglycemia; Insulin; Insulin Resistance; Glycerol; Fatty Acids; Ketone Bodies; Cohort Studies

Mahendran, Yuvaraj
Tyypin 2 diabetesta ennustavat biomarkkerit
Itä-Suomen yliopisto, terveystieteiden tiedekunta
Publications of the University of Eastern Finland. Dissertations in Health Sciences 214. 2014. 56 s.

ISBN: 978-952-61-1367-8 (print)
ISBN: 978-952-61-1368-5 (PDF)
ISSN: 1798-5706 (print)
ISSN: 1798-5714 (PDF)
ISSNL: 1798-5706

TIIVISTELMÄ

Puutteellinen insuliinin eritys ja heikentynyt insuliinin vaikutus kohdekudoksissa (insuliiniresistenssi) ovat tärkeimmät tyypin 2 diabeteksen aineenvaihdunnan häiriöt. Tyypin 2 diabetes on perinnöllinen sairaus, mutta sen puhkeamiseen tarvitaan geenien lisäksi ympäristötekijöitä. Useita tyypin 2 diabetekseen liittyviä geenejä on löydetty viimeisten vuosien aikana, mutta geenien funktio on useimmissa tapauksissa edelleenkin tuntematon. Tyypin 2 diabeteksen esiintyvyys on nopeasti lisääntynyt viimeisten vuosikymmenien aikana johtuen ylipainon lisääntymisestä, liikunnan vähenemisestä sekä epäterveellisestä ruokavaliosta. Tyypin 2 diabeteksen varhainen diagnoosi on tärkeää, koska tähän sairauteen liittyy pitkäaikaiskomplikaatioiden riski. Tutkimuksen tärkein tavoite oli tyypin 2 diabetesta ja hyperglykemiaa ennustavien geneettisten ja ei-geneettisten tekijöiden (biomarkkereiden) löytäminen. Aineistona oli METSIM (METabolic Syndrome In Men) –kohortti, johon kuului 10,197 Kuopiossa ja sen lähikunnissa asuvaa miestä. Kohortin viiden vuoden seuruututkimuksessa lisääntynyt glyserolin, vapaiden rasvahappojen, tyydyttyneiden rasvahappojen, sekä omega 7- ja omega 9- rasvahappojen pitoisuus ennusti hyperglykemian ja tyypin 2 diabeteksen kehittymistä. Omega 6-rasvahappojen lisääntynyt pitoisuus puolestaan suojasi hyperglykemian ja tyypin 2 diabeteksen kehittymiseltä, joka johtui osittain vaikutuksesta insuliiniherkkyyteen. Punasolumembraanien rasvahapoista palmitoleiinihapon ja dihomo-gamma-linoleenihapon lisääntynyt pitoisuus sekä eripituisten rasvahappojen lisääntyneet suhteet (16:1n-7/16:0 ja 20:3n-6/18:2n-6) ennustivat hyperglykemian ja tyypin 2 diabeteksen riskiä. Lisääntynyt linoleenihapon pitoisuus ja lisääntynyt 18:1n-7/16:1n-7 – suhde vähensivät puolestaan hyperglykemian riskiä. Nämä tulokset olivat riippumattomia insuliiniresistenssistä, insuliinin erityksestä ja glukoositasoista. Asetoasetaatin ja β-hydroksibutyraatin lisääntynyt pitoisuus ennusti hyperglykemian kehittymistä ja asetoasetaation lisääntynyt pitoisuus myös tyypin 2 diabetesta johtuen insuliinierityksen huononemisesta. GCKR-geenin yleinen polymorfia (rs780094) liittyi merkitsevästi β-hydroksibutyraatin pitoisuuteen. Yhteenvetona voidaan todeta, että tutkimussarjassa löydettiin useita uusia biomarkkereita, jotka ennustavat hyperglykemian ja tyypin 2 diabeteksen riskiä ja joita voidaan käyttää myös kliinisessä diagnostiikassa.

Luokitus: QU 84, QU 90, WK 810, WK 820, WK 880

Yleinen suomalainen asiasanasto: aikuistyypin diabetes; markkerit; merkkiaineet; geenit; metabolomiikka; hyperglykemia; insuliini; insuliiniresistenssi; glyseroli; rasvahapot; ketoaineet; kohorttitutkimus

To my parents Mr. Harry Klinker and Mrs.Tiny Klinker

Acknowledgements

This study was performed in the Institute of Clinical Medicine, University of Eastern Finland and the Kuopio University Hospital

All praise and thanks to the Lord Jesus Christ!

It is my privilege to express my wholehearted gratitude and utmost respect to my principle supervisor Academy Prof. Markku Laakso for giving me the opportunity to carry out this study under his splendid guidance. His impressive intelligence, vision, enthusiasm in science and experience has supported this study and encouraged my research interests. His trust, patience, help and support go far beyond the confines of this work and also I thank him for providing me with all facilities possible for finishing my doctorate training successfully.

I would like to extend my thank you note to Prof. Johanna Kuusisto for her invaluable guidance, and support which helped me to pursue my studies successfully.

I would like to express my gratitude to the thesis examiners, Prof. Risto Kaaja and Docent Olavi Ukkola for their valuable comments to improve the thesis., I also thank Ewen MacDonald for his language revisions.

I am much obliged to Dr. Jagadish Vangipurapu for all his intellectual and technical guidance right from the beginning of my studies, until I submit my thesis. I am lucky to have been able to obtain his intellectual contribution to this study, which helped me to understand and expand my knowledge. I thank him for his generosity, unstinting encouragement, and bountiful support.

I am grateful to Dr. Henna Cederberg, Dr. Alena Stančáková, Mr. Teemu Kuulasmaa and Dr. Jussi Paananen for their co-operation and constant guidance which helped me to successfully finish this study., I am also indebted to Ms. Seija Laitinen for her kindness and compassion throughout the duration of my PhD work.

I thank all the co-authors and technicians for their valuable contributions to this study. A special thanks to Ms. Arja Afflekt for her continuous help and support.

I express sincere thanks to my colleagues, Shalem Modi Raju, a friend who helped me in so many different ways during my initial days in Kuopio and Niyas K. Saleem, with whom I shared lot of information. Heartfelt thanks to Maykel López Rodriguez, for sharing a good time both inside and outside the office and for participating in research discussions. I also thank Dorota Kaminska and Maria Fizelova for sharing their thoughts and conversation with me.

I am also appreciative of the unending support of my loving friends - Alok Jaiswal, Ganesh Chauhan, Om Prakash, Rubina Tabassum, Symantas Ragauskas, Javkhlanbayar, Ashok Matte, Ashik Jawahar Deen, Suresh Raju, Haja Sherief, Appu Etcherla, Bhima Mitkari, Yajuvinder Singh, Rolls John V, Balamuralikrishna Jayaprakash, Uma Thanigai and Krishnaveni with whom I enjoyed my days while

sharing knowledge and experiences. In addition, my thank you note is extended to Dr. Narasinha Shurpali, Varsha, Sudipta, Jani Paukkonen, Jayendra, Bhavini, Merlin, Sireesha, Nesna for their care and concern.

I offer my tribute to Dr. Mausumi Bharadwaj and Dr. Shirly George Panicker, who introduced me to the field of science, their trust and kindness helped me to pursue my interest in research., I would also like to thank Dr. Showket Hussain and Dr. Neha Singh for their love and encouragement in both my research and personal life.

My acknowledgement could never be complete without thanking my family members, sister Kodeeswari Sherin and Nagarajan and all Klinker family members. I am particularly grateful to Ruby Ponnudurai for her enduring love, care and emotional support. I am humbly indebted to Uncle Soma Sundaram for his constant prayer and love towards me.

Finally, I thank my Lord for giving me such excellent opportunities and for the nice people who have made a deep impression in my heart.

The study has been funded by grants from the National Institute of Health, Academy of Finland, European Union, Finnish Heart Foundation, and Sigrid Juselius Foundation.

Kuopio, March 2014

Yuvaraj Mahendran

List of Original Publications

This dissertation is based on the following original publications:

I Mahendran Y*, Cederberg H*, Vangipurapu J, Kangas AJ, Soininen P, Uusitupa M, Kuusisto J, Ala-Korpela M, Laakso M. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. *Diabetes Care 36: 3732-3738, 2013*

II Mahendran Y, Ågren J, Uusitupa M, Cederberg H, Vangipurapu J, Stančáková A, Schwab U, Kuusisto J & Laakso M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. *Am J Clin Nutr 99:79-85, 2014*

III Mahendran Y, Vangipurapu J, Cederberg H, Stancáková A, Pihlajamäki J, Soininen P, Kangas AJ, Paananen J, Civelek M, Saleem NK, Pajukanta P, Lusis AJ, Bonnycastle LL, Morken MA, Collins FS, Mohlke KL, Boehnke M, Ala-Korpela M, Kuusisto J, Laakso M. Association of ketone body levels with hyperglycemia and type 2 diabetes in 9,398 Finnish men. *Diabetes 62:3618-3626, 2013*

*equal contribution

Contents

1 INTRODUCTION..... 1

2 REVIEW OF THE LITERATURE.....2

2.1 Pathophysiology of type 2 diabetes.....2

2.1.1 Insulin secretion.....2

2.1.2 Insulin resistance.....3

2.1.2.1 Skeletal muscle insulin sensitivity.....4

2.1.2.2 Liver insulin sensitivity.....5

2.1.2.3 Adipose tissue insulin sensitivity.....5

2.2 Dietary fat as a risk factor for type 2 diabetes.....6

2.3 Genetics of type 2 diabetes.....7

2.3.1 Heritability of type 2 diabetes.....7

2.4 Approach for genetic studies in type 2 diabetes.....7

2.4.1 Linkage and candidate gene approach.....7

2.4.2 Genome wide association studies …….....8

2.4.3 Exome wide association studies..... 8

2.5 Hyperglycemia and type 2 diabetes risk loci identified by GWAs.....8

2.5.1 Gene variants affecting insulin secretion.....9

2.5.2 Gene variants affecting insulin sensitivity.....13

2.5.3 Gene variants affecting obesity.....13

2.6 Metabolomics.....14

2.6.1 Untargeted metabolomics.....14

2.6.2 Targeted metabolomics for biomarker discovery.....14

2.6.2.1 Glycerol, free fatty acids and fatty acids.....15

2.6.2.2 Erythrocyte membrane fatty acids……..…….....16

2.6.2.3 Ketone bodies.....17

3 AIMS OF THE STUDY.....19

4 SUBJECTS, MATERIALS AND METHODS.....20

4.1 Subjects..... 20

4.1.1 Baseline study.....20

4.1.2 Follow-up study.....22

4.2 Materials and Methods.....23

5 RESULTS..... 25

5.1 Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes (*Study I*)…….25

5.2 Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes (*Study II*)…….30

5.3 Association of ketone body levels with hyperglycemia and type 2 diabetes (*Study III*).....33

6 DISCUSSION..... 37

6.1 Representativeness of the study population…..…….....37

6.2 Glycerol and fatty acids as predictors of hyperglycemia and incident type 2 diabetes (*Study I*)……..…….....38

6.3 Erythrocyte membrane fatty acids as predictors of hyperglycemia and incident type 2 diabetes (*Study II*).....40

6.4 Ketone body levels as predictors of hyperglycemia and incident type 2 diabetes (*Study III*)….…….....40

6.5 Concluding remarks…….....42

7 SUMMARY.....44

8 REFERENCES..... 45

APPENDICES: ORIGINAL PUBLICATIONS (I-III)

Abbreviations

2hPG	2-hour plasma glucose during an OGTT
ACC	Acetyl-CoA carboxylase
AcAc	Acetoacetate
ADA	American Diabetes Association
ADAMTS9	A disintegrin and metalloproteinase with thrombospondin motifs 9
ADCY5	Adenylate cyclase 5
ADP	Adenosine diphosphate
ADRA2A	Adrenergic alpha-2A receptor
Akt	Protein kinase B
ANOVA	Analysis of variance
ANKRD55	Ankyrin repeat domain 55
ANK1	Ankyrin 1
ARAP1	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1
ATP	Adenosine triphosphate
ATGL	Adipose triglyceride lipase
AUC	Area under the curve
BCAAs	Branched-chain amino acids
BCL11A	B-cell CLL/lymphoma 11A
BHB	3-hydroxybutyrate
BMI	Body mass index
C2CD4B	C2 calcium-dependent domain containing 4B
CAMK1D	Calcium/calmodulin-dependent protein kinase 1 D
CAPN10	Calpain 10
CDC123	Cell division cycle 123 homolog
CDK	Cyclin-dependent kinase
CDKAL1	Cyclin-dependent kinase 5 regulatory sub-unit associated protein 1-like 1
CDKN2	Cyclin-dependent kinase inhibitor 2
CENTD2	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1 (ARAP1)
CHCHD9	Coiled-coil-helix-coiled-coil-helix domain containing 2 pseudogene 9
CRP	C-reactive protein
CRY2	Cryptochrome 2
D5D	Δ^5 desaturase
D6D	Δ^6 desaturase
DAGs	Diacylglycerols
DI30	Disposition Index 30
DGKB	Diacylglycerol kinase beta
EMFA	Erythrocyte membrane fatty acid
FA	Fatty acid
FADS	Fatty acid desaturase
FAS	Fatty acid synthase
FFAs	Free fatty acids

FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FTO	Fat mass and obesity associated
G6PC2	Glucose-6-phosphatase catalytic 2
GCK	Glucokinase
GCKR	Glucokinase regulator
GIPR	Gastric inhibitory polypeptide receptor
GLIS3	GLIS family zinc finger 3
GLUT	Facilitated glucose transporter
GWA	Genome-wide association
HapMap	Haplotype map of the human genome
HDL	High-density lipoprotein
HHEX	Hematopoietically expressed homeobox
HMGA2	High mobility group AT-hook 2
HNF1A	Hepatocyte nuclear factor 1 homeobox A
HNF1B	Hepatocyte nuclear factor 1 homeobox B
HOMA-B	Homeostasis model assessment of insulin secretion
HOMA-IR	Homeostasis model assessment of insulin resistance
HSL	Hormone sensitive lipase
ICAM-1	Intercellular adhesion molecule-1
IFG	Impaired fasting glucose
IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2
IGT	Impaired glucose tolerance
IIFG	Isolated impaired fasting glucose
IIGT	Isolated impaired glucose tolerance
IR	Insulin receptor
IRS	Insulin receptor substrate
ISI	Insulin sensitivity index
JAZF1	Juxtaposed with another zinc finger gene 1
KANK1	KN motif and ankyrin repeat domains 1
KATP	ATP-sensitive potassium channel
KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
KLF14	Kruppel-like factor 14
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholine
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein-kinase
Matsuda ISI	Matsuda Insulin Sensitivity Index
METSIM	Metabolic Syndrome In Men
MGL	Monoacylglycerol
mRNA	messenger Ribonucleic acid
MTNR1B	Melatonin receptor 1B

MUFA	Monounsaturated fatty acid
NGT	Normal glucose tolerance
NMR	Nuclear Magnetic Resonance
NOTCH2	Notch homolog 2 [Drosophila]
OGTT	Oral glucose-tolerance test
OR	Odds ratio
PAM1	Peptidylglycine alpha-amidating monooxygenase
PDK1	Phophoinositide-dependent protein kinase-1
PDX1	Pancreatic and duodenal homeobox 1
PI3-K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
PKB	Protein kinase B
PKC	Protein kinase C
PPARG	Peroxisome proliferator-activated receptor gamma
PRC1	Protein regulator of cytokinesis 1
PROX1	Prospero homeobox 1
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
SCD	Stearoyl coenzyme A desaturase
SE	Standard error
SFA	Saturated fatty acid
SLC2A2	Solute carrier family 2 member 2
SLC30A8	Solute carrier family 30 (zinc transporter), member 8
SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes
TCF7L2	Transcription factor 7-like 2
TG	Triglycerides
TAG	Triacylglycerol
TBC1D30	TBC1 domain family, member 30
THADA	Thyroid adenoma associated
TNFa	Tumor necrosis factor alpha
TP53INP1	Tumor protein p53 inducible nuclear protein 1
TSPAN8	Tetraspanin 8
VLDL	Very-low density lipoprotein
WFS1	Wolfram syndrome 1
WHO	World Health Organization
Wnt	Wingless-type MMTV integration site family

1 Introduction

Type 2 diabetes (T2D) is characterized by chronic hyperglycemia attributable to insulin resistance and pancreatic β-cell dysfunction. These metabolic disturbances lead to microvascular (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) (1). T2D is usually diagnosed in adulthood, although it is becoming prevalent in children and adolescents (2). Early onset of T2D will subsequently increase the burden of long-term complications of this disease (3).

T2D is a multi-factorial disease caused by the complex interaction between risk genes and environmental/lifestyle risk factors. The development of T2D is clearly linked with a family history of diabetes. The heritability of T2D is stronger in monozygotic twins than in dizygotic twins (4), and the prevalence of T2D is especially high in certain ethnic groups suggesting that T2D has a strong genetic basis. Recent genome-wide association studies (GWAs) have identified several loci associated with T2D and hyperglycemia, but the biological role of these variants found is often unknown. Studies of rare variants are likely to increase our knowledge on the missing heritability of T2D. Lifestyle factors, including smoking, aging, obesity, heavy alcohol consumption, low physical activity, and diet including low amount of fiber and high amount of saturated fat also contribute to the risk of T2D (5).

Increasing incidence and prevalence of T2D is a global health burden for all societies. The number of patients with T2D continues to increase, as economic development and urbanization lead to changes in lifestyles. According to the International Diabetes Federation, 371 million people have diabetes worldwide in 2012 and it is expected that this number will increase substantially up to 552 million by 2030. About 183 million people with diabetes are undiagnosed and diabetes is likely to become the fourth or fifth leading cause of death in the near future (www.idf.org). Therefore, the global epidemic of T2D clearly highlights the importance of changes in lifestyle and diet over the last several decades. It is apparent that early assessment and diagnosis would prevent or delay the incidence of T2D and also to minimize the occurrence of micro- and macrovascular complications.

The aim of present study was to identify metabolic and genetic biomarkers that could predict hyperglycemia and incident T2D. Furthermore, this series of studies has investigated the role of insulin sensitivity and insulin secretion as mediators for the association of metabolites with the deterioration of hyperglycemia and incident T2D.

2 Review of the Literature

2.1 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

The development of T2D is characterized by a progressive deterioration of glucose tolerance from normal glucose tolerance to abnormal glucose tolerance and finally to diabetes. T2D is diagnosed on the basis of elevated glucose levels and/or HbA1c level. According to the American Diabetes Association (ADA) criteria, the diagnosis of diabetes is based on elevated fasting plasma glucose (FPG) (≥ 7.0 mmol/L) or elevated 2-hour plasma glucose (2hPG) level (≥ 11.1 mmol/L) in an oral glucose tolerance test (OGTT) or elevated HbA1c levels (≥ 6.5 %) (6).

The two major pathophysiological abnormalities in T2D are impaired β-cell function and insulin resistance.

2.1.1 Insulin secretion

Insulin secretion is a highly dynamic process regulated by complex mechanisms. The pancreatic β-cell secretes a peptide hormone, insulin, the only blood glucose-lowering hormone in human metabolism. The insulin mRNA is translated as a precursor called proinsulin and inserted into the endoplasmic reticulum, and further processed to the biological active form inside secretory granules. Several intracellular signals, such as Ca^{2+}, ATP, cAMP, and diacylglycerol and inositol 1,4,5-triphosphate are involved in insulin secretion. Glucose is transported into the β-cells by *GLUT1* (encoded by *SLC2A1*) and *GLUT3* (encoded by *SLC2A3*) transporters (7). Upon transportation, an increase in glucose metabolism in the β-cell occurs. This involves an enhancement in the activity of glucokinase and generates a high concentration of the intracellular adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio. The resulting increase in the ATP/ADP ratio triggers the closure of the ATP-sensitive K^+ (K_{ATP}) channels and depolarizes the cell membrane. The activation of voltage-dependent Ca^{2+} channels causes an increase in Ca^{2+} entry into the β-cells, and the rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) which in turn stimulates insulin release (Figure 1) (8).

Insulin secretion in response to glucose exhibits a characteristic biphasic pattern, this consists of a rapid initiated and transient first phase followed by a sustained second phase during which insulin secretion continues at a somewhat lower rate but is still enhanced (9). Only a fraction of the β-cell insulin content is released during stimulation. The complete loss of the first phase insulin secretion and a marked reduction of the second phase insulin release in non-diabetic individuals are the early markers for the risk of T2D (10).

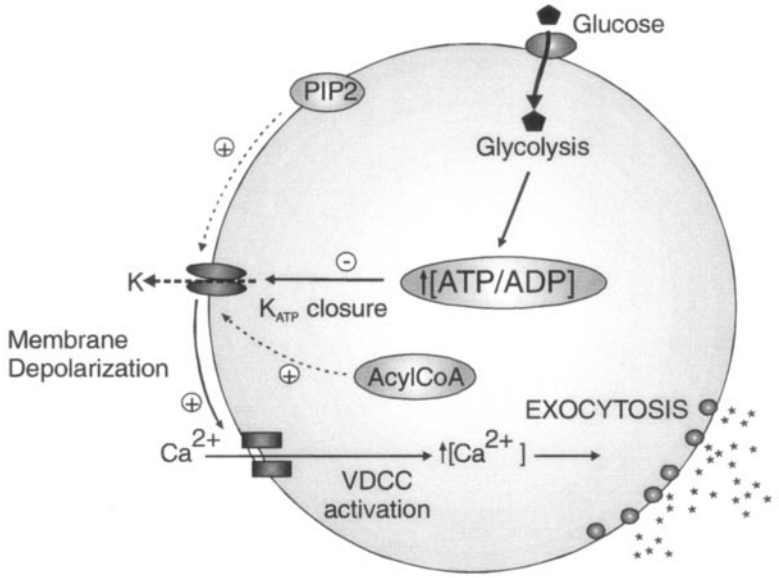

Figure 1. Regulation of insulin secretion (8). VDCC, voltage-dependent calcium channel; PIP2, phosphatidylinositol 4,5-bisphosphate.

Impaired insulin secretion is the major abnormality encountered in the pathogenesis of T2D. One consequence of the decline of early phase insulin secretion is impaired glucose tolerance, which leads to the development of post-prandial hyperglycemia which in turn impairs insulin secretion *via* glucotoxicity. Finally, the impairment of insulin secretion causes overproduction of endogenous glucose and this ultimately leads to the development of frank diabetes. Early phase insulin secretion is impaired in individuals with disturbed glucose homeostasis and in those at high risk for T2D. Impaired insulin secretion predicts T2D independent of insulin resistance in normoglycemic subjects (11). Defects in insulin secretion are attributable to a decrease in the sensitivity of the glucose receptor which transmits the glucose signal to trigger insulin release in the pancreatic β-cell (12). Genetic and environmental factors are the main determinants of insulin secretion. The offspring of patients with T2D show a defect in the first-phase insulin secretion in addition to insulin resistance (13). High levels of saturated fatty acids (FAs) impair insulin secretion and this leads to the deterioration of glucose tolerance (14).

2.1.2 Insulin resistance

Insulin resistance is defined as a reduction in insulin's ability to stimulate glucose uptake in peripheral insulin sensitive tissues. Insulin mediates its action by binding to the insulin receptor (IR) in three major tissues, skeletal muscle, liver and the adipose tissues. The IR undergoes autophosphorylation and it enhances tyrosine kinase activity. Activated receptors lead to the binding of various scaffold proteins, including the insulin receptor substrates (IRS). This, in turn, results in the activation of the insulin signalling pathway, where the phosphorylation of IRS proteins leads to their association with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-K). This results in the activation of p100 catalytic subunit of PI3-K that catalyzes the production of phosphoinositol lipids, including phosphatidylinositol 3,4,5-trisphosphates

[PI(3,4,5)P3], which further activates the Ser/Thr kinase 3-phosphoinositide-dependent protein kinase (PDK)-1. PDK-1 phosphorylates and activates other downstream kinases, including Akt and protein kinase C (PKC), which mediates translocation of GLUT4. This series of signals stimulates glucose uptake into the cells (Figure 2) (15).

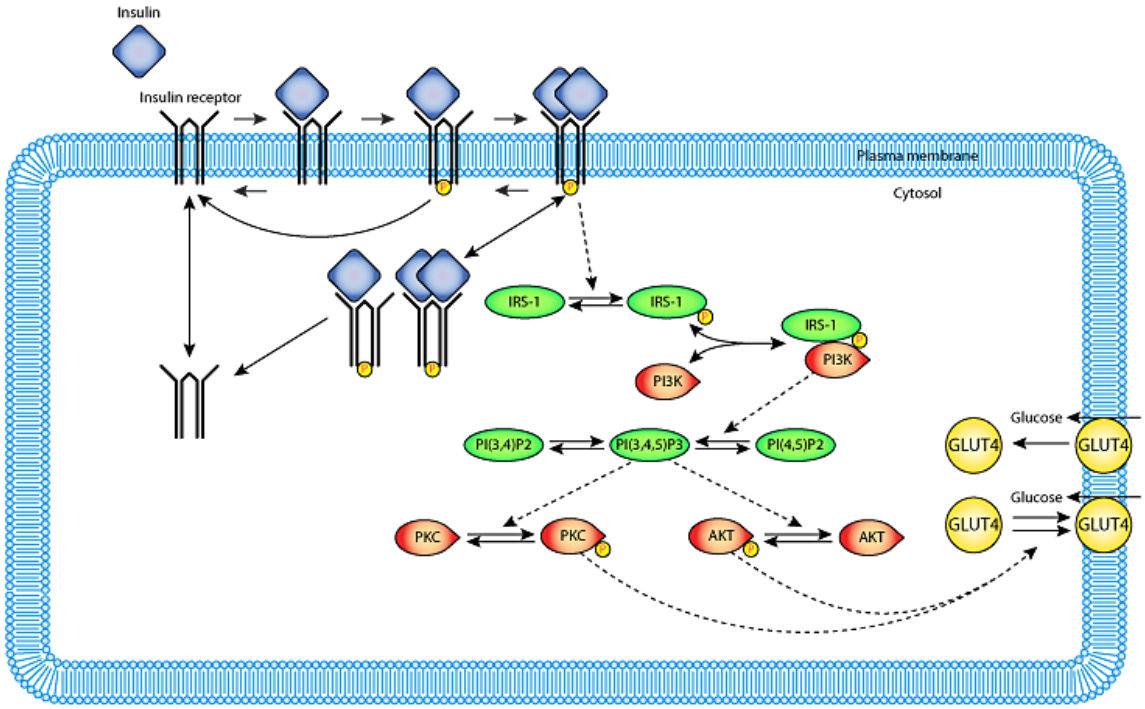

Figure 2. Insulin signaling pathway (15). PI(3,4)P2, phosphatidylinositol (3, 4)-bisphosphate; AKT, protein kinase B; GLUT4, glucose transporter type 4.

Since IRS molecules are key mediators in the insulin signaling pathway, IR-deficient mice develops severe diabetes resulting in FA infiltration of the liver and increased production of ketone bodies (16). The lack of *IRS-1* gene in primary adipocytes of mice showed decreased glucose transport and GLUT4 translocation in the membrane (17). GLUT4 is the main insulin-responsive glucose transporter, and mice deficient of GLUT4 exhibit moderate insulin resistance and impaired glucose tolerance but do not develop diabetes. However, the GLUT4 deficiency resulted in growth retardation, reduced fat tissue, cardiac hypertrophy and shortened lifespan (18). Defects in the insulin signaling pathway, such as impaired IRS tyrosine phosphorylation and reduced activation of PI 3-kinase/Akt signaling have also been demonstrated to be responsible for reduced glucose transport and glucose utilization in skeletal muscle and adipocytes (19).

In addition to the defects in the insulin signaling pathway, genetic predisposition, unhealthy diet, physical inactivity, accumulation of lipids in the liver and skeletal muscle contribute to insulin resistance.

2.1.2.1 Skeletal muscle insulin sensitivity

Glucose uptake into skeletal muscle is insulin dependent, and skeletal muscle accounts for about 60-70% of whole body glucose uptake (20). GLUT4-mediated glucose transport into

skeletal muscle is essential for the maintenance of normal glucose homeostasis, and it is activated by insulin and muscle contraction (21). Muscle specific inactivation of the insulin receptor results in severe insulin resistance and glucose intolerance (21). Insulin resistance in skeletal muscle is attributable to defects in the insulin signalling pathway, such as IRS-1 and PI3-K and Akt activation (22). Individuals with T2D exhibit reduced IR and IRS-1 phosphorylation and lowered PI3-K activity in skeletal muscle (23, 24). Skeletal muscle insulin resistance in genetically predisposed individuals manifests itself as impaired activation of PI3-K, IRS-1 and AKT (25).

2.1.2.2 Liver insulin sensitivity

Liver accounts for ~30% of whole body insulin-mediated glucose uptake and plays a key role in the maintenance of glucose homeostasis. Approximately 85% of glucose produced in the liver is derived from glycogen breakdown and gluconeogenesis. Insulin regulates both glycogenolysis and gluconeogenesis. Impaired insulin mediated suppression of hepatic glucose production leads to increased levels of plasma glucose and contributes to the pathogenesis of T2D. In addition, hepatic insulin resistance results in other abnormalities including hyperinsulinemia, increased β-cell stress, hyperglycemia, dyslipidemia and increased levels of inflammatory factors.

The accumulation of triglycerides (TGs) in the liver is responsible for hepatic insulin resistance (26). In the liver, an excess formation of diacylglycerols (DAGs) leads to the activation and translocation of PKCε in the membrane and consequently to the inhibition of insulin-stimulated insulin receptor kinase phosphorylation of IRS proteins which in turn downregulates the downstream insulin-signalling cascade. Intrahepatic accumulation of diacylglycerol mediates hepatic insulin resistance (27). It is known that defects in the IRS-1 and IRS-2 insulin receptor signalling pathways directly contribute to hyperglycemia and hepatic insulin resistance (28).

2.1.2.3 Adipose tissue insulin sensitivity

Adipose tissue accounts for ~10% of whole body glucose uptake. The primary role of adipose tissue is to store free fatty acids (FFAs) and release FFAs to ensure adequate energy level in the body. In the fed state, the upregulation of lipoprotein lipase (LPL) in adipose tissue hydrolyses chylomicron-associated TGs, and stimulates the uptake of FAs by adipose tissue. In the fasting state, FFAs originate almost entirely from the hydrolysis of TGs within adipocytes. Stored TGs are rapidly mobilized by the action of the three main lipases in the adipocyte: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL). HSL catalyzes the first and rate-limiting step in the mobilization of FFAs from adipose tissue (29, 30). In adipose tissue, HSL is activated by several hormones such as catecholamines, adrenocorticotropic hormone and glucagon via cAMP-dependent protein kinase A and inhibited by insulin (31, 32).

Insulin is the major regulator of LPL and HSL activity in the adipose tissue. Insulin upregulates LPL activity and promotes gene expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (33). Additionally, insulin prevents HSL activity in the adipose tissue through inhibition of its phosphorylation (34). In insulin resistant conditions, the responses of both LPL and HSL activities to insulin are blunted. LPL is associated with increased TG synthesis, and the ineffective suppression of HSL-mediated lipolysis in the adipose tissue causes an abnormal release of FFAs in plasma (35).

Adipose tissue releases hormones and cytokines that are involved in glucose metabolism, inflammation, and lipid metabolism. In obese subjects, levels of FFAs, TNF-alpha, IL-6, plasminogen activator inhibitor type 1 and C-reactive protein (CRP) are significantly increased, and the levels of adiponectin are low (36). Adiponectin increases insulin sensitivity by stimulating FA oxidation and inhibiting hepatic glucose production (37, 38). In obese individuals, the levels of TNF-alpha in the adipose tissue are increased and these contribute to insulin resistance (39) by inhibiting the genes involved in insulin signaling and adipocyte differentiation including CAAT-enhancer-binding protein-α, PPARG, GLUT4, IRS-1 protein, protein kinase B (PKB), adiponectin, and long-chain FA acyl-CoA synthase (38). Adipose tissue specific downregulation of GLUT4 can cause insulin resistance and thereby increase the risk of developing diabetes (40). Adipocytes from diabetic and insulin resistant individuals exhibit reduced GLUT4 translocation, reduced IRS-1 expression, impaired insulin-stimulated PI3-K and Akt/PKB (20). Elevated levels of TNF-alpha, combined with the elevation of IL-6, IL-1β, and CRP proteins are associated with incident diabetes (41). During fasting state, high levels of FFAs in plasma, a subsequent increase in the intake of FA by muscle and FA flux to the liver also increases and contributes to hepatic gluconeogenesis and ketogenesis.

2.2 DIETARY FAT AS A RISK FACTOR FOR TYPE 2 DIABETES

Essential FAs, such as omega-3 and omega-6 polyunsaturated FAs, trans-fatty acids, and saturated FAs (15:0, 17:0) are considered as reliable FA biomarkers. These FAs are derived only from diet and cannot be synthesized endogenously. Other saturated and monounsaturated FAs are derived either from the diet or are endogenously synthesized. The levels of these FAs vary considerably from day to day within an individual. Therefore, they cannot be considered as reliable biomarkers of dietary intake (42). The distribution of individual FAs can be measured in plasma, cholesterol esters, TGs, phospholipids, erythrocytes, platelets, various lipoprotein sub-fractions and adipose tissue.

Type of dietary fat intake might be a more important factor for the risk of diabetes than the total dietary fat intake (43). The increased intake of saturated FAs increases LDL cholesterol concentration. The replacement of saturated FAs by mono-or polyunsaturated FAs has been reported to lower the level of LDL cholesterol and to increase that of HDL cholesterol (44). A diet high in saturated FAs worsens insulin resistance (45). Randomized clinical studies have demonstrated that low-carbohydrate, low-glycemic index, Mediterranean, and high-protein diets can improve glycemic control and increase the HDL cholesterol level (46). In non-diabetic

men, a high intake of saturated FAs increases the risk of T2D (47). Similarly, a western diet and a high fat intake are also associated with incident T2D (48, 49).

2.3 GENETICS OF TYPE 2 DIABETES

T2D is a genetically heterogeneous disease and about 30-70% of the risk can be attributed to genetic factors. The pattern of inheritance suggests that multiple genes and different combination of genes are involved in T2D.

2.3.1 Heritability of type 2 diabetes

There is undisputed evidence that T2D is inherited. The prevalence of diabetes varies widely across different ethnic groups, due to underlying genes and different frequencies of predisposing alleles (50). T2D aggregates in the families. The concordance of T2D in monozygotic twins is ~70% compared with 20-30% in dizygotic twins (4). The risk of developing T2D is ~40% in the offspring if one parent has diabetes and 70% if both parents have diabetes (51, 52). A prospective study has demonstrated a twofold increased risk of incident T2D in subjects with a family history of diabetes (53).

2.4 APPROACH FOR GENETIC STUDIES IN TYPE 2 DIABETES

2.4.1 Linkage and candidate gene approach

Linkage analysis is undertaken to identify the regions of the genome that harbour genes which predispose to different diseases. Linkage analysis requires a large pedigree with many affected and unaffected individuals from several consecutive generations in the same homogenous population (54). With linkage studies, several loci for T2D have been identified on chromosome 20q and chromosome 1q (q21 – q23) (55-57). The exploration of chromosome 1q revealed the gene encoding transcription factor 7-like 2 (*TCF7L2*). This locus has been reported to confer the strongest effect on the risk of T2D, and this association has been replicated in several ethnic groups (58, 59).

The candidate gene approach focuses on the search for an association between disease and variants in or near biologically defined candidate genes which have been chosen based on their inferred physiological role in disease, especially in pathways involved in insulin secretion and insulin resistance. *PPARG*, *KCNJ11*, *WFS1*, *HNF1B* genes have been identified as risk genes for T2D using the candidate gene approach (60).

2.4.2 Genome wide association studies

Genome wide association studies (GWAs) are designed to find loci that fit the common disease-common variant hypothesis of human disease. This approach is unbiased with respect to the genome structure and previous knowledge of the disease etiology. The completion of the Human Genome Project in 2003 and the International HapMap Project in 2005 led to the identification of several million SNPs.

The first GWAs identified a zinc transporter and member of solute carrier family, *SLC30A8,* and *HHEX* as the first confirmed candidate genes for T2D and confirmed the association of *TCF7L2* and *KCNJ11* with T2D (61). These results provided evidence that the GWA approach was useful for identifying functionally relevant loci. Another three GWAs studies identified *CDKAL1*, *IGF2BP2*, and a variant near *CDKN2A-B* as novel T2D loci and confirmed the known T2D loci of *TCF7L2, KCNJ11, PPARG, SLC30A8*, and *HHEX (62-64).* The association of *FTO* with T2D was identified and subsequently confirmed in replication studies (65, 66). Additionally, meta-analyses have found six new loci *JAZF1, CDC123, TSPAN8, THADA, ADAMTS9*, and *NOTCH2* for T2D (67). Most of the T2D loci identified with GWAs were common variants conferring small effects.

2.4.3 Exome wide association studies

The exome sequencing approach is based on a common disease-rare variant hypothesis. It postulates that multiple rare variants with large effects sizes are the main determinants of heritability of the disease. Exome sequencing has been successful in the identification of mutations for rare Mendelian disorders (68). A few studies have demonstrated the benefit of applying large-scale exome sequencing approach for discovering variations associated with complex metabolic traits (69, 70). A recent study in Finnish individuals using the exome chip approach identified three new low-frequency loci in *TBC1D30, KANK1,* and *PAM* that were associated with insulin processing or insulin secretion (71). This study provided the first proof that exome-wide association studies are a powerful way for identifying low-frequency functional variants for complex diseases.

2.5 HYPERGLYCEMIA AND TYPE 2 DIABETES RISK LOCI IDENTIFIED BY GWAs

Altogether GWAs have identified >65 genetic variants associated with T2D (51). Figure 3 illustrates the year of discovery of all 65 loci associated with T2D and their effect sizes. However, for several of these variants, the biological role of the specific variant is unknown. Most of the common gene variants of T2D have been associated with β-cell function and not with insulin sensitivity (72).

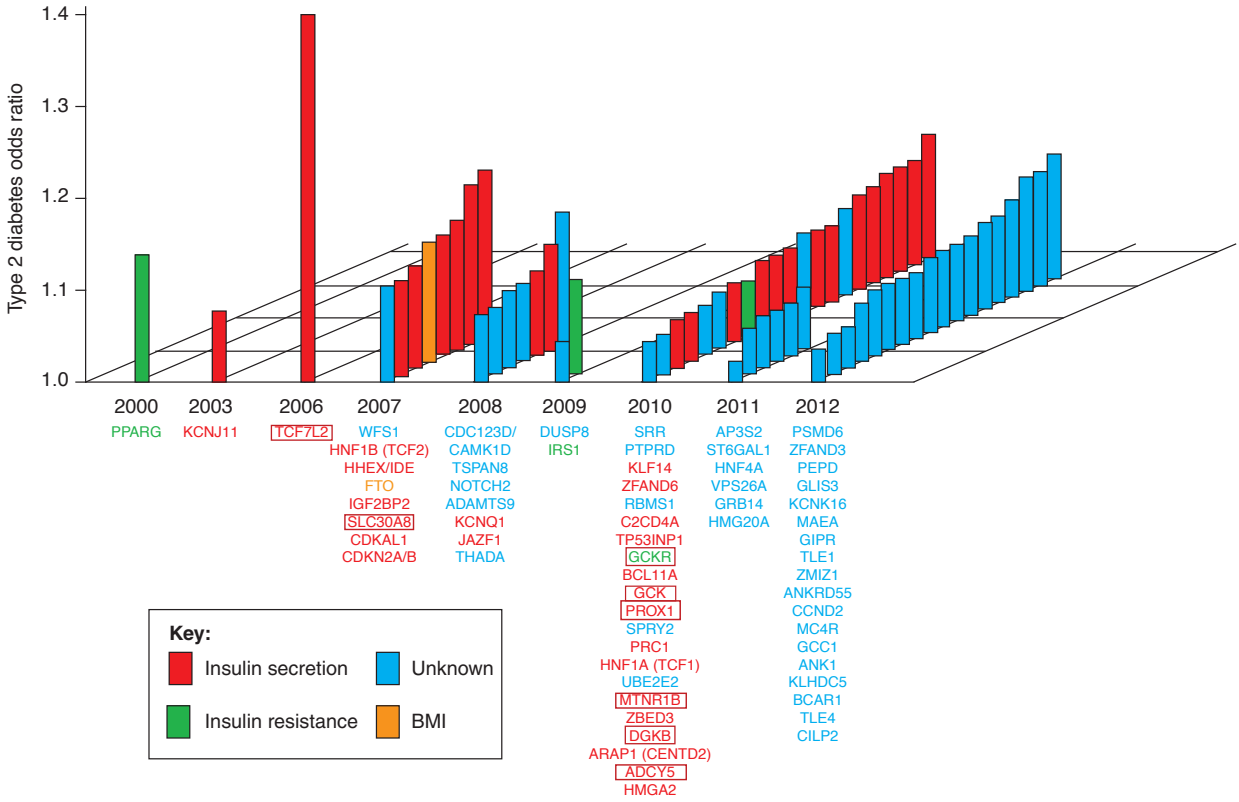

Figure 3. 65 T2D variants identified with GWAs (modified from (72)), the red rectangle represents genetic loci that are associated with both fasting glucose and T2D.

So far GWAs have reported 36 variants associated with fasting glucose levels (73-75), but the association of these variants with T2D has remained unclear. For example, *GCK*, *MTN1RB* and *G6PC2* are associated with fasting glucose with strong effect sizes, but they do not have any significant effects on the risk of T2D. In contrast, *TCF7L2* is the strongest candidate gene for T2D, but its effect on fasting glucose level is limited. Individual SNPs associated with hyperglycemia and T2D are summarized in detail in Table 1. Only the most important genes regulating the risk of T2D and hyperglycemia are discussed below.

2.5.1 Gene variants affecting insulin secretion

ARAP1 also known as *CENTD2* encodes ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1. *ARAP1* gene is known to regulate EGF-R trafficking and the signaling also involved in apoptosis (76, 77). The variant rs1552224 in *ARAP1* is associated with an increased risk of T2D and decreased glucose-stimulated insulin release (78, 79).

Table 1. Single nucleotide polymorphisms associated with T2D, insulin secretion, insulin sensitivity, obesity and other glycemic traits

Gene	SNP	Chromosomal Location	GWA trait	Other reported associations with phenotypes	References
Gene variants affecting insulin secretion					
ADCY5	rs11708067	3q21.1	FG, T2D	Decreased HOMA-B Decreased birth weight	(73) (80)
ADRA2A	rs10885122	10q25.2	FG		(81)
ARAP1/ CENTD2	rs1552224	11q13.4	T2D		(79)
BCL11A	rs10490072	2p21	T2D		(82)
CDKAL1	rs7754840	6p22.3	T2D	Impaired conversion of proinsulin to insulin Decreased birth weight	(83-85) (86)
CDKN2A/B	rs10811661	9p21	T2D		(87)
C2CD4A	rs7172432	15q22.2	T2D		(88)
C2CD4B	rs11071657	15q22.2	FG	Decreased HOMA-B	(73, 81)
DGKB	rs2191349	7p21.2	FG, T2D	Decreased HOMA-B	(73, 81)
FADS1	rs174550	11q12.2-q13.1	FG	Decreased HOMA-B	(73, 81)
GCK	rs4607517 rs1799884	7p15.3-p15.1	FG, T2D FG, T2D	Decreased HOMA-B	(73, 81)
G6PC2	rs560887	2q24.3	FG	Decreased HOMA-B Decreased T2D risk	(73, 81)
GLIS3	rs7034200	9p24.2	FG	Decreased HOMA-B	(73, 81)
HMGA2	rs1531343	12q15	T2D		(78)
HNF1A/ TCF1	rs7957197	12q24.31	T2D		(78)
HNF1B	rs7501939	17q12	T2D		(84)(89)
HHEX	rs1111875	10q23.33	T2D	Decreased birth weight	(86, 87, 90)
IGF2BP2	rs4402960	3q27.2	T2D		(63, 87, 91-93)
JAZF1	rs864745	7p15	T2D		(67, 94)
KCNJ11	rs5219	11p15.1	T2D	Increased glucagon level	(92, 95, 96)
KCNQ1	rs2237895	11p15	T2D		(97, 98)
KLF14	rs972283	7q32	T2D		(78)
MTNR1B	rs10830963	11q21–q22	FG, T2D		(92, 99)
MADD	rs7944584	11p11.1	FG	Impaired proinsulin to insulin conversion Decreased HOMA-B	(100) (73)
PROX1	rs340874	1q41	FG, T2D	Decreased Insulin sensitivity Decreased HOMA-B	(73, 81)
PRC1	rs8042680	15q26.1	T2D		(78)
SLC30A8	rs13266634	8q12.11	FG, T2D	Impaired proinsulin to insulin conversion	(61, 83, 101, 102)
SLC2A2	rs11920090	3q26.1-q26.2	T2D	Decreased HOMA-B	(73)
TP53INP1	rs896854	8q22	T2D		(78)
TCF7L2	rs7903146	10q25.3	FG, T2D	Impaired proinsulin to insulin conversion Decreased incretin effect Decreased glucagon level	(58, 83, 102, 103) (104)
ZBED3	rs4457053	5q13.3	T2D		(78)
ZFAND6	rs11634397	15q25.1	T2D		(78)
Gene variants affecting insulin sensitivity					
GCKR	rs780094	2p23	FG, T2D, FI, TGs		(73, 102)
IRS1	rs2943641	2q36	T2D		(105)
PPARG	rs1801282	3p25	T2D		(106)

Gene variants affecting obesity					
FTO	rs9939609	16q12.2	BMI,T2D	Decreased insulin sensitivity Increased fasting insulin	(66, 78)
Gene variants with unknown function					
ADAMTS9	rs4607103	3p14.3-2	T2D	Decreased insulin sensitivity Increased insulin secretion	(107)
ANKRD55	rs459193	5q11.2	T2D	Decreased insulin sensitivity	(108)
ANK1	rs516946	8p11.1	T2D	Decreased insulin secretion	(108)
AP3S2	rs2028299	15q26.1	T2D, BMI		(109, 110)
BCAR1	rs7202877	16q23.1	T2D	Decreased disposition index	(108)
CCND2	rs11063069	12p13	T2D		(111)
CDC123D/ CAMK1D	rs12779790	10p13	T2D	Decreased insulin secretion Decreased arginine stimulated insulin secretion	(82, 94)
CLIP2	rs10401969	19p13.11	T2D, TGs, LDL		(111)
CRY2	rs11605924	11p11.2	FG, T2D		(73)
DUSP8	rs5945326	11p15.5	T2D		(78)
GCC1	rs6467136	7q32.1	T2D		(112)
GIPR	rs10423928	19q13.3	2hPG, T2D	Decreased insulin secretion Increased fasting proinsulin	(75) (102)
GLIS3	rs7034200	9p24.2	FG, T2D,	Decreased HOMA-B Decreased fasting insulin Decreased insulin secretion	(73) (81)
GRB14	rs13389219	2q22-q24	T2D	Decreased Matsuda ISI Increased HOMA-B Increased fasting insulin	(108)
HMG20A	rs7178572	15q24	FG, T2D, BMI		(110)
HNF4A	rs4812829	20q13.12	T2D	Decreased β-cell function	(110)
KLHDC5	rs10842994	12p11.22	T2D		(111)
KCNK16	rs1535500	6p21.2-p21.1	T2D		(112)
MAEA	rs6815464	4p16.3	T2D		(112)
MC4R	rs12970134	18q22	T2D, TGs, BMI	Increased insulin resistance	(108, 111)
NOTCH2	rs10923931	1p13-p11	T2D	Decreased fasting insulin	(113)
PEPD	rs3786897	19q13.11	T2D		(112)
PSMD6	rs831571	3p14.1	T2D		(112)
PTPRD	rs17584499	9p23-p24.3	T2D		(114)
RBMS1	rs7593730	2q24.2	T2D	Decreased HOMA-IR	(115)
SPRY2	rs1359790	13q31.1	T2D		(116)
SRR	rs391300	17p13.3	T2D		(114)
ST6GAL1	rs16861329	3q27-q28	T2D, TGs, HDL-C	Decreased β-cell function	(110, 117)
TLE1	rs2796441	9q21.32	T2D		(78)
TLE4	rs13292136	9q21.32	T2D		(111)
TSPAN8	rs7961581	12q14.1-q21.1	T2D	Decreased insulin secretion	(94)
THADA	rs7578597	2p21	T2D	Decreased insulin secretion	(92)
UBE2E2	rs7612463	3p24.2	T2D	Decreased HOMA-B	(118)
VPS26A	rs1802295	10q21.1	T2D		(110)
WFS1	rs10010131	4p16.1	FG, T2D, HbA1c	Decreased insulin secretion	(53, 119, 120)
ZFAND3	rs9470794	15q25.1	T2D		(112)
ZMIZ1	rs12571751	10q22.3	2hPG, T2D		(108, 111)

FG, Fasting glucose; FI, Fasting insulin; 2hPG, 2 hour postprandial glucose; BMI, Body mass index; LDL, low density lipoprotein; HDL-C, High density lipoprotein-cholesterol; TGs; Triglycerides

CDKAL1 encodes a cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1. It is expressed in pancreatic islets, skeletal muscle and brain (63, 65). Pancreatic β-cell specific knockout of *CDKAL1* in mice displayed pancreatic islet hypertrophy, a reduction in insulin secretion, and impaired blood glucose control (121). Stančáková *et al*. reported that *CDKAL1* variant rs7754840 was associated with reduced first phase insulin secretion (84). GWAs and meta-analysis have revealed significant associations of *CDKAL1* variant rs7754840 with T2D in various ethnic groups (85, 122).

DGKB encodes diacylglycerol kinase beta (DGK) and it belongs to the intracellular lipid kinase family. DGK phosphorylates diacylglycerol to produce phosphatidic acid (PA), where PA regulates PKC. Activation of PKC leads to increased serine phosphorylation of the IRS-1 and this subsequently resulted in decreased insulin-stimulated glucose transport activity (123). The variant rs2191349 in *DGKB* was associated with decreased insulin secretion, increased fasting glucose and risk of T2D (73).

FADS1 encodes the fatty acid deta-5 desaturase (D5D), a key enzyme involved in the metabolism of long-chain polyunsaturated omega-3 and omega-6 FAs. The D5D enzyme is expressed mainly in the liver, brain, heart and lung (1). The SNP rs174548 was found to be associated with *FADS1* mRNA expression in the liver and rs174550 with low insulin secretion (73, 102), fasting glucose, insulin, HOMA-IR, HOMA-B, TGs and T2D (73).

HHEX encodes the transcription factor hematopoietically expressed homeobox protein which is essential for pancreatic and liver development and is also involved in the Wnt signaling pathway (124, 125). The variant rs1111875 in the 3'flanking region of *HHEX* has been associated with T2D risk and pancreatic β-cell dysfunction (87, 90).

IGF2BP2 encoding insulin-like growth factor II mRNA–binding protein 2, plays an important role in embryogenesis and pancreatic development (126). *IGF2BP2* belongs to a family of mRNA-binding proteins (IMP1, IMP2, and IMP3) and regulates the translation of IGF2 mRNA-binding protein family. This family is known to play a vital role in growth and insulin-signaling (127). A common variant rs4402960 in intron 2 of *IGF2BP2* has been associated with reduced early phase insulin secretion, impaired pancreatic β-cell function and an increased risk of T2D (63, 87, 91-93).

KCNJ11 encodes the potassium inwardly-rectifying channel, subfamily J, member 11 gene. It encodes protein K_{ATP} (Kir 6.2) and is highly expressed in the liver. Mutations in *KCNJ11* influence the K_{ATP} channel activity and impair insulin secretion in β-cells (128). A *KCNJ11* E23K (rs5219) variant was found to be associated with an increased risk of T2D, BMI and impaired glucose-induced insulin secretion (95).

KCNQ1 encoding potassium voltage-gated channel, KQT-like subfamily member 1 gene, is expressed in the heart, pancreas, kidneys and intestine (97, 129). The encoded protein plays a role in the electrical depolarization of the cell membrane in the heart and possibly also in pancreatic β-cells (130). Variants rs2283228 and rs2237895 of *KCNQ1* have been associated with T2D in Asian and European individuals (129).

MTNR1B encodes the melatonin receptor type 1B. The circadian rhythm of melatonin hormone influences insulin secretion and glucose homeostasis through its islet-specific receptor (131). The T allele of rs1387153 has been shown to be associated with increased FPG level and an increased risk of T2D (99, 132). The risk G allele of SNP rs10830963 has been associated with impaired insulin secretion (99). Exome sequencing has revealed that 36 rare variants (minor allele frequency < 0.1) of this gene were associated with T2D (133).

SLC30A8 encodes solute carrier family 30 (zinc transporter), member 8. It is highly expressed in the pancreatic islet β-cells (134). Deletion of *SLC30A8* exon 3 in mice has resulted in marked reduction of the zinc content in islets, reduced fasting insulin and impaired insulin secretion (135). The non-synonymous variant rs13266634 in *SLC30A8* causes an arginine to tryptophan change (Arg325Trp) and this has been associated with a decrease in the first phase insulin release and increased susceptibility for T2D (61, 101).

TCF7L2 encodes transcription factor 7-like 2. It is a member of the T-cell-specific high-mobility group (HMG) box-containing transcription factor, a key component of the Wnt-signaling pathway. Depletion of *TCF7L2* has resulted in increased β-cell apoptosis, decreased β-cell proliferation and glucose-stimulated insulin secretion. Variant rs7903146 in the third intron of *TCF7L2* has been shown to be significantly associated with low levels of insulin secretion and an increased risk of T2D (58, 103).

2.5.2 Gene variants affecting insulin sensitivity

PPARG encodes for peroxisome proliferator activated receptor gamma, one of the members of nuclear hormone regulating transcription factors. It is moderately expressed in skeletal muscle, liver, macrophages, brain and highly expressed in adipose tissue (136). *PPARG* is important for adipocyte differentiation and the expression of adipocyte-specific genes. Adipose tissue and muscle specific deletion of *PPARG* resulted in glucose intolerance and progressive insulin resistance in the adipose tissue, liver and skeletal muscle (137, 138). The missense variant Pro12Ala of *PPARG* has been associated with the risk of T2D (139, 140).

IRS1 encodes for insulin receptor substrate 1 and plays a major role in insulin signaling. Variant rs2943641, located adjacent to *IRS1*, has been associated with insulin resistance, hyperinsulinemia and T2D (105).

GCKR encodes for glucokinase regulatory protein and plays an important role in whole body glucose homeostasis in the liver (141). *GCKR* inhibits glucokinase (GCK) in competition with glucose substrate. A variant rs780094 in *GCKR* has been shown to be associated with several phenotypes including fasting glucose and insulin level, impaired fasting glucose, insulin secretion, reduced HOMA-IR, FFAs, serum TGs and the risk of T2D (62, 73, 142).

2.5.3 Gene variants affecting obesity

FTO encodes for fat mass and obesity associated gene. *FTO* is expressed in the hypothalamus, liver, muscle, adipose tissue and pancreatic β-cell (143). The rs9939609 variant resides within

the first intron of *FTO* has been shown to be associated with obesity, reduced insulin sensitivity, and T2D (66, 78).

2.6 METABOLOMICS

Metabolomics is the study of global metabolite profile in the various cells, tissues, organs or biological fluids. It measures the chemical phenotypes that are the net results of genomic, transcriptomic and proteomic variability, therefore providing the most integrated profile of biological status. Metabolomics is used to discover new diagnostic markers and to enhance better understanding of disease mechanisms.

2.6.1 Untargeted metabolomics

The untargeted metabolomics approach has the ability to detect and quantify a broad range of both known and unknown metabolites and reveal potential metabolites linking cellular pathways to biological mechanisms. This approach makes it possible to assess a large number of metabolites that are substrates and products in different metabolic pathways. Using this approach, differences in concentrations of a wide range of metabolite profiles, such as bile acids, urea cycle intermediates, purine degradation products, glutamine, glutamate, FFAs, acylcarnitines, lysophosphatidylcholines and other small molecules can been measured e.g. before and after a glucose load (144, 145). In obese individuals, several metabolites (FFAs, TGs, amino acids, C3 and C5 acylcarnitine, glutamate, pyruvate) have been reported to be elevated as compared to those of lean subjects indicating that branched-chain amino acids (BCAAs) contribute to the development of obesity-associated insulin resistance (146).

Longitudinal studies have revealed that plasma branched-chain and aromatic amino acids are new predictors of the development of T2D (147). A 12-year follow-up study on lipid profiling during a 2-hour glucose tolerance test found that TGs of low carbon number and the double bond content were associated with an increased risk of T2D. These lipids were elevated in insulin resistance whereas TGs of high carbon number and the double bond content were poorly correlated with insulin resistance (148). The TwinsUK Study assessed metabolites before and after hyperglycemia and identified that glucose, mannose, FFAs, and amino acids (BCAAs, valine, isoleucine, leucine, and their branched-chain-keto-acid, 3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate and 3-methyl-2-oxobutyrate) were associated with IFG and T2D. Adrenate and arachidonate levels were elevated in IFG whereas dodecenoate, heptanoate and pelargonate were decreased in subjects with T2D (149).

2.6.2 Targeted metabolomics for biomarker discovery

Targeted metabolomics has an excellent potential in the identification of new biomarkers as well as in the validation of identified biomarkers. By using this approach, specific metabolites levels which are chemically characterized and biochemically annotated can be measured by the liquid chromatography, nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry.

These techniques have their own advantages but some disadvantages in capturing the metabolites of interest. In the candidate biomarker discovery study, glycine, lysophosphatidylcholine (LPC) (18:2) and acetylcarnitine were found to be significant predictors of impaired glucose tolerance and T2D (150). A recent targeted metabolomics study reported that sugar metabolites, amino acids and choline-containing phospholipids were associated with the risk of T2D (151).

Glucose, HbA1c and insulin are well known biomarkers for T2D. Recently several new and emerging metabolic biomarkers for T2D and glycemia were reported, including ferritin, leptin, adiponectin, CRP, interleukin-2 receptor A, interleukin 6, interleukin-18, plasminogen activator inhibitor-1, apolipoprotein B, serum γ-glutamyl transferase, plasma fetuin-A, plasma levels of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and tissue plasminogen activator (152-160). A recent longitudinal study has indicated that plasma levels of alanine, leucine, isoleucine, tyrosine, and glutamine predict incident T2D (161). Furthermore, the concentration of leucine, valine, and phenylalanine predicted insulin resistance (162), and alanine, lactate, and pyruvate predicted the levels of 2-hour glucose (163).

2.6.2.1 Glycerol, free fatty acids and fatty acids

In the 1950's Gordon, reported that plasma FFAs are mainly released from the adipose tissue and are utilized by metabolically active tissues, such as skeletal muscle and liver (164). Glycerol and FFAs in the plasma are the two main components released from TGs by lipolysis in the adipose tissue (Figure 4). Glycerol acts as a gluconeogenic substrate and thus regulates glucose homeostasis. In the fasting state, elevated levels of FFAs almost entirely originate from the hydrolysis of TGs in the adipose tissue. The stored TGs are rapidly mobilized by the action of the three main lipases of the adipocyte: ATGL, HSL and MGL.

Circulating glycerol and FFAs levels are also regulated by obesity, physical activity, starvation, hormonal factors, short- and long term dietary intake and multiple pathological conditions, e.g. abnormal glucose tolerance and metabolic syndrome (165, 166). Insulin plays a vital role in regulating the levels of glycerol and FFAs by inhibiting lipolysis in the adipose tissue, explaining why glycerol and FFA concentrations are reduced after a meal that contains carbohydrate, which stimulate insulin secretion. In insulin resistance states, an increased amount of lipolysis of stored TG molecules in the adipose tissue produces high amount of glycerol and FAs (167, 168). Glycerol is a gluconeogenic substrate and stimulates gluconeogenesis (169). Elevated levels of glycerol and FFAs have been shown to be associated with hyperglycemia and T2D (170, 171). A few prospective studies have reported that high levels of fasting FFAs and TGs are predictors of incident T2D (172-176).

Polyunsaturated omega-3 and omega-6 fatty acids (PUFAs) are derived from the diet and cannot be synthesized *de novo* in humans. In contrast, monounsaturated omega-7 and omega-9 fatty acids (MUFAs) are derived from the *de novo* synthesized saturated FAs. Thus, the serum FA profile is determined by both diet and endogenous FA metabolism (42). The serum lipid

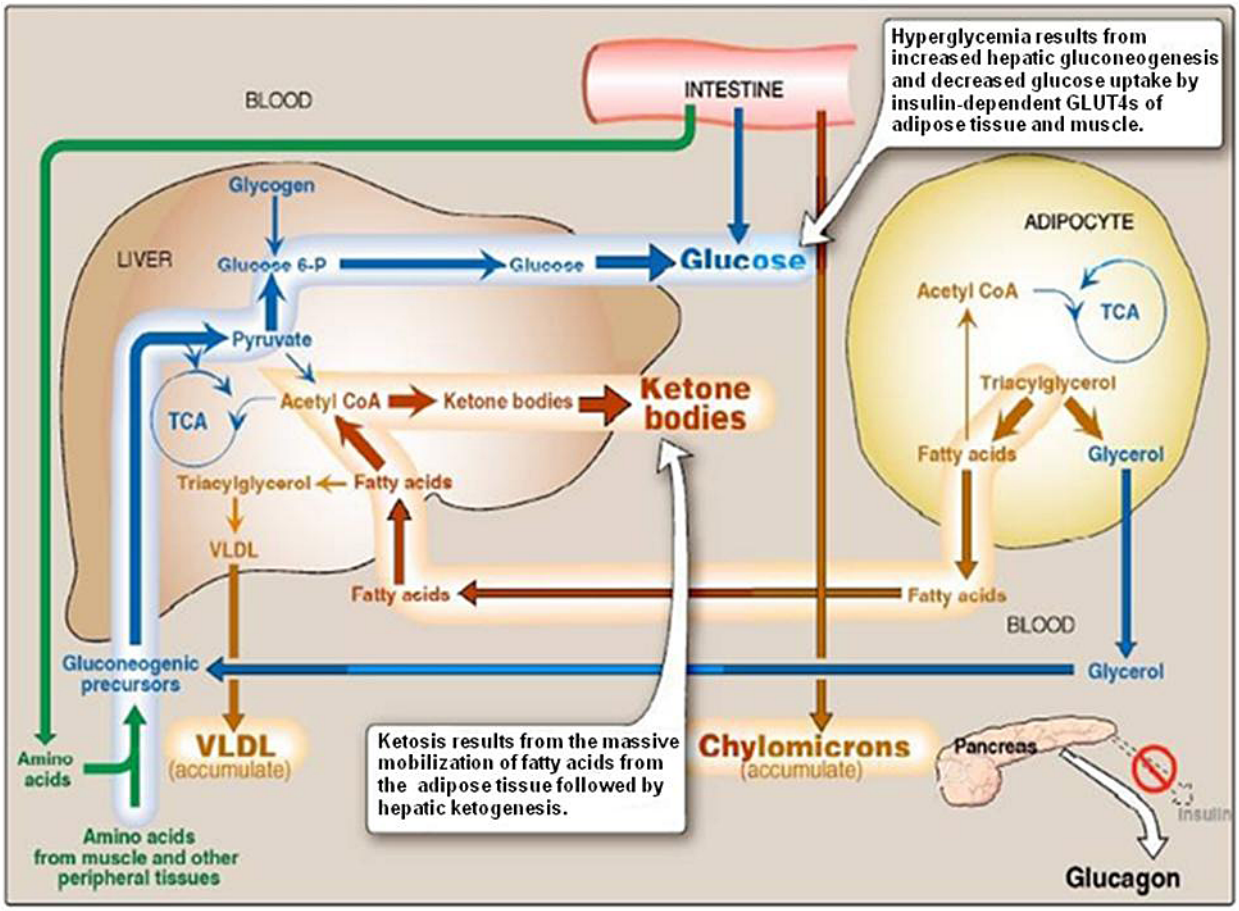

Figure 4. Metabolic pathways (adapted from richsen.wordpress.com). Glucose 6-P, glucose 6-phosphate; TCA, tricarboxylic acid cycle; VLDL, very low-density lipoprotein; Acetyl CoA, acetyl coenzyme A.

esters reflect the intake of individual FAs over the last few weeks. Endogenously synthesized serum FA pattern is mainly influenced by genetic disposition and intrauterine programming (177). A high intake of saturated FAs increases the risk of hyperglycemia and T2D (178). However, high concentrations of polyunsaturated omega-3 FAs have not predicted the lowering of the risk of T2D (179-181). A meta-analysis including 26 studies concluded that marine omega-3 FAs (including docosahexaenoic acid, DHA) in the diet did not exert beneficial effects on the prevention of T2D, with the exception of benefits for Asian populations (182). Omega-3 and omega-6 FAs have been associated with improved insulin sensitivity in individuals with T2D. In contrast, saturated FAs have been reported to impair the action of insulin (183, 184). Monounsaturated omega-7 and omega-9 FAs have been previously shown to increase the risk of T2D (185-187).

2.6.2.2 Erythrocyte membrane fatty acids

The FA composition is predominantly determined by long term dietary FAs intake of the order of 120 days and endogenous synthesis of FA. It can be measured in various tissues and lipid pools and erythrocyte membrane (188, 189). Membrane phospholipids reflect serum FA profiles of saturated and monounsaturated FAs but they may also contain lower levels of omega-3 and omega-6 FAs (190). Erythrocyte membrane lacks *de novo* FA synthesis and modification by

desaturation or elongation, and it mirrors long-term FA intake, whereas plasma FAs and TG fractions represent dietary intake only of the past few days (191, 192). Delta-6 desaturases (D6D) and D5D are required for the synthesis of the highly unsaturated FAs by introducing a double bond in the long chain FAs. Stearoyl coenzyme A desaturase (SCD) catalyzes the synthesis of monounsaturated FAs (MUFA) from saturated FAs (SFAs) (193).

The FA synthesis pathway produces saturated FAs, which can then be elongated and desaturated to generate FAs such as palmitoleic acid, oleic acid, or vaccenic acid. Activities of desaturases are difficult to measure directly in large-scale epidemiological studies, and therefore as an alternative, enzymatic conversions are estimated by the FA product-to-precursor ratios (194). Most of the case-control studies have reported that the levels of individual erythrocyte membrane fatty acids (EMFAs), desaturase and elongase activities are elevated in individuals with T2D than in control subjects (195-201). Furthermore, prospective studies have identified EMFAs and desaturase activities as predictors for incident T2D (181, 202, 203).

2.6.2.3 Ketone bodies

The two main ketone bodies (KBs), 3-hydroxybutyrate (BHB) and acetoacetate (AcAc) play a vital role in serving as a major source of body fuel in the fasting state. The low availability of carbohydrates enables fat-derived energy to be generated in the liver which is utilized by many organs, such as brain, heart, kidney and skeletal muscle. For instance, after an overnight fast, KBs supply 2-6% of the body energy requirement, whereas after a 3-day fast, they supply 30-40% of energy requirements (204).

During fasting, increased lipolysis in the adipose tissue results in the release of FFAs into the plasma (Figure 4). FFAs are degraded through β-oxidation in the liver mitochondria, resulting in the production of acetyl-CoA. Acetyl-CoA is then either incorporated into the tricarboxylic acid cycle or channeled into the ketogenesis pathway. Ketogenesis takes place in the liver, stimulated by an excess FFA availability in the liver. Insulin plays a central role in regulating KB levels and inhibiting ketogenesis by triggering dephosphorylation of HSL and hindering the breakdown of TGs to FFAs and glycerol. Ketogenesis takes place mainly during a state of insulin deficiency and glucagon excess (205, 206).

Abnormal KB levels have been implicated in diabetic ketoacidosis due to the impairment in insulin secretion (207, 208). Elevated levels of KBs have been shown to be associated with insulin resistance and T2D (209). In contrast, a recent study using a metabolomics approach revealed that the levels of KBs were associated with increased insulin sensitivity (210). Furthermore, a strong correlation has been found between plasma glucose and FFA with KB levels (211). Several small studies reported that KB levels were decreased in obese women compared to lean women, and KBs levels were higher in obese individuals with abnormal glucose tolerance than in obese individuals with normal glucose tolerance (212, 213).

3 Aims of the Study

The main aim of this study was to identify biomarkers for the development of hyperglycemia and incident type 2 diabetes based on a 5-year follow-up of the population-based METSIM cohort, and to investigate the significance of insulin sensitivity and insulin secretion in these associations.

The specific aims were:

1) To investigate the levels of fasting glycerol, FFAs and serum FAs as predictors for the worsening of hyperglycemia and incident T2D

2) To investigate the proportions of EMFAs and their ratios as predictors for the worsening of hyperglycemia and incident T2D

3) To investigate the levels of ketone bodies as predictors for the worsening of hyperglycemia and incident T2D, and to investigate the association of single nucleotide polymorphisms regulating hyperglycemia or the risk of T2D with the levels of KBs.

4 Subjects, Materials and Methods

4.1 SUBJECTS

The original METSIM cohort includes 10,197 Finnish men with varying degrees of glucose tolerance at baseline. Subjects included in this study were randomly selected from the population register of Kuopio town, Eastern Finland. The cross-sectional Studies I-III included only non-diabetic subjects and individuals with newly diagnosed T2D (none of the participants were receiving antidiabetic medication) and the follow-up study included only non-diabetic individuals at baseline (Figure 5).

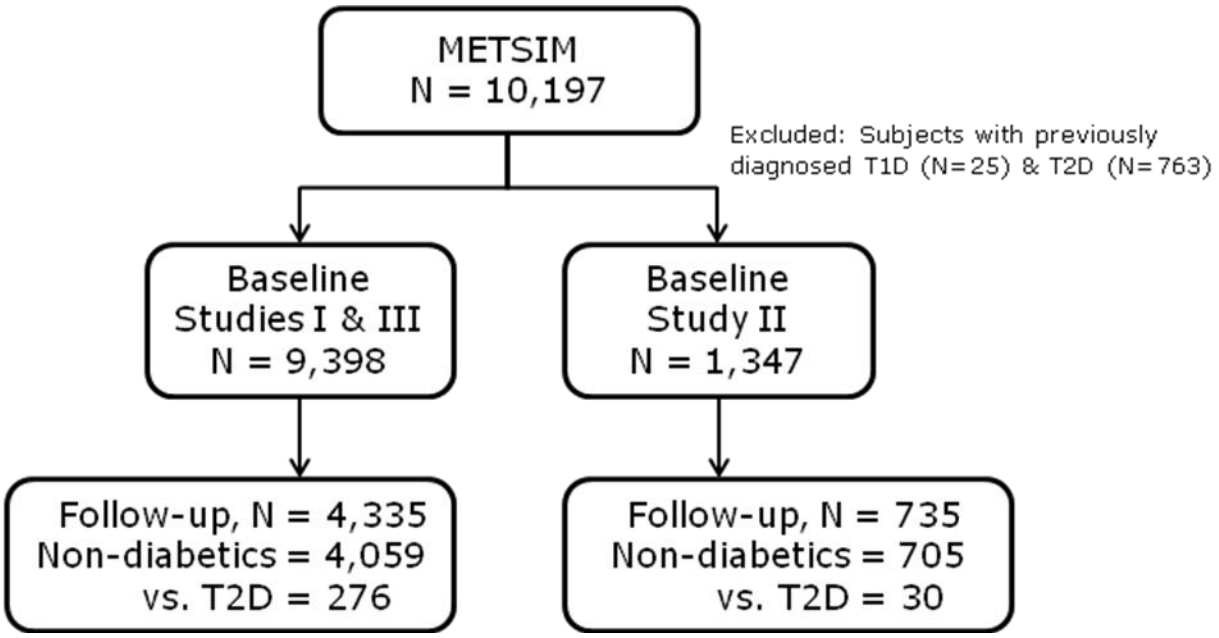

Figure 5. Study subjects

4.1.1 Baseline study

Studies I & III. The cross-sectional analysis included 9,398 Finnish men from a population-based METSIM (METabolic Syndrome In Men) study performed during 2005-2010 (age 57±7 years, BMI, 27.0±4.0 kg/m^2, mean ± SD). Characteristics of the subjects included in the baseline studies are given in Table 2. Glucose tolerance was classified according to the ADA criteria (6). Among participants 3,034 (32.3%) had normal glucose tolerance, NGT; 4,344 (46.2%) had isolated impaired fasting glucose, IFG; 312 (3.3%) had isolated impaired glucose tolerance, IGT; 1,059 (11.3%) had both IFG and IGT, and 649 (6.9%) had newly diagnosed T2D). Individuals with previously diagnosed type 1 or type 2 diabetes were excluded from all statistical analyses.

Table 2. Characteristics of the participants included in the METSIM study

Variable	No of Cases	Mean ± SD	Range
Age, years	9,398	57.3 ± 7.1	45 - 74
Body mass index, kg/m^2	9,395	27.0 ± 4.0	16.2 - 55.4
Fasting glucose, mmol/L	9,398	5.8 ± 0.7	3.5 - 20.0
2h plasma glucose, mmol/L	9,396	6.4 ± 2.4	1.4 - 38.2
Fasting insulin, pmol/L	9,394	52.3 ± 39.3	6.0 - 611.4
2h insulin, pmol/L	9,386	334.9 ± 345.8	10.8 - 5191.2
Matsuda Insulin Sensitivity Index, mg/dL, mU/L	9,337	6.7 ± 4.2	0.5 - 42.5
Insulin AUC_{0-30} / Glucose AUC_{0-30}, pmol/mmol	9,343	30.7 ± 21.3	1.95 - 313.3
Glycerol x 100, mmol/L	9,349	6.1 ± 2.6	0.0 - 27.3
Fasting free fatty acids x 10, mmol/L	9,395	3.7 ± 1.5	0.6 - 17.8
Total triglycerides, mmol/L	9,397	1.4 ± 1.0	0.3 - 37.6
Omega-3 fatty acids, percentage of total FAs	9,285	4.5 ± 1.4	1.5 - 16.3
Docosahexaenoic acid, percentage of total FAs	9,282	1.9 ± 0.7	0.0 - 6.1
Omega-6 fatty acids, percentage of total FAs	9,285	32.9 ± 4.3	12.8 - 47.7
Linoleic acid, percentage of total FAs	9,280	27.9 ± 4.4	8.9 - 42.3
Monounsaturated fatty acids, percentage of total FAs	9,285	30.3 ± 4.1	11.0 - 53.2
Saturated fatty acids and omega-7&-9 fatty acids, percentage of total FAs	9,285	62.6 ± 4.5	49.5 - 85.3
Acetoacetate, mmol/L	9,243	0.06 ± 0.04	0.0 - 0.58
β-hydroxybutyrate, mmol/L	9,307	0.14 ± 0.11	0.0 – 2.67

SD, standard deviation; AUC, area under the curve; FAs, fatty acid.

Study II. EMFAs were measured in 1,346 Finnish men (age 55 ± 6years, BMI, 26.5 ± 3.5 kg/m^2, mean ± SD). A total of 1,346 men for the EMFA analysis were selected randomly with equivocal percentages of each glucose category as compared to the original METSIM cohort of 10,197 men. Baseline characteristics of the study participants are shown by means and SDs (Table 3). Glucose tolerance category was classified according to the ADA criteria (6) as follows: 456 (33.9%) had normal glucose tolerance, NGT; 681 (50.6%) had isolated impaired fasting glucose, IIFG; 32 (2.4%) had isolated impaired glucose tolerance, IIGT; 118 (8.8%) had both IFG and IGT, and 59 (4.4%) had newly diagnosed T2D. Individuals with previously diagnosed type 1 or type 2 diabetes at baseline were excluded from this study.

Table 3. Clinical and laboratory characteristics of the cross-sectional METSIM cohort

Variable	No of Cases	Mean ± SD	Range
Age, years	1,347	54.9 ± 5.7	45 - 70
BMI, kg/m^2	1,346	26.5 ± 3.5	17.4 - 48.1
Fasting glucose, mmol/L	1,347	5.8 ± 0.6	4.1 - 13.7
2h-glucose, mmol/L	1,346	6.0 ± 2.1	2.2 - 21.1
Fasting Insulin, pmol/L	1,346	45.5 ± 31.2	6.6 - 370.8
2h insulin, pmol/L	1,344	268.7 ± 268.5	19.2 - 2111.4
Matsuda Insulin Sensitivity Index	1,341	7.5 ± 4.4	0.5 - 27.7
Insulin $AUC_{0\text{-}30}$ / Glucose $AUC_{0\text{-}30}$, pmol/mmol	1,343	28.1 ± 18.0	2.4 - 166.6
Disposition index (DI30)	1,341	164.7 ± 72.8	16 - 588.3
16:0 (palmitic acid), %	1,346	22.5 ± 0.9	19.6 - 25.9
18:0 (stearic acid), %	1,346	15.5 ± 0.5	13.4 - 17.5
Total SFAs, %	1,346	46.0 ± 1.0	43.2 - 49
16:1n-7 (palmitoleic acid), %	1,346	0.4 ± 0.2	0.1 - 1.9
18:1n-7 (vaccenic acid), %	1,346	1.1 ± 0.1	0.8 - 2.6
18:1n-9 (oleic acid), %	1,346	11.9 ± 0.8	9.6 - 15.4
Total MUFAs, %	1,346	19.5 ± 1.0	16.3 - 26.4
18:2n-6 (linoleic acid), %	1,346	8.3 ± 1.1	4.0 - 12.9
20:3n-6 (dihomo-gamma-linolenic acid), %	1,346	1.5 ± 0.3	0.8 - 3.2
20:4n-6 (arachidonic acid), %	1,346	11.9 ± 1.1	8.1 - 15.1
20:5n-3 (eicosapentaenoic acid), %	1,346	1.5 ± 0.6	0.4 - 4.9
22:4n-6 (adrenic acid), %	1,346	1.9 ± 0.4	0.6 - 3.5
22:6n-3 (docosahexaenoic acid), %	1,346	6.2 ± 1.1	2.4 - 10.6
Total PUFAs, %	1,346	34.5 ± 1.2	27.9 - 38.2
Ratio (16:1n-7/16:0) (SCD1)	1,346	0.018 ± 0.006	0.007 - 0.078
Ratio (20:3n-6/18:2n-6) (Δ^6 desaturase)	1,346	1.7 ± 0.3	1.0 - 3.2
Ratio (20:4n-6/20:3n-6) (Δ^5 desaturase)	1,346	8.1 ± 1.6	3.5 - 13.7
Ratio (18:1n-7/16:1n-7) (elongase)	1,346	3.0 ± 0.9	0.8 - 8.5

SD, standard deviation; AUC, area under the curve; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCD1, stearoyl coenzyme A desaturase 1.

4.1.2 Follow-up study

Studies I & III. In the period 2010-2013, a total of 4,335 non-diabetic subjects from the original METSIM cohort of 10,197 men had been so far re-examined (mean follow-up time of 5 years); 4,059 were non-diabetic and 276 had newly diagnosed T2D at follow-up. The diagnosis of new diabetes was based either on an OGTT at the follow-up study or drug treatment for diabetes started between the baseline and follow-up studies.

Study II. The analysis of the prospective ongoing 5-year follow-up study (between 2010-2013) included only men who were non-diabetic at the baseline study. Thus, individuals with

previously diagnosed T2D were excluded. A total of 735 non-diabetic individuals with EMFA measurements at baseline have so far participated in the follow-up study of whom 705 remained non-diabetic and 30 developed newly diagnosed T2D (7 of them were diagnosed with T2D between the baseline and the follow-up studies and all of them were receiving anti-diabetic medication; 23 had newly detected T2D in an OGTT performed at the 5-year follow-up visit).

All the studies were approved by the Ethics Committee of the University of Eastern Finland and Kuopio University Hospital, and were conducted in accordance with the principles of the Helsinki Declaration. All study participants provided written informed consent.

4.2 MATERIALS AND METHODS

Anthropometric measurements

Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (kg) divided by height (m) squared. Waist and hip circumference were measured to the nearest 0.5 cm.

Oral glucose tolerance test

A 2-hr OGTT (75 g of glucose) was performed and samples for plasma glucose and insulin were drawn at 0, 30, and 120 min.

Laboratory measurements

Plasma glucose was measured by enzymatic hexokinase photometric assay and plasma insulin was determined by immunoassay. In Study I, serum total TG levels and FFAs were measured by enzymatic colorimetric methods. NMR spectroscopy was used to measure fasting glycerol, FFAs and serum FA profile (omega-3 FAs, omega-6 FAs, omega-7 and -9 FAs, saturated FAs, total FAs, linoleic acid (LA), other polyunsaturated FAs, docosahexaenoic acid (DHA), and monounsaturated FAs, relative to total FAs) (214). The results for FAs are expressed relative to total FAs and given as percentages in all tables and figures. In Study II**,** EMFAs were determined as previously described (215), with modifications (216). EMFAs were measured by gas chromatography. The proportion of each FA is expressed as mole percentage of total FAs in all tables. In Study III**,** NMR spectroscopy was used to measure fasting levels of acetoacetate (AcAc) and β-hydroxybutyrate (BHB) levels (mmol/L) in serum samples. NMR methods have been previously described in detail (214).

Calculations

The trapezoidal method was used to calculate the glucose and insulin areas under the curve (AUC) in an OGTT based on samples collected at 0, 30, and 120 min. The calculation of insulin sensitivity (Matsuda ISI), insulin secretion ($InsAUC_{0\text{-}30}/GlucAUC_{0\text{-}30}$) and disposition (DI30) indices have been previously described (217, 218). In Study II, desaturase and elongase enzyme

activities were evaluated as EMFA product to precursor ratios as follows: palmitoleic acid 16:1n-7/palmitic acid 16:0 as a marker of stearoyl coaenzyme A desaturase 1 (SCD1) activity, dihomo-gamma-linolenic acid 20:3n-6/linoleic acid 18:2n-6 as a marker of Δ^6 desaturase (D6D) activity, arachinodic acid 20:4n-6/20:3n-6 as a marker of Δ^5 desaturase (D5D) activity, and vaccenic acid 18:1n-7/16:1n-7 as a marker of elongase activity.

Genotyping

In Study III, the genotyping of 62 SNPs associated with the risk of T2D or hyperglycemia (63, 73, 111, 219) was primarily based on Illumina HumanExome-12v1_A Beadchip (71). SNPs that were not available from the exome array were genotyped using either the Applied Biosystems TaqMan Allelic Discrimination Assay or Sequenom iPlex Gold SBE assay. All SNPs were in Hardy-Weinberg equilibrium at the significance level corrected for multiple testing by the Bonferroni method ($P < 0.0012$).

Gene expression analysis

In Study III, subcutaneous fat biopsy samples (N = 200) were obtained from a random sample of the participants of the METSIM baseline study (age 55.6 ± 4.9 years; BMI 26.6 ± 3.3 kg/m^2). Total RNA was isolated from these samples using Qiagen miRNeasy Kit. Only high-quality samples were used for transcriptional profiling with the Illumina Human HT-12 v3 Expression BeadChip.

Statistical analysis

Statistical analyses were conducted using SPSS version 19 (SPSS, Chicago, IL). All traits, except for age were log-transformed to correct for their skewed distributions. In Studies I-III, glycerol, FAs, EMFAs proportions and KBs were compared across the FPG and 2hPG categories using the general linear model. The linear regression model was used to evaluate fasting levels of glycerol, FAs, EMFAs proportions and KBs measured at baseline as predictors for changes in Glucose AUC in an OGTT at the 5 year-follow-up study. Unstandardized effect sizes (B [SE]) per copy of the risk alleles of the SNPs investigated were estimated by linear regression analysis. Logistic regression analysis was used to assess the association of the levels of glycerol, FAs, EMFAs proportions and KBs with incident T2D during the follow-up.

The thresholds of statistical significance in linear and logistic models were $P < 5.5 \times 10^{-3}$ (Study I), $P < 2.8 \times 10^{-3}$ (Study II), $P < 4.0 \times 10^{-4}$ (Study III). $P < 0.05$ was considered as nominally significant in all studies. Statistical power calculation was performed in all studies using Bioconductor's GeneticsDesign package version 1.14. Pearson correlation coefficients for adipose tissue mRNA expression levels of major enzymes involved in the synthesis and degradation of KBs were calculated with insulin sensitivity and insulin secretion.

5 Results

5.1 GLYCEROL AND FATTY ACIDS IN SERUM PREDICT THE DEVELOPMENT OF HYPERGLYCEMIA AND TYPE 2 DIABETES (*Study I*)

This study evaluated first the association of glycerol, FFAs and serum FAs in non-diabetic individuals (N=8,749) and individuals with newly diagnosed T2D (N=649) in the FPG and 2hPG categories in a cross-sectional setting. Secondly, these biomarkers were evaluated as predictors for the worsening of hyperglycemia and the conversion to type 2 diabetes among non-diabetic individuals at baseline in a 4.5-year follow-up study.

Glycerol and FFAs across the categories of glucose tolerance at baseline

Fasting glycerol levels were significantly higher across the FPG (P = 4.5x10^{-28}) and 2hPG (P = 1.2x10^{-99}) categories. Similarly, fasting FFAs levels were significantly higher across the entire range of FPG (P = 4.3x10^{-51}) and 2hPG (P = 2.2x10^{-217}) (Figure 6).

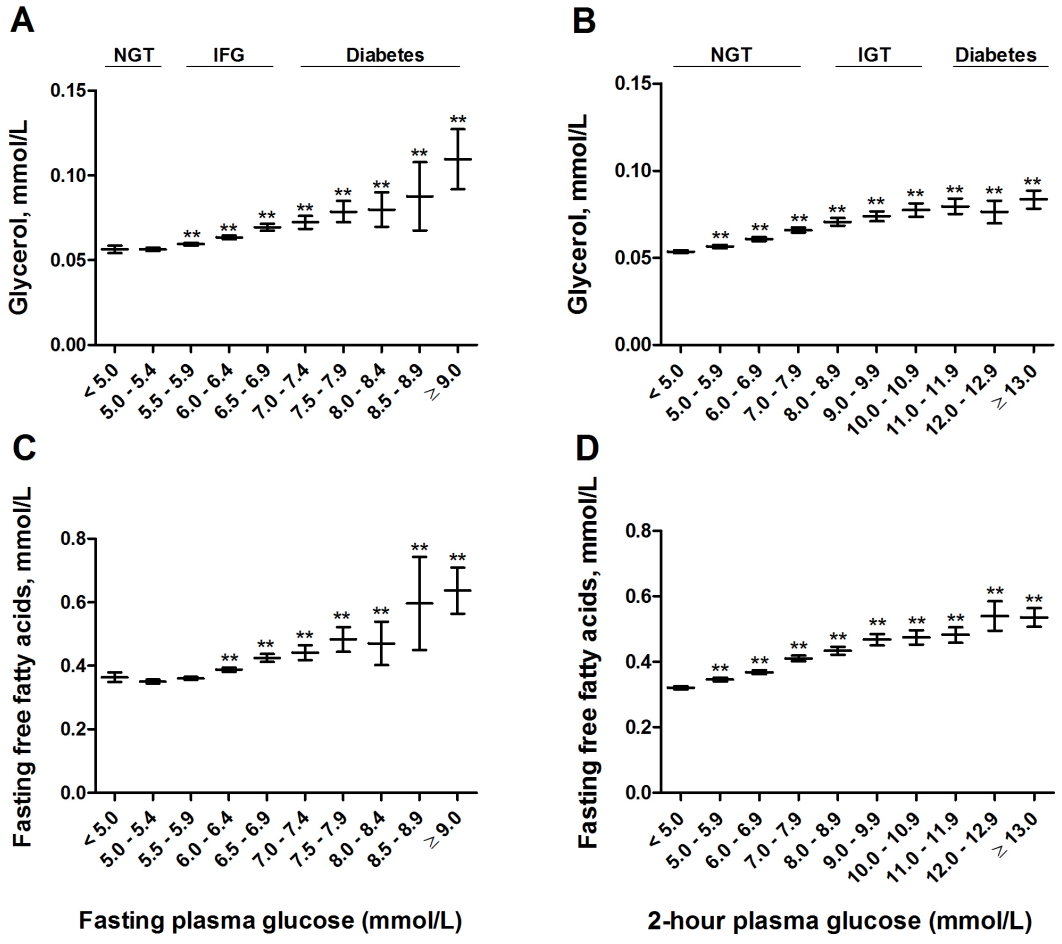

Figure 6. Mean values and their 95% CIs of fasting levels of glycerol (A, B) and FFAs (C, D) across the entire range of fasting and 2hPG categories. *P* values (from ANOVA post hoc tests) indicating statistical significance with respect to the reference category (fasting or 2hPG <5.0 mmol/L) are coded as follows: **P*<0.05, ***P*<0.01. *P* values for trends, adjusted for age and BMI, were as follows: A) 4.5 x 10^{-28}, B) 1.2 x 10^{-99}, C) 4.3 x 10^{-51}, D) 2.2 x 10^{-217}

Serum fatty acids

The levels of omega-3 FAs were significantly lower across the FPG ($P = 1.0x10^{-3}$) and 2hPG ($P = 1.9x10^{-4}$) categories, however the levels were slightly higher within the NGT category. Levels of omega-6 FAs were also lower across the FPG ($P = 4.3x10^{-63}$) and 2hPG ($P = 1.2x10^{-146}$) categories especially in participants with newly diagnosed diabetes (-20 and -16%, in the FPG and 2hPG categories, respectively) (Figure 7).

Fasting levels of monounsaturated FAs were significantly higher across the FPG ($P = 3.9 \times 10^{-41}$) and 2hPG ($P = 2.0 \times 10^{-78}$) categories. Also fasting levels of saturated FAs, and omega-7 and -9 FAs were significantly higher across the FPG ($P = 5.1x10^{-52}$) and 2hPG ($P = 6.1x10^{-132}$) categories (Figure 7).

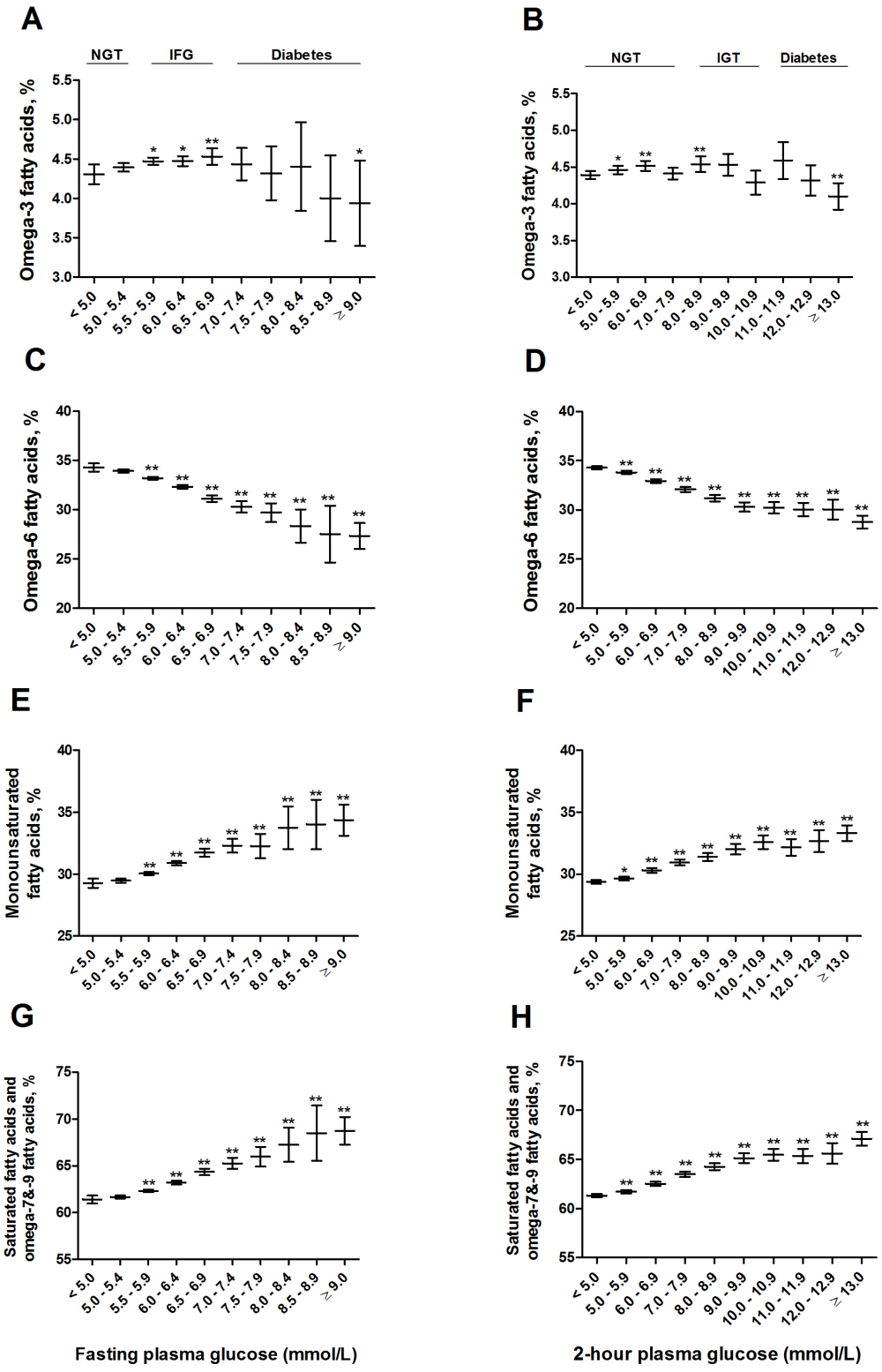

Figure 7. Mean values and their 95% CIs of omega-3 FAs of total FAs (A, B), omega-6 FAs (C, D), monounsaturated FAs (E, F), and saturated FAs and omega-7 and -9 FAs (G, H) across the entire range of fasting and 2hPG categories. *P* values (from ANOVA post hoc tests) indicating statistical significance with respect to the reference category (fasting or 2hPG <5.0 mmol/L) are coded as follows: **P*<0.05, ***P*<0.01. *P*-values for trends, adjusted for age and BMI, were as follows: A) 1.0×10^{-3}, B) 1.9×10^{-4}, C) 4.3×10^{-63}, D) 1.2×10^{-146}, E) 3.9×10^{-41}, F) 2.0×10^{-78}, G) 5.1×10^{-52}, H) 6.1×10^{-132}

Glycerol and FAs as predictors for hyperglycemia and incident type 2 diabetes

During a mean 4.5-year follow-up (range 2.5-6.2 years) a total of 276 from 4,335 men developed incident T2D. Fasting levels of glycerol ($P = 9.1x10^{-39}$), FFAs ($P = 4.6x10^{-42}$), total TGs ($P= 3.4x10^{-21}$), monounsaturated FAs ($P = 6.4x10^{-16}$), and saturated FAs, and omega-7 and -9 FAs ($P= 3.3x10^{-26}$), adjusted for age, BMI, smoking and physical activity, predicted an increase in the Glucose AUC (Table 5). In contrast, levels of omega-6 FAs (including LA) were associated significantly with lower Glucose AUC at follow-up ($P = 1.8x10^{-26}$). In the logistic regression analysis, fasting levels of glycerol (OR 1.18, 95% CI, 1.12-1.24), FFAs (OR 1.19, 95% CI, 1.10-1.29), total TGs (OR 1.26, 95% CI, 1.11-1.44), monounsaturated FAs (OR 1.09, 95% CI, 1.06-1.12), and saturated FAs, and omega-7 and -9 FAs (OR 1.09, 95% CI, 1.06-1.12) significantly predicted an increase in the risk of incident T2D. In contrast, fasting levels of omega-6 FAs significantly predicted a decrease in incident T2D (OR 0.92, 95% CI, 0.89-0.95). Omega-3 FAs (including DHA) did not predict changes in Glucose AUC or incident T2D.

Adjustment for Matsuda ISI slightly weakened most of the associations of glycerol, FFAs, total TGs, omega-6 FAs, monounsaturated FAs, saturated and omega-7 and -9 FAs with Glucose AUC and incident diabetes (Table 5). In contrast, adjustment for insulin secretion (Insulin AUC_{0-30}/Glucose AUC_{0-30}) did not have any major effect on these associations. However, adjustment for DI30 (Matsuda ISI x Insulin AUC_{0-30}/Glucose AUC_{0-30}) had an effect on all the variables with incident T2D but not with Glucose AUC.

Table 5. Association of baseline levels of ketone bodies, glycerol, fasting free fatty acids, total triglycerides and omega fatty acids with Glucose AUC and incident type 2 diabetes at the 5-year follow-up of the METSIM cohort

Variable	Glucose AUC at follow-up						Incident type 2 diabetes				
	B	SE	*P*	*P**	*P*†	*P*§	OR 95% CI	*P*	*P**	*P*†	*P*§
Glycerol, mmol/L	153.6	12.0	**9.1×10^{-39}**	**1.9×10^{-24}**	**1.9×10^{-42}**	**1.4×10^{-22}**	1.18 (1.12-1.24)	**5.8×10^{-11}**	**1.1×10^{-6}**	**3.5×10^{-12}**	**3.3×10^{-4}**
Fasting free fatty acids, mmol/L	173.9	13.0	**4.6×10^{-42}**	**2.5×10^{-36}**	**8.7×10^{-40}**	**1.3×10^{-15}**	1.19 (1.10-1.29)	**3.0×10^{-5}**	**4.6×10^{-4}**	**1.0×10^{-4}**	0.924
Total triglycerides, mmol/L	109.2	11.7	**3.4×10^{-21}**	**2.2×10^{-4}**	**7.4×10^{-31}**	**1.0×10^{-11}**	1.26 (1.11-1.44)	**3.9×10^{-4}**	**5.2×10^{-3}**	**2.8×10^{-5}**	0.013
Omega-3 fatty acids, percentage of total FAs	-21.9	17.8	0.353	0.781	0.214	0.067	0.91 (0.82-1.00)	0.045	0.107	0.033	0.034
Docosahexaenoic acid, percentage of total FAs	-14.3	14.1	0.454	0.931	0.247	0.064	0.86 (0.71-1.04)	0.115	0.244	0.088	0.062
Omega-6 fatty acids, percentage of total FAs	-442.8	42.0	**1.8×10^{-26}**	**2.3×10^{-11}**	**6.3×10^{-31}**	**1.1×10^{-10}**	0.92 (0.89-0.95)	**1.8×10^{-7}**	**2.0×10^{-3}**	**1.3×10^{-8}**	0.037
Linoleic acid, percentage of total FAs	-413.1	34.3	**1.5×10^{-34}**	**5.5×10^{-19}**	**6.7×10^{-38}**	**9.6×10^{-15}**	0.92 (0.89-0.95)	**6.3×10^{-8}**	**3.9×10^{-4}**	**1.3×10^{-8}**	0.033
Monounsaturated fatty acids, percentage of total FAs	329.1	40.8	**6.4×10^{-16}**	**1.9×10^{-6}**	**6.9×10^{-19}**	**4.3×10^{-7}**	1.09 (1.06-1.12)	**1.1×10^{-7}**	**3.7×10^{-4}**	**2.3×10^{-8}**	9.0×10^{-3}
Saturated and omega-7&-9 fatty acids, percentage of total FAs	853.4	81.2	**3.3×10^{-26}**	**9.4×10^{-11}**	**2.3×10^{-31}**	**6.1×10^{-12}**	1.09 (1.06-1.12)	**8.5×10^{-9}**	**3.9×10^{-4}**	**3.3×10^{-10}**	6.0×10^{-3}

N= 4,205 for Glucose AUC (excluding participants started on anti-diabetic medication between baseline and follow-up) and N= 4,335 for incident T2D. $P < 5.5 \times 10^{-3}$ was considered as statistically significant given the nine traits tested. B, unstandardized regression coefficient, OR, odds ratio; *P*, adjusted for age, BMI, smoking and physical activity. *P**, adjusted for age, BMI, smoking, physical activity and Matsuda insulin sensitivity index (ISI). *P*†, adjusted for age, BMI, smoking, physical activity and Insulin $AUC_{0\text{-}30}$/Glucose $AUC_{0\text{-}30}$. *P*§, adjusted for age, BMI, smoking, physical activity and Matsuda ISI x Insulin $AUC_{0\text{-}30}$/Glucose $AUC_{0\text{-}30}$. Statistically significant results are marked in bold.

5.2 ASSOCIATION OF ERYTHROCYTE MEMBRANE FATTY ACIDS WITH CHANGES IN GLYCEMIA AND RISK OF TYPE 2 DIABETES (*Study II*)

Proportions of EMFAs across different glucose tolerance categories at baseline

This study investigated the proportions of EMFAs and their ratios across the different glucose tolerance categories in non-diabetic individuals (N=1,287) and in individuals with newly diagnosed T2D (N=59). In comparison with the NGT reference category, saturated FAs did not vary significantly across the glucose tolerance categories. In contrast, age and BMI adjusted levels of monounsaturated FAs 16:1n-7 were nominally higher from +2 to +24% (P=0.016) across the abnormal glucose tolerance categories. Among the PUFAs 18:2n-6 levels were significantly lower (P=3.7x10^{-8}), and 22:4n-6 levels were nominally higher (P= 0.044) across the glucose tolerance categories. Among the FA ratio's, 18:1n-7/16:1n-7 was nominally lower (P= 0.027) and 16:1n-7/16:0 (P=7.2x10^{-3}) and 20:3 n-6/18:2n-6 (P=3.9x10^{-3}) were nominally higher across the glucose tolerance categories.

Association of EMFAs with insulin secretion and insulin sensitivity in the 5-year follow-up study

Insulin sensitivity

Palmitic acid (P=4.9x10^{-4}) and vaccenic acid (P=3.7x10^{-6}) were significantly associated with increased insulin sensitivity, and dihomo-gamma-linolenic acid (P=6.5x10^{-6}) with decreased insulin sensitivity. With respect to the EMFA ratios, 16:1n-7/16:0 (SCD1 activity, P=3.1x10^{-4}) and 20:3n-6/18:2n-6 (D6D activity, P=1.1x10^{-7}) were significantly associated with reduced insulin sensitivity, whereas 20:4n-6/20:3n-6 (D5D activity, P=3.6x10^{-4}) and 18:n-7/16:1n-7 (elongase activity, P=1.2x10^{-5}) were significantly associated with increased insulin sensitivity.

Insulin secretion

Palmitoleic acid (P=3.9x10^{-4}), and the 16:1n-7/16:0 ratio (SCD1 activity, P=4.3x10^{-5}) were significantly associated with decreased insulin secretion (DI30), whereas linoleic acid (P=1.6x10^{-4}) and the 18:1n-7/16:1n-7 ratio (elongase activity, P=4.3x10^{-5}) were significantly associated with increased insulin secretion.

EMFAs proportions as predictors for hyperglycemia and incident type 2 diabetes

SFAs did not predict changes in Glucose AUC or in incident T2D (Table 6). The levels of palmitoleic acid (P=2.8x10^{-7}), dihomo-gamma-linoleic acid (P=2.3x10^{-4}), the 16:1n-7/16:0 ratio (SCD1 activity, P=1.6x10^{-8}) and the 20:3n-6/18:2n-6 ratio (D6D activity, P=9.4x10^{-7}) significantly predicted an increase in Glucose AUC at follow-up after the adjustment for confounding factors, whereas linoleic acid (P=0.0015), and the 18:1n-7/16:1n-7 ratio (elongase activity, P=1.5x10^{-9}) significantly predicted a decrease in Glucose AUC. Palmitoleic acid (OR 1.35, 95% CI, 1.07, 1.69, P=0.010) and the 16:1n-7/16:0 ratio (OR 2.23, 95% CI, 1.29, 3.85, P=0.004) nominally increased, and linoleic acid (OR 0.54, 95% CI, 0.35, 0.82, P=0.004) nominally decreased the risk of incident diabetes.

All statistically significant associations persisted after further adjustment for baseline insulin sensitivity, insulin secretion, FPG, 2hPG, or the Glucose AUC.

Table 6. Association of baseline proportions of EMFAs with Glucose AUC and incident type 2 diabetes at the 5-year follow-up

Fatty acid	Glucose AUC at follow-up (N=724)						Incident type 2 diabetes (N=30)				
	B*	SE*	*P**	*P*†	*P*§	*P*‡	OR (95 % CI)*	*P**	*P*†	*P*§	*P*‡
16:0 (palmitic acid), %	-316.2	308.6	0.272	0.874	0.128	0.790	0.74 (0.48, 1.12)	0.152	0.239	0.098	0.649
18:0 (stearic acid), %	458.1	345.9	0.164	0.426	0.103	0.294	1.25 (0.63, 2.50)	0.520	0.638	0.394	0.650
Total SFAs, %	922.4	558.0	0.087	0.097	0.098	0.078	0.96 (0.66, 1.41)	0.836	0.834	0.859	0.804
16:1n-7 (palmitoleic acid), %	216.1	41.4	**2.8×10^{-7}**	**3.6×10^{-7}**	**4.7×10^{-7}**	**1.7×10^{-7}**	1.35 (1.07, 1.69)	0.010	0.010	0.015	0.028
18:1n-7 (vaccenic acid), %	-348.5	126.9	0.004	0.054	**1.0×10^{-3}**	0.011	0.99 (0.71, 1.40)	0.966	0.809	0.810	0.525
18:1n-9 (oleic acid), %	40.7	194.2	0.827	0.472	0.988	0.238	0.98 (0.59, 1.62)	0.925	0.988	0.870	0.740
Total MUFAs, %	-12.2	238.9	0.814	0.876	0.774	0.350	1.26 (0.86, 1.85)	0.232	0.189	0.259	0.099
18:2n-6 (linoleic acid), %	-284.0	97.2	**1.5×10^{-3}**	0.008	**1.0×10^{-3}**	0.036	0.54 (0.35, 0.82)	0.004	0.005	0.004	0.013
20:3n-6 (dihomo-gamma-linolenic acid), %	238.7	67.6	**2.3×10^{-4}**	**1.9×10^{-3}**	**4.7×10^{-5}**	**3.6×10^{-4}**	1.04 (0.92, 1.18)	0.485	0.632	0.374	0.733
20:4n-6 (arachidonic acid), %	88.9	130.6	0.436	0.848	0.407	0.529	1.24 (0.87, 1.77)	0.235	0.293	0.195	0.706
20:5n-3 (eicosapentaenoic acid), %	-39.3	32.3	0.267	0.563	0.189	0.419	1.04 (0.58, 1.87)	0.898	0.811	0.919	0.903
22:4n-6 (adrenic acid), %	125.9	50.7	0.011	0.070	0.004	0.085	1.09 (0.99, 1.19)	0.073	0.112	0.045	0.183
22:6n-3 (docosahexaenoic acid), %	-74.1	64.8	0.302	0.284	0.328	0.282	1.00 (0.71, 1.41)	0.992	0.991	0.970	0.980
Total PUFAs, %	-436.0	330.1	0.222	0.133	0.260	0.028	0.89 (0.66, 1.20)	0.441	0.394	0.448	0.136
Ratio (16:1n-7/16:0) (SCD1)	253.0	44.1	**1.6×10^{-8}**	**4.5×10^{-8}**	**2.0×10^{-8}**	**2.4×10^{-7}**	2.23 (1.29, 3.85)	0.004	0.005	0.006	0.020
Ratio (20:3n-6/18:2n-6) (Δ^6 desaturase)	324.1	69.3	**9.4×10^{-7}**	**3.4×10^{-5}**	**9.9×10^{-8}**	**1.0×10^{-5}**	1.11 (0.99, 1.23)	0.070	0.119	0.042	0.191
Ratio (20:4n-6/20:3n-6) (Δ^5 desaturase)	-165.2	59.5	0.004	0.009	**1.0×10^{-3}**	**1.1×10^{-3}**	0.95 (0.75, 1.21)	0.685	0.791	0.603	0.735
Ratio (18:1n-7/16:1n-7) (elongase)	-248.1	40.7	**1.5×10^{-9}**	**1.3×10^{-8}**	**8.9×10^{-10}**	**2.2×10^{-8}**	0.63 (0.39, 1.03)	0.065	0.080	0.067	0.198

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCD1, stearoyl coenzyme A desaturase 1. B and SE were obtained from multiple linear regression. Odds ratios (OR) and their 95% confidence intervals were obtained from logistic regression analyses. *P**, adjusted for age, BMI, smoking and physical activity. *P*†, adjusted for age, BMI, smoking, physical activity and Matsuda insulin sensitivity index (ISI). *P*§, adjusted for age, BMI, smoking, physical activity and Insulin $AUC_{0\text{-}30}$/Glucose $AUC_{0\text{-}30}$. *P*‡, adjusted for age, BMI, smoking, physical activity and DI30 (Matsuda ISI x Insulin $AUC_{0\text{-}30}$/Glucose $AUC_{0\text{-}30}$). $P < 2.8\times10^{-3}$ was considered as statistically significant given the 18 traits tested. Statistically significant results are marked in bold.

5.3 ASSOCIATION OF KETONE BODY LEVELS WITH HYPERGLYCEMIA AND TYPE 2 DIABETES (*Study III*)

Levels of KBs across the categories of glucose tolerance at the baseline study

This study examined the association of AcAc and BHB, in non-diabetic individuals (N=8,749) and individuals with newly diagnosed T2D (N=649) in the FPG and 2hPG categories (Figure 8). In the FPG category, AcAc levels were significantly lower ($P < 0.01$) in individuals with IFG and significantly higher ($P < 0.01$) in individuals with newly detected diabetes. In the 2hPG category, AcAc levels were significantly higher ($P < 0.01$) in subjects with IGT as well as in newly diagnosed diabetes as compared with the reference category.

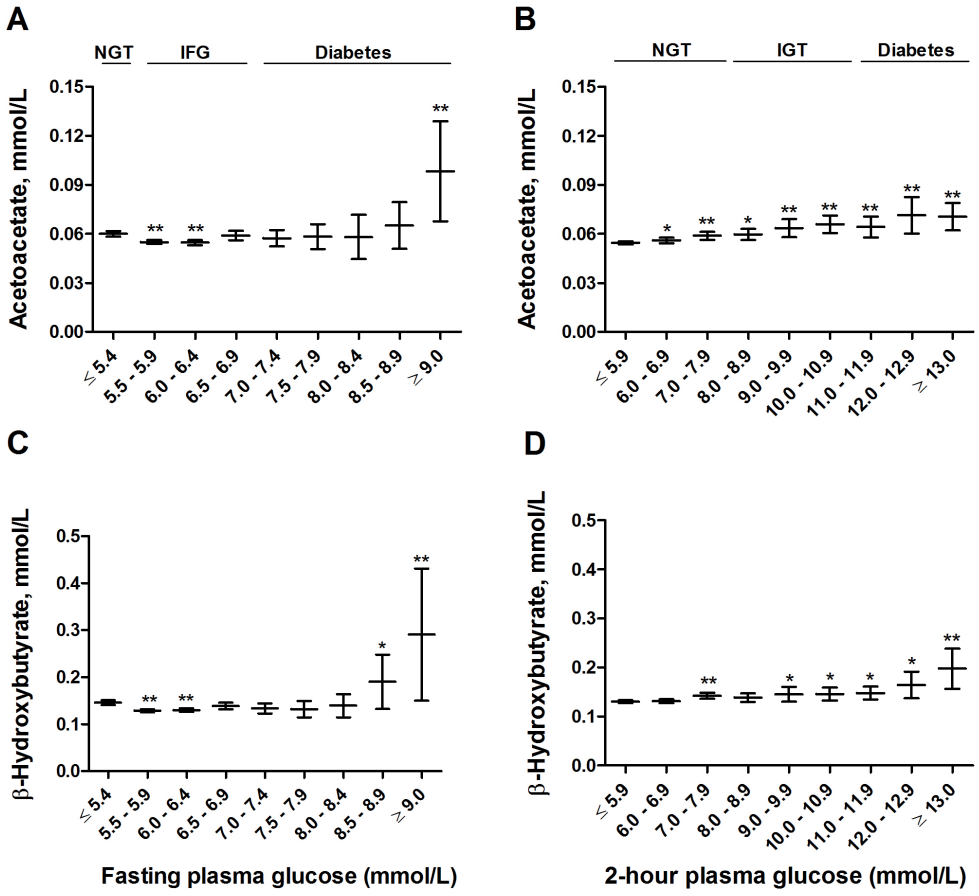

Figure 8. Mean values and their 95% confidence intervals of fasting levels of AcAc (A, B) and BHB (C, D) across the fasting and 2hPG categories. *P* values (from ANOVA post-hoctests) indicate statistical significance with respect to the reference category (FPG ≤5.4 mmol/L, 2hPG ≤5.9 mmol/L). **P*<0.05, ***P*<0.01

In the FPG category, BHB levels were significantly lower ($P < 0.01$) in subjects with IFG and significantly higher ($P < 0.01$) in the diabetic range. Considering 2hPG, BHB levels were nominally higher in IGT ($P < 0.05$) and in newly diagnosed T2D ($P < 0.01$), as compared with the reference category.

KBs as predictors for hyperglycemia and incident type 2 diabetes

Follow-up data of 4,335 participants were available from the ongoing prospective METSIM 5-year follow-up study. A total of 276 participants developed incident diabetes between the baseline and follow-up studies. AcAc and BHB levels adjusted for confounding factors known to increase the risk of diabetes (age, BMI, smoking, and physical activity) predicted an increase in the Glucose AUC evaluated as a continuous variable at follow-up (P=2.3×10^{-4}, P=5.7×10^{-6}, respectively (Table 7).

Table 7. Association of baseline levels of acetoacetate and β-hydroxybutyrate as predictors of Glucose AUC and with incident type 2 diabetes at 5-year follow-up. Statistical analyses were performed with Glucose AUC as a continuous variable and as the highest quartile (Q4) *vs*. the three lowest quartiles (Q1-Q3) combined and type 2 diabetes as a categorical variable (yes/no)

Glucose AUC at follow-up as a continuous variable	**B**	**SE**	***P****	***P*†**	***P*§**
Acetoacetate, mmol/L	39.5	10.1	**2.3x10^{-4}**	**9.9x10^{-7}**	**9.3x10^{-4}**
β-hydroxybutyrate, mmol/L	51.6	11.1	**5.7x10^{-6}**	**2.7x10^{-9}**	**6.2x10^{-5}**
Glucose AUC at follow-up as a categorical variable (Q4 vs. Q1-Q3)	**OR**	**95% CI**	***P****	***P*†**	***P*§**
Acetoacetate, mmol/L	1.56	1.33-1.84	**7.9x10^{-8}**	**9.1x10^{-11}**	**5.5x10^{-7}**
β-hydroxybutyrate, mmol/L	1.46	1.25-1.72	**3.4x10^{-6}**	**2.1x10^{-8}**	**1.8x10^{-5}**
No diabetes vs. Newly diagnosed type 2 diabetes	**OR**	**95% CI**	***P****	***P*†**	***P*§**
Acetoacetate, mmol/L	1.32	1.00-1.74	**0.047**	**0.012**	0.125
β-hydroxybutyrate, mmol/L	1.03	0.77-1.36	0.864	0.085	0.345

B and SE were obtained from multiple linear regression. Odds ratios (OR) and their 95% confidence intervals were obtained from logistic regression analyses. *P**, adjusted for age, BMI, smoking, and physical activity. *P*†, adjustment for age, BMI, smoking, physical activity and Matsuda ISI. *P*§, adjustment for age, BMI, smoking, physical activity and $InsAUC_{0\text{-}30}/GlucAUC_{0\text{-}30}$ .Statistically significant results are marked in bold.

The highest quartile of AcAc adjusted for age, BMI, smoking and physical activity predicted conversion to T2D, OR 1.32 (95% CIs, 1.00, 1.74; P=0.047; Table 7), and also after further adjustment for FPG (OR 1.41, 95% CIs 1.06-1.89, P=0.019). Adjustment for 2hPG, instead of FPG, abolished statistical significance (P=0.423). When analyzed in the glucose tolerance categories, AcAc predicted incident diabetes in individuals with IFG (OR 1.49, 95% CI 1.12-1.99, P=0.007) after the adjustment for confounding factors.

Additional adjustment for insulin sensitivity strengthened the association of KBs with development of hyperglycemia and conversion to T2D, whereas insulin secretion weakened/abolished these associations.

Association of risk SNPs for type 2 diabetes or hyperglycemia with the levels of ketone bodies

After correction for multiple testing (threshold of statistical significance, $P<4.0\times10^{-4}$), the glucose increasing C allele of rs780094 of *GCKR* showed a significant association with elevated levels of BHB (effect size +5.6% per the C allele, $P=3.7\times10^{-6}$ after adjusting for age and BMI) and a nominally significant association with AcAc (+3.9%, $P=0.003$). Additionally, there were nominally significant associations for SNPs of *ANK1*, *GIPR*, *HMGA2*, *SLC2A2* and *FADS1* with the levels of both AcAc and BHB. Also several other SNPs were nominally associated with either AcAc or BHB alone (Table 8).

Table 8. Risk SNPs for type 2 diabetes or hyperglycemia associated ($P<0.05$) with fasting acetoacetate and β-hydroxybutyrate

Gene / SNP	N	Allele maj/min	Risk allele frequency	Acetoacetate %B	Acetoacetate *P*	Acetoacetate *P**	β-hydroxybutyrate %B	β-hydroxybutyrate *P*	β-hydroxybutyrate *P**
ADAMTS9 rs4607103	8120	C/T	74.1	-3.7	**0.007**	**0.009**	-3.3	0.067	0.079
ANK1 rs516946	8120	C/T	80.5	+3.4	**0.042**	**0.036**	+3.4	**0.014**	**0.011**
CENTD2 rs1552224	8119	A/C	74.7	1.9	**0.022**	**0.025**	1.8	0.099	0.133
CRY2 rs11605924	8108	A/C	52.9	1.7	0.124	0.097	3.4	**0.002**	**0.001**
DGKB rs2191349	8120	G/T	42.8	1.3	0.152	0.160	2.3	**0.038**	**0.047**
FADS1 rs174550	8119	T/C	57.7	-3.2	**6.7×10^{-4}**	**5.5×10^{-4}**	-3.1	0.151	0.112
FAM148B/C2CD4B, rs11071657	8118	A/G	69.1	-0.1	0.446	0.505	-2.7	**0.032**	**0.019**
GCKR rs780094	8120	C/T	62.2	3.9	**0.005**	**0.003**	5.6	**8.3×10^{-6}**	**3.7×10^{-6}**
GIPR rs10423928	8302	T/A	21.6	-3.8	**0.001**	**0.001**	-3.7	**0.004**	**0.003**
HMGA2 rs2612067	8353	T/G	6.90	-5.9	**0.005**	**0.006**	-6.0	**0.009**	**0.010**
KCNQ1 rs231362	8388	G/A	51.9	-1.8	**0.050**	**0.041**	-1.7	0.261	0.199
KLF14 rs972283	8120	G/A	57.5	-2.8	0.055	0.066	-2.7	**0.009**	**0.014**
MC4R rs12970134	8120	G/A	17.5	-3.6	**0.006**	**0.005**	-2.0	0.129	0.100
PPARG rs1801282	8119	C/G	84.9	3.0	**0.036**	**0.037**	-0.03	0.996	0.918
SLC2A2 rs11920090	8120	T/A	86.7	-3.8	**0.011**	**0.011**	-4.9	**0.018**	**0.017**

Major/minor (maj/min) alleles of each SNP are shown. Risk alleles for hyperglycemia or T2D are underlined. Effect sizes (indicated as % of B from the mean) per risk allele. Significant *P*-values are given in bold ($P<0.05$) or bold and underlined ($P< 4.0\times10^{-4}$). *P* is unadjusted. *P** is adjusted for age and BMI. Of 62 risk SNPs for T2D or hyperglycemia were studied, only SNPs that are associated with single or both traits presented in this table.

Gene expression of genes involved in ketone body metabolism

Significant correlations were found with adipose tissue mRNA expression levels of several genes associated with ketolysis with glucose metabolism parameters (Table 9). Of these genes, *ACAT1* expression had the most significant correlations with Glucose AUC (r=-0.314, P=6.1×10^{-6}), Matsuda ISI (r=0.479, P=7.1×10^{-13}), and insulin secretion (r=-0.444, P=7.0×10^{-11}). Similarly, the expressions of other genes regulating ketolysis, *BDH1* (β-hydroxybutyrate dehydrogenase, type 1), *OXCT1* (3-oxoacid CoA transferase 1), and *ACSS2* (acyl-CoA synthetase short-chain family member 2) were inversely correlated with Glucose AUC and insulin secretion and positively correlated with Matsuda ISI.

Table 9. Pearson correlations of adipose tissue mRNA expression of major enzymes involved in fatty acid oxidation, ketogenesis and ketolysis with Glucose AUC, Matsuda ISI and Matsuda ISI-adjusted $InsAUC_{0\text{-}30}$/$GlucAUC_{0\text{-}30}$

Function /Gene	**Glucose AUC**		**Matsuda ISI**		**$InsAUC_{0\text{-}30}$/ $GlucAUC_{0\text{-}30}$**	
	r	***P***	***r***	***P***	***r***	***P***
Fatty acid oxidation						
CPT1A	0.198	4.9x10^{-3}	-0.229	1.1x10^{-3}	0.168	0.019
CPT2	-0.068	0.340	0.249	3.7x10^{-4}	-0.274	1.0x10^{-4}
Ketogenesis						
HMGCS2	0.078	0.273	-0.013	0.851	0.006	0.936
HMGCS1	-0.042	0.557	0.088	0.217	-0.068	0.342
Ketolysis						
BDH1	-0.222	1.6x10^{-3}	0.425	3.4x10^{-10}	-0.408	3.0x10^{-9}
OXCT1	-0.121	0.088	0.232	9.4x10^{-4}	-0.182	0.011
ACAT1	-0.314	6.1x10^{-6}	0.479	7.1x10^{-13}	-0.444	7.0x10^{-11}
ACSS2	-0.108	0.130	0.307	9.7x10^{-6}	-0.274	1.0x10^{-4}

CPT1A, carnitine palmitoyltransferase 1A; *CPT2*, carnitine palmitoyltransferase II; *HMGCS2,* 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial); *HMGCS1,* 3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble); *BDH1,* 3-hydroxybutyrate dehydrogenase, type 1; *OXCT1,* 3-oxoacid CoA transferase 1; *ACAT1,* acetyl-CoA acetyltransferase 1; *ACSS2,* acyl-CoA synthetase short-chain family member 2.

6 Discussion

6.1 REPRESENTATIVENESS OF THE STUDY POPULATION

This study was based on a large population-based well-characterized METSIM cohort having a relatively long follow-up period (around 5 years) to ensure good statistical power.

Studies I and III included a total of 9,398 non-diabetic men from the cross-sectional METSIM study examined in 2005-2010. Subjects, aged from 45 to 73 years, were randomly selected from the population register of the Kuopio town in Eastern Finland. In Study II, 1,346 non-diabetic men were selected randomly with equivocal percentages from each glucose category compared to the original METSIM cohort. All subjects included in Studies I-III had a 1-day outpatient visit to the Clinical Research Unit at the University of Eastern Finland, including an interview on their history of previous chronic diseases and current drug treatment, physical exercise, smoking, alcohol intake and cardiovascular risk factors. The diagnosis of T2D was based on an OGTT. Insulin sensitivity and insulin secretion were evaluated using validated OGTT-derived indices.

Detailed phenotyping using the NMR method was applied to all individuals involved in METSIM study, and EMFAs were determined in a subset of the METSIM study population using gas chromatography. Measuring individual metabolites with these techniques carries a high potential in detecting and validating new biomarkers. All risk SNPs for hyperglycemia/T2D known at the time of the study were genotyped in the entire METSIM cohort, ensuring the identification of those SNPs associated with biomarkers of interest.

Additional advantage of the METSIM study is that it includes a long-term follow-up (mean follow-up about 5 years), which permits an evaluation of the prospective significance of the identified biomarkers as predictors of worsening of hyperglycemia and incident T2D. The diagnosis of new diabetes was based on an OGTT. The main limitation of this series of studies is that the METSIM cohort includes only Finnish men and we did not have detailed dietary data which somewhat limits the conclusions drawn from Studies I-III. Study II, had a relatively small number of individuals who developed diabetes during the follow-up. Although the METSIM Study is large in size, the power was limited to detect genetic association with KB levels (Study III).

6.2 GLYCEROL AND FATTY ACIDS AS PREDICTORS OF HYPERGLYCEMIA AND INCIDENT TYPE 2 DIABETES (*Study I*)

The phenotype represents both genetic predisposition and environmental influences, including diet, physical activity and smoking, and therefore assessing the concentration of different metabolites provides information on individual profile of physiological and pathophysiological markers.

Glycerol is an important intermediate in glucose and lipid metabolism. Study I, revealed that fasting levels of glycerol and FFAs were higher not only in newly diagnosed diabetes but also in IFG and IGT in a cross-sectional analysis of the METSIM Study. Glycerol has a direct effect on glucose levels and gluconeogenesis and its levels have been shown to be elevated in obese individuals and in patients with type 2 diabetes (211, 220), highlighting the potential importance of glycerol homeostasis. We observed that fasting levels of glycerol, total TGs and FFAs predicted an increase in Glucose AUC and the development of new-onset T2D during this prospective 4.5-year follow-up of the METSIM cohort, independent of known risk factors for T2D. None of the previous studies have indicated glycerol to be a significant predictor of hyperglycemia and incident T2D.

By measuring insulin sensitivity and insulin secretion with validated indices (217) we were able to evaluate statistically the possible mechanisms by which glycerol, total TGs and FFAs predict hyperglycemia and T2D. Our follow-up study of the METSIM cohort suggested for the first time that insulin resistance was the most important mediator for the association of levels of glycerol, total TGs and FFAs with the development of hyperglycemia. This conclusion is supported by our statistical evaluation revealing that adjustment for insulin sensitivity (Matsuda ISI) attenuated and/or abolished the association of glycerol, total TGs and FFAs with Glucose AUC and with incident T2D. High levels of glycerol and FFAs have been shown to increase insulin resistance in skeletal muscle (171). In contrast, adjustment for insulin secretion did not alter these associations, although previous studies have shown that long-term exposure of β-cells to FFAs can lead to impaired insulin secretion (172).

Elevated levels of omega-3, omega-6 FAs, monounsaturated FAs, and saturated and omega-7 and -9 FAs have been linked to hyperglycemia in some previous studies (179, 180, 185-187), although the evidence is conflicting. Human intervention trials have also provided somewhat contradictory results, but they suggest that saturated FAs induce insulin resistance (221). We demonstrated that the fasting levels of monounsaturated FAs, saturated FAs, and omega-7 and -9 FAs were increased in IFG, IGT and in newly diagnosed diabetes in this cross-sectional analysis of the METSIM cohort, whereas the levels of omega-3 and omega-6 FAs were reduced in individuals with newly diagnosed T2D.

Monounsaturated FAs and saturated and omega-7 and -9 FAs predicted the worsening of hyperglycemia and the development of T2D in the METSIM cohort, independent of known risk factors for T2D. Three previous studies are in line with the present findings and have linked the

elevated risk of T2D with the levels of omega-7 FAs (palmitoleic acid) (185-187), but not with omega-9 FA (oleic acid). Studies published on the association of omega-3 FAs and omega-6 FAs with incident T2D are somewhat conflicting and inconclusive (179, 180, 185). The present study detected a significant association of omega-6 FAs (mainly linoleic acid) with reduction in the development of both hyperglycemia and T2D. Linoleic acid is derived mainly from the diet, but it can be metabolized to longer chain unsaturated FAs. In the current study, the monounsaturated FAs, especially palmitoleic and oleic acids, were associated with an increased risk of abnormal glucose metabolism and T2D. This may be explained by the fact that the major saturated FAs are desaturated to monounsaturated FAs, and that in the Western diet, the levels of saturated FAs and monounsaturated FAs are positively correlated (222-224).

To investigate the mechanisms underlying the associations of hyperglycemia with FAs, we evaluated their associations with insulin sensitivity and insulin secretion. We observed that insulin sensitivity was positively correlated with the levels of omega-6 FAs explaining at least in part the preventive effect of omega-6 FAs on the development of hyperglycemia and incident diabetes. In contrast, levels of monounsaturated FAs and saturated and omega-7 and -9 FAs showed negative correlations with insulin sensitivity, which is in agreement with previous findings of an inverse association of omega-7 FAs with insulin sensitivity (223, 225). Moreover, omega-9 FAs (dietary oleic acid) influence fat oxidation (226) suggesting that they may have negative effects on insulin sensitivity.

Additional evidence that insulin resistance is a potent mediator for the association of omega FAs with hyperglycemia emerges from our multivariate models. The adjustment for Matsuda ISI, but not for insulin secretion, attenuated the associations of omega-6 FAs (including linoleic acid), monounsaturated FAs and saturated FAs, and omega-7 and -9 FAs with Glucose AUC and incident T2D. These results might imply that insulin sensitivity is the major causal mechanism explaining the association of these FAs with hyperglycemia and the risk of incident T2D. Similarly, the preventive effect of omega-6 FAs was mediated *via* high insulin sensitivity. FAs are important structural components of cell membranes, and they are precursors of long chain FA derived molecules, which may affect insulin sensitivity. Furthermore, FAs modify gene expression and receptor binding (227), thus making them an important candidate in the search of risk factors for T2D and related glucose abnormalities.

In summary, Study I suggested that high levels of glycerol, FFAs, serum monounsaturated FAs, saturated FAs, and omega-7 and -9 FAs are not only indicators of diabetic hyperglycemia but are also markers of disturbed glucose metabolism in the prediabetic state. However, this study does not necessarily imply that insulin resistance is a causal mechanism linking elevated levels of glycerol and FAs with the worsening of hyperglycemia and incident T2D, since dietary and other factors could also play an important role in these associations.

6.3 ERYTHROCYTE MEMBRANE FATTY ACIDS AS PREDICTORS OF HYPERGLYCEMIA AND INCIDENT TYPE 2 DIABETES (*Study II*)

Previous studies have not investigated the association of EMFA proportions and their product to precursor ratios with hyperglycemia. This present cross-sectional analysis of the METSIM cohort demonstrated that the levels of palmitoleic acid (C16:1n-7), SCD1 (16:1n-7/16:0) and D6D (20:3n-6/18:2n-6) were significantly higher not only in individuals with newly diagnosed T2D but also in individuals with IFG, IGT and both. In contrast, linoleic acid (C18:2n-6) was significantly lower in newly diagnosed T2D.

In our prospective follow-up study, high levels of palmitoleic acid, dihomo-gamma-linolenic acid and the ratios of 16:1n-7/16:0 (SCD1 activity) and 20:3n-6/18:2n-6 (D6D activity) significantly predicted the worsening of hyperglycemia, whereas linoleic acid and the 18:1n-7/16:1n-7 ratio (elongase activity) predicted a decrease in Glucose AUC at follow-up. Moreover, palmitoleic acid and the 16:1n-7/16:0 ratio (SCD1 activity) nominally increased the risk of incident T2D, independent of known confounding factors, whereas linoleic acid was preventive of diabetes. Our conclusions are in line with three previously published longitudinal studies (181, 202, 203). The ratios of 20:3n-6/18:2n-6 (D6D activity) and 18:1n-7/16:1n-7 (elongase activity) predicted incident T2D in our study, but these associations were abolished after adjusting for confounding factors. In contrast, the levels of n-3 polyunsaturated FAs were not associated with the worsening of glycemia or the risk of diabetes, in line with a recent meta-analysis (228).

We also evaluated the role of insulin sensitivity and insulin secretion as potential mediators for the associations of EMFAs proportions and their ratios with incident T2D. Previous studies have hinted that the FA composition especially in skeletal muscle could alter membrane fluidity, ion permeability, and insulin receptor binding and affinity, or insulin action (184, 227). Cross-sectional studies have reported that the altered D5D and D6D activities could be related to insulin resistance (177, 229). In our study, adjustment for insulin sensitivity somewhat weakened *P* values but did not abolish statistical significance. Similarly, adjustment for insulin secretion did not essentially change these results suggesting that the role of genes could be important in these associations. However, the ratios of 20:3n-6/18:2n-6 (D6D activity) and 16:1n-7/16:0 (SCD1 activity) which were associated with abnormal glucose tolerance at baseline, also predicted decreases in insulin sensitivity and insulin secretion at the follow-up study. Therefore, EMFAs may decrease insulin sensitivity and insulin secretion, although it is not possible to conclude the direction of causality because dietary and other factors can also influence insulin sensitivity.

6.4 KETONE BODY LEVELS AS PREDICTORS OF HYPERGLYCEMIA AND INCIDENT TYPE 2 DIABETES (*Study III*)

High levels of KBs are a characteristic finding in individuals with diabetes (230), but there is a lack of information about the KB levels in the non-diabetic glucose range. We observed an

increase in KB levels with increasing glucose levels in an OGTT in a cross-sectional analysis of the METSIM study cohort. These results agree with a previous finding indicating that elevated glucose levels are associated with increased levels of KBs (211).

In the prospective analysis of the METSIM cohort we observed that the levels of AcAc and BHB significantly predicted the worsening of hyperglycemia in non-diabetic individuals, but these associations were abolished after adjustment for Glucose AUC at baseline. This could point to an important link between the levels of KBs and glucose metabolism. AcAc, but not BHB, predicted the development of new T2D in our prospective follow-up of the METSIM cohort, independent of known risk factors for T2D. The reason why KBs predicted very significantly the worsening of glycemia, but not so clearly incident T2D, are FPG and 2hPG levels at the diagnosis of T2D which were often only marginally elevated (FPG in the range of 7.0-7.5 mmol/L, 2hPG in the range of 11.1-12.0 mmol/L), whereas the levels of KBs were significantly increased at higher glucose levels (FPG levels exceeding 8.0 mmol/L, and 2hPG levels exceeding 12.0 mmol/L, Figure 8).

To study the mechanisms by which KBs increase the risk of hyperglycemia and T2D, we investigated the association of KB levels with insulin sensitivity and insulin secretion. Surprisingly, we found that high levels of KBs were associated with high insulin sensitivity in the non-diabetic glucose range at baseline, similar to recent findings in young Finnish adults (210). Furthermore, it was noted that insulin sensitivity was significantly correlated with the key enzymes of ketolysis, which suggests that in insulin sensitive individuals KBs are rapidly converted to acetyl-CoA, which stimulates oxidative phosphorylation and mitochondrial generation of ATP. However, it is not likely that insulin resistance is an important mechanism in the prediction of hyperglycemia by elevated KB levels. This was clearly demonstrated by our METSIM follow-up data which showed that adjustment for Matsuda ISI did not weaken the association of KBs with the development of hyperglycemia. In contrast, including a marker of insulin secretion in the model substantially weakened or abolished the association of KBs with the development of hyperglycemia and the conversion to T2D. These findings emphasize the crucial role of impaired insulin secretion as a regulator of hyperglycemic effects of KBs. Adequate insulin secretion relative to insulin sensitivity maintains low levels of KBs by suppressing the expression of hormone sensitive lipase and thus prevents the release of FFAs from adipose tissue which is the major source of hepatic ketogenesis and high circulating levels of KBs (206, 231).

We also investigated the association of risk SNPs for hyperglycemia and T2D with KB levels. Of the 62 SNPs analyzed, only the glucose increasing major C allele of rs780094 of *GCKR* (encoding glucokinase regulatory protein) was significantly associated with increased BHB levels and nominally associated with AcAc levels. Glucokinase (GCK) is 'a sensor' of the glucose level which plays a crucial role in whole body glucose homeostasis. The activity of GCK is regulated by *GCKR* in the liver (141). The C allele of rs780094 of *GCKR* has been previously reported to be associated with fasting glycemia, risk of T2D, insulin resistance, and decreased levels of total

and VLDL, TGs, decreased levels of alanine and isoleucine, and elevated levels of glutamine (62, 142, 161, 232, 233). The significant association of rs780094 with KB levels adds further to the pleiotropic effects of *GCKR*.

6.5 CONCLUDING REMARKS

There is still a lack of reliable biomarkers for the detection of the metabolic alterations associated with T2D highlighting the need for the development of early diagnostic and prognostic markers for T2D. A detailed understanding of the pathophysiology of T2D and identification of early metabolic alterations is essential for identifying individuals at high risk of this disease. Recent advancements in the application of high throughput methodologies, including deep metabolic phenotyping and genotyping in a large, well powered and characterized population cohorts has made possible the rapid progress in the field of biomarker discovery.

Previous studies and recent studies based on the application of metabolomics have identified several biomarkers predicting incident T2D including total TGs, HDL cholesterol, inflammatory markers, adiponectin, liver enzymes, fetuin-A, aromatic amino acids and branch-chain amino acids which have been measured from biofluids or tissue samples (151). In the present studies, NMR and gas chromatography were utilized and the analysis revealed that levels of glycerol, serum FAs, proportions of EMFAs and KBs can be considered as biomarkers for the development of hyperglycemia and incident T2D. However, the clinical importance of these biomarkers needs to be validated in other populations and also in women.

Our series of studies show that it is possible to obtain important information on the mechanisms how different metabolites can impair glucose tolerance and further to incident T2D if reliable markers of insulin sensitivity and insulin secretion are applied. It is important to measure both of these pathophysiological abnormalities since the mechanisms by which an individual metabolite increases the risk of hyperglycemia and T2D are likely to differ. For example, insulin resistance seems to be a more likely causal mechanism how high concentrations of certain serum FAs increase the risk of T2D, whereas impaired insulin secretion is likely to be a causal mechanism how KBs lead to the worsening of hyperglycemia and elevated risk of T2D. However, several other factors are likely to play also a significant role in these associations, namely dietary factors and gene variants.

GWAs and meta-analyses have uncovered several novel risk loci for T2D that are consistent across all ethnic groups. Revealing biological functions of these common variants has been challenging. Recently exome sequencing has accelerated the potential to identify new low-frequency and rare variants in complex diseases, including T2D. However even taking the applications of exome sequencing and exome chip into account, studies on the genetics of T2D need other approaches. Gene-gene and gene-environment/lifestyle interaction analyses are

urgently needed as well as studies on epigenetics (methylation of promoters and histone modifications).

In conclusion, we have identified novel biomarkers for the estimation of the risk for the development of hyperglycemia and incident diabetes beyond classical clinical indicators and laboratory measurements. Our findings indicate that plasma metabolites predict the onset of T2D and provide important information beyond standard clinical markers.

7 Summary

The main findings of Studies I – III were as follows:

***Study I*:** High fasting levels of glycerol, FFAs, monounsaturated FAs, and saturated FAs, and omega-7 and -9 FAs predicted the worsening of hyperglycemia and incident type 2 diabetes, whereas high levels of omega-6 FAs were associated with a reduced risk of hyperglycemia and type 2 diabetes. Insulin resistance explained these associations, at least in part.

Study II: High levels of palmitoleic acid, dihomo-gamma-linolenic acid, 16:1n-7/16:0 and 20:3n-6/18:2n-6 ratios predicted the worsening of hyperglycemia, whereas linoleic acid and 18:1n-7/16:1n-7 ratio predicted the improvement of hyperglycemia. The high levels of palmitoleic acid and 16:1n-7/16:0 nominally predicted incident type 2 diabetes, whereas linoleic acid prevented type 2 diabetes. These associations were largely independent of insulin sensitivity, insulin secretion and glucose levels.

Study III: High levels of acetoacetate and β-hydroxybutyrate predicted the worsening of hyperglycemia, and acetoacetate predicted incident type 2 diabetes. Impaired insulin secretion, but not insulin resistance, explained these associations. One common variant of *GCKR* was significantly associated with β-hydroxybutyrate levels.

8 References

1. Lin Y, Sun Z. Current views on type 2 diabetes. J Endocrinol 2010; 204:1-11
2. Pinhas-Hamiel O, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. J Pediatr 2005; 146:693-700
3. Rosenbloom AL, Silverstein JH, Amemiya S, Zeitler P, Klingensmith GJ. Type 2 diabetes in children and adolescents. Pediatr Diabetes 2009; 10 Suppl 12:17-32
4. Kaprio J, Tuomilehto J, Koskenvuo M, Romanov K, Reunanen A, Eriksson J, Stengard J, Kesaniemi YA. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 1992; 35:1060-1067
5. Bi Y, Wang T, Xu M, Xu Y, Li M, Lu J, Zhu X, Ning G. Advanced research on risk factors of type 2 diabetes. Diabetes Metab Res Rev 2012; 28 Suppl 2:32-39
6. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2004; 27 Suppl 1:S15-S35
7. McCulloch LJ, van de Bunt M, Braun M, Frayn KN, Clark A, Gloyn AL. GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications for understanding genetic association signals at this locus. Mol Genet Metab 2011; 104:648-653
8. Koster JC, Permutt MA, Nichols CG. Diabetes and insulin secretion: the ATP-sensitive K+ channel (K ATP) connection. Diabetes 2005; 54:3065-3072
9. Curry DL, Bennett LL, Grodsky GM. Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology 1968; 83:572-584
10. Hosker JP, Rudenski AS, Burnett MA, Matthews DR, Turner RC. Similar reduction of first- and second-phase B-cell responses at three different glucose levels in type II diabetes and the effect of gliclazide therapy. Metabolism 1989; 38:767-772
11. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 1993; 329:1988-1992
12. Cerasi E, Luft R, Efendic S. Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. A glucose dose-response study. Diabetes 1972; 21:224-234
13. Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Jarvinen H, Evron W, Dailey G, Gerich J. Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. JAMA 1995; 273:1855-1861
14. Swinburn BA, Boyce VL, Bergman RN, Howard BV, Bogardus C. Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians. J Clin Endocrinol Metab 1991; 73:156-165
15. Sedaghat AR, Sherman A, Quon MJ. A mathematical model of metabolic insulin signaling pathways. Am J Physiol Endocrinol Metab 2002; 283:E1084-E1101
16. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, Asico LD, Jose PA, Taylor SI, Westphal H. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 1996; 12:106-109
17. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, Sekihara H, Yoshioka S, Horikoshi H, Furuta Y, Ikawa Y, Kasuga M, Yazaki Y, Aizawa S. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 1994; 372:182-186
18. Katz EB, Stenbit AE, Hatton K, DePinho R, Charron MJ. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 1995; 377:151-155
19. Giorgino F, Laviola L, Leonardini A. Pathophysiology of type 2 diabetes: rationale for different oral antidiabetic treatment strategies. Diabetes Res Clin Pract 2005; 68 Suppl 1:S22-S29
20. Smith U. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue? Int J Obes Relat Metab Disord 2002; 26:897-904
21. Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JF, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, Kahn B. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nat Med 2000; 6:924-928
22. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000; 105:311-320
23. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR. Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. Diabetes 1997; 46:524-527
24. Maegawa H, Shigeta Y, Egawa K, Kobayashi M. Impaired autophosphorylation of insulin receptors from abdominal skeletal muscles in nonobese subjects with NIDDM. Diabetes 1991; 40:815-819
25. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 2009; 32 Suppl 2:S157-S163
26. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 2008; 134:1369-1375
27. Magkos F, Su X, Bradley D, Fabbrini E, Conte C, Eagon JC, Varela JE, Brunt EM, Patterson BW, Klein S. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. Gastroenterology 2012; 142:1444-1446
28. Suzuki R, Tobe K, Aoyama M, Inoue A, Sakamoto K, Yamauchi T, Kamon J, Kubota N, Terauchi Y, Yoshimatsu H, Matsuhisa M, Nagasaka S, Ogata H, Tokuyama K, Nagai R, Kadowaki T. Both insulin signaling defects in the liver and obesity contribute to insulin resistance and cause diabetes in Irs2(-/-) mice. J Biol Chem 2004; 279:25039-25049

29. Holm C, Kirchgessner TG, Svenson KL, Fredrikson G, Nilsson S, Miller CG, Shively JE, Heinzmann C, Sparkes RS, Mohandas T, Lusis AJ, Belfrage P, Schotz MC. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. Science 1988; 241:1503-1506
30. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008; 29:2959-2971
31. Holm C. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Biochem Soc Trans 2003; 31:1120-1124
32. Kraemer FB, Shen WJ. Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. J Lipid Res 2002; 43:1585-1594
33. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2001; 2:282-286
34. Anthonsen MW, Ronnstrand L, Wernstedt C, Degerman E, Holm C. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. J Biol Chem 1998; 273:215-221
35. Coppack SW, Evans RD, Fisher RM, Frayn KN, Gibbons GF, Humphreys SM, Kirk ML, Potts JL, Hockaday TD. Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. Metabolism 1992; 41:264-272
36. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003; 107:671-674
37. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999; 257:79-83
38. Ruan H, Lodish HF. Regulation of insulin sensitivity by adipose tissue-derived hormones and inflammatory cytokines. Curr Opin Lipidol 2004; 15:297-302
39. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995; 95:2409-2415
40. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 2001; 409:729-733
41. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003; 52:812-817
42. Baylin A, Campos H. The use of fatty acid biomarkers to reflect dietary intake. Curr Opin Lipidol 2006; 17:22-27
43. Howard BV. Dietary fat as a risk factor for type 2 diabetes. Ann N Y Acad Sci 2002; 967:324-328
44. Gumbiner B, Low CC, Reaven PD. Effects of a monounsaturated fatty acid-enriched hypocaloric diet on cardiovascular risk factors in obese patients with type 2 diabetes. Diabetes Care 1998; 21:9-15
45. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nälsén C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH; KANWU Study. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 2001; 44:312-319
46. Ajala O, English P, Pinkney J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. Am J Clin Nutr 2013; 97:505-516
47. Feskens EJ, Kromhout D. Habitual dietary intake and glucose tolerance in euglycaemic men: the Zutphen Study. Int J Epidemiol 1990; 19:953-959
48. Williams DE, Knowler WC, Smith CJ, Hanson RL, Roumain J, Saremi A, Kriska AM, Bennett PH, Nelson RG. The effect of Indian or Anglo dietary preference on the incidence of diabetes in Pima Indians. Diabetes Care 2001; 24:811-816
49. Marshall JA, Hamman RF, Baxter J. High-fat, low-carbohydrate diet and the etiology of non-insulin-dependent diabetes mellitus: the San Luis Valley Diabetes Study. Am J Epidemiol 1991; 134:590-603
50. Barroso I. Genetics of Type 2 diabetes. Diabet Med 2005; 22:517-535
51. Lyssenko V, Laakso M. Genetic Screening for the Risk of Type 2 Diabetes: Worthless or valuable? Diabetes Care 2013; 36 Suppl 2:S120-S126
52. Groop LC, Tuomi T. Non-insulin-dependent diabetes mellitus--a collision between thrifty genes and an affluent society. Ann Med 1997; 29:37-53
53. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, Berglund G, Altshuler D, Nilsson P, Groop L. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008; 359:2220-2232
54. Ott J, Kamatani Y, Lathrop M. Family-based designs for genome-wide association studies. Nat Rev Genet 2011; 12:465-474
55. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Leprêtre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet 2000; 67:1470-1480
56. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, O'Rahilly S, Frayling TM, Bell JI, Lathrop GM, Bennett A, Dhillon R, Fletcher C, Groves CJ, Jones E, Prestwich P, Simecek N, Rao PV, Wishart M, Bottazzo GF, Foxon R, Howell S, Smedley D, Cardon LR, Menzel S, McCarthy MI. A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. Am J Hum Genet 2001; 69:553-569

57. Zouali H, Hani EH, Philippi A, Vionnet N, Beckmann JS, Demenais F, Froguel P. A susceptibility locus for early-onset non-insulin dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. Hum Mol Genet 1997; 6:1401-1408
58. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006; 38:320-323
59. Cauchi S, El Achhab Y, Choquet H, Dina C, Krempler F, Weitgasser R, Nejjari C, Patsch W, Chikri M, Meyre D, Froguel P. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J Mol Med 2007; 85:777-782
60. Grant RW, Moore AF, Florez JC. Genetic architecture of type 2 diabetes: recent progress and clinical implications. Diabetes Care 2009; 32:1107-1114
61. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007; 445:881-885
62. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Boström K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Råstam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjögren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007; 316:1331-1336
63. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007; 316:1341-1345
64. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447:661-678
65. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS; Wellcome Trust Case Control Consortium (WTCCC), McCarthy MI, Hattersley AT. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007; 316:1336-1341
66. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007; 316:889-894
67. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PI, Abecasis GR, Almgren P, Andersen G, Ardlie K, Boström KB, Bergman RN, Bonnycastle LL, Borch-Johnsen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren WL, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hitman GA, Hughes TE, Isomaa B, Jackson AU, Jørgensen T, Kong A, Kubalanza K, Kuruvilla FG, Kuusisto J, Langenberg C, Lango H, Lauritzen T, Li Y, Lindgren CM, Lyssenko V, Marvelle AF, Meisinger C, Midthjell K, Mohlke KL, Morken MA, Morris AD, Narisu N, Nilsson P, Owen KR, Palmer CN, Payne F, Perry JR, Pettersen E, Platou C, Prokopenko I, Qi L, Qin L, Rayner NW, Rees M, Roix JJ, Sandbaek A, Shields B, Sjögren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ; Wellcome Trust Case Control Consortium, Illig T, Hveem K, Hu FB, Laakso M, Stefansson K, Pedersen O, Wareham NJ, Barroso I, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008; 40:638-645
68. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 2010; 42:30-35
69. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, Walker MF, Ober GT, Teran NA, Song Y, El-Fishawy P, Murtha RC, Choi M, Overton JD, Bjornson RD, Carriero NJ, Meyer KA, Bilguvar K, Mane SM, Sestan N, Lifton RP, Günel M, Roeder K, Geschwind DH, Devlin B, State MW. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012; 485:237-241
70. Albrechtsen A, Grarup N, Li Y, Sparsø T, Tian G, Cao H, Jiang T, Kim SY, Korneliussen T, Li Q, Nie C, Wu R, Skotte L, Morris AP, Ladenvall C, Cauchi S, Stančáková A, Andersen G, Astrup A, Banasik K, Bennett AJ, Bolund L, Charpentier G, Chen Y, Dekker JM, Doney AS, Dorkhan M, Forsen T, Frayling TM, Groves CJ, Gui Y, Hallmans G, Hattersley AT, He K, Hitman GA, Holmkvist J, Huang S, Jiang H, Jin X, Justesen JM, Kristiansen K, Kuusisto J, Lajer M, Lantieri O, Li W, Liang H, Liao Q, Liu X, Ma T, Ma X, Manijak MP, Marre M, Mokrosiński J, Morris AD, Mu B, Nielsen AA, Nijpels G, Nilsson P, Palmer CN, Rayner NW, Renström F, Ribel-Madsen R, Robertson N, Rolandsson O, Rossing P, Schwartz TW; D.E.S.I.R. Study Group, Slagboom PE, Sterner M; DIAGRAM Consortium, Tang M, Tarnow L, Tuomi T, van't Riet E, van Leeuwen N, Varga TV, Vestmar MA, Walker M, Wang B, Wang Y, Wu H, Xi F, Yengo

L, Yu C, Zhang X, Zhang J, Zhang Q, Zhang W, Zheng H, Zhou Y, Altshuler D, 't Hart LM, Franks PW, Balkau B, Froguel P, McCarthy MI, Laakso M, Groop L, Christensen C, Brandslund I, Lauritzen T, Witte DR, Linneberg A, Jørgensen T, Hansen T, Wang J, Nielsen R, Pedersen O. Exome sequencing-driven discovery of coding polymorphisms associated with common metabolic phenotypes. Diabetologia 2013; 56:298-310

71. Huyghe JR, Jackson AU, Fogarty MP, Buchkovich ML, Stančáková A, Stringham HM, Sim X, Yang L, Fuchsberger C, Cederberg H, Chines PS, Teslovich TM, Romm JM, Ling H, McMullen I, Ingersoll R, Pugh EW, Doheny KF, Neale BM, Daly MJ, Kuusisto J, Scott LJ, Kang HM, Collins FS, Abecasis GR, Watanabe RM, Boehnke M, Laakso M, Mohlke KL. Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. Nat Genet 2013; 45:197-201

72. Yaghootkar H, Frayling TM. Recent progress in the use of genetics to understand links between type 2 diabetes and related metabolic traits. Genome Biol 2013; 14:203

73. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Mägi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparsø T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proença C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jørgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martínez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orrù M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvänen AC, Tanaka T, Thorand B, Tichet J, Tönjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC; DIAGRAM Consortium; GIANT Consortium; Global BPgen Consortium, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Ríos M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF; Anders Hamsten on behalf of Procardis Consortium; MAGIC investigators, Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC, Barroso I. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010; 42:105-116

74. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Mägi R, Strawbridge RJ, Rehnberg E, Gustafsson S, Kanoni S, Rasmussen-Torvik LJ, Yengo L, Lecoeur C, Shungin D, Sanna S, Sidore C, Johnson PC, Jukema JW, Johnson T, Mahajan A, Verweij N, Thorleifsson G, Hottenga JJ, Shah S, Smith AV, Sennblad B, Gieger C, Salo P, Perola M, Timpson NJ, Evans DM, Pourcain BS, Wu Y, Andrews JS, Hui J, Bielak LF, Zhao W, Horikoshi M, Navarro P, Isaacs A, O'Connell JR, Stirrups K, Vitart V, Hayward C, Esko T, Mihailov E, Fraser RM, Fall T, Voight BF, Raychaudhuri S, Chen H, Lindgren CM, Morris AP, Rayner NW, Robertson N, Rybin D, Liu CT, Beckmann JS, Willems SM, Chines PS, Jackson AU, Kang HM, Stringham HM, Song K, Tanaka T, Peden JF, Goel A, Hicks AA, An P, Müller-Nurasyid M, Franco-Cereceda A, Folkersen L, Marullo L, Jansen H, Oldehinkel AJ, Bruinenberg M, Pankow JS, North KE, Forouhi NG, Loos RJ, Edkins S, Varga TV, Hallmans G, Oksa H, Antonella M, Nagaraja R, Trompet S, Ford I, Bakker SJ, Kong A, Kumari M, Gigante B, Herder C, Munroe PB, Caulfield M, Antti J, Mangino M, Small K, Miljkovic I, Liu Y, Atalay M, Kiess W, James AL, Rivadeneira F, Uitterlinden AG, Palmer CN, Doney AS, Willemsen G, Smit JH, Campbell S, Polasek O, Bonnycastle LL, Hercberg S, Dimitriou M, Bolton JL, Fowkes GR, Kovacs P, Lindström J, Zemunik T, Bandinelli S, Wild SH, Basart HV, Rathmann W, Grallert H; DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium, Maerz W, Kleber ME, Boehm BO, Peters A, Pramstaller PP, Province MA, Borecki IB, Hastie ND, Rudan I, Campbell H, Watkins H, Farrall M, Stumvoll M, Ferrucci L, Waterworth DM, Bergman RN, Collins FS, Tuomilehto J, Watanabe RM, de Geus EJ, Penninx BW, Hofman A, Oostra BA, Psaty BM, Vollenweider P, Wilson JF, Wright AF, Hovingh GK, Metspalu A, Uusitupa M, Magnusson PK, Kyvik KO, Kaprio J, Price JF, Dedoussis GV, Deloukas P, Meneton P, Lind L, Boehnke M, Shuldiner AR, van Duijn CM, Morris AD, Toenjes A, Peyser PA, Beilby JP, Körner A, Kuusisto J, Laakso M, Bornstein SR, Schwarz PE, Lakka TA, Rauramaa R, Adair LS, Smith GD, Spector TD, Illig T, de Faire U, Hamsten A, Gudnason V, Kivimaki M, Hingorani A, Keinanen-Kiukaanniemi SM, Saaristo TE, Boomsma DI, Stefansson K, van der Harst P, Dupuis J, Pedersen NL, Sattar N, Harris TB, Cucca F, Ripatti S, Salomaa V, Mohlke KL, Balkau B, Froguel P, Pouta A, Jarvelin MR, Wareham NJ, Bouatia-Naji N, McCarthy MI, Franks PW, Meigs JB, Teslovich TM, Florez JC, Langenberg C, Ingelsson E, Prokopenko I, Barroso I. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet 2012; 44:991-1005

75. Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, Kao WH, Li M, Glazer NL, Manning AK, Luan J, Stringham HM, Prokopenko I, Johnson T, Grarup N, Boesgaard TW, Lecoeur C, Shrader P, O'Connell J, Ingelsson E, Couper DJ, Rice K, Song K, Andreasen CH, Dina C, Köttgen A, Le Bacquer O, Pattou F, Taneera J, Steinthorsdottir V, Rybin D, Ardlie K, Sampson M, Qi L, van Hoek M, Weedon MN, Aulchenko YS, Voight BF, Grallert H, Balkau B, Bergman RN, Bielinski SJ, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher Y, Brunner E, Buchanan TA, Bumpstead SJ, Cavalcanti-Proença C, Charpentier G, Chen YD, Chines PS, Collins FS, Cornelis M, J Crawford G, Delplanque J, Doney A, Egan JM, Erdos MR, Firmann M, Forouhi NG, Fox CS, Goodarzi MO, Graessler J, Hingorani A, Isomaa B, Jørgensen T, Kivimaki M, Kovacs P, Krohn K, Kumari M, Lauritzen T, Lévy-Marchal C, Mayor V, McAteer JB, Meyre D, Mitchell BD, Mohlke KL, Morken MA, Narisu N, Palmer CN, Pakyz R, Pascoe L, Payne F, Pearson D, Rathmann W, Sandbaek A, Sayer AA, Scott LJ, Sharp SJ, Sijbrands E, Singleton A, Siscovick DS, Smith NL, Sparsø T, Swift AJ, Syddall H, Thorleifsson G, Tönjes A, Tuomi T, Tuomilehto J, Valle TT, Waeber G, Walley A, Waterworth DM, Zeggini E, Zhao JH; GIANT consortium; MAGIC investigators, Illig T, Wichmann HE, Wilson JF, van Duijn C, Hu FB, Morris AD, Frayling TM, Hattersley AT, Thorsteinsdottir U, Stefansson K, Nilsson P, Syvänen AC, Shuldiner AR, Walker M, Bornstein SR, Schwarz P, Williams GH, Nathan DM, Kuusisto J, Laakso M, Cooper C, Marmot M, Ferrucci L, Mooser V, Stumvoll M, Loos RJ, Altshuler D, Psaty BM, Rotter JI, Boerwinkle E, Hansen T, Pedersen O, Florez JC, McCarthy MI, Boehnke M, Barroso I, Sladek R, Froguel P, Meigs JB, Groop L, Wareham NJ, Watanabe RM. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 2010; 42:142-148
76. Simova S, Klima M, Cermak L, Sourkova V, Andera L. Arf and Rho GAP adapter protein ARAP1 participates in the mobilization of TRAIL-R1/DR4 to the plasma membrane. Apoptosis 2008; 13:423-436
77. Daniele T, Di Tullio G, Santoro M, Turacchio G, De Matteis MA. ARAP1 regulates EGF receptor trafficking and signalling. Traffic 2008; 9:2221-2235
78. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, McCulloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, McCarroll SA, Langenberg C, Hofmann OM, Dupuis J, Qi L, Segrè AV, van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bengtsson Boström K, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, Chines PS, Cornelis M, Couper DJ, Crawford G, Doney AS, Elliott KS, Elliott AL, Erdos MR, Fox CS, Franklin CS, Ganser M, Gieger C, Grarup N, Green T, Griffin S, Groves CJ, Guiducci C, Hadjadj S, Hassanali N, Herder C, Isomaa B, Jackson AU, Johnson PR, Jørgensen T, Kao WH, Klopp N, Kong A, Kraft P, Kuusisto J, Lauritzen T, Li M, Lieverse A, Lindgren CM, Lyssenko V, Marre M, Meitinger T, Midthjell K, Morken MA, Narisu N, Nilsson P, Owen KR, Payne F, Perry JR, Petersen AK, Platou C, Proença C, Prokopenko I, Rathmann W, Rayner NW, Robertson NR, Rocheleau G, Roden M, Sampson MJ, Saxena R, Shields BM, Shrader P, Sigurdsson G, Sparsø T, Strassburger K, Stringham HM, Sun Q, Swift AJ, Thorand B, Tichet J, Tuomi T, van Dam RM, van Haeften TW, van Herpt T, van Vliet-Ostaptchouk JV, Walters GB, Weedon MN, Wijmenga C, Witteman J, Bergman RN, Cauchi S, Collins FS, Gloyn AL, Gyllensten U, Hansen T, Hide WA, Hitman GA, Hofman A, Hunter DJ, Hveem K, Laakso M, Mohlke KL, Morris AD, Palmer CN, Pramstaller PP, Rudan I, Sijbrands E, Stein LD, Tuomilehto J, Uitterlinden A, Walker M, Wareham NJ, Watanabe RM, Abecasis GR, Boehm BO, Campbell H, Daly MJ, Hattersley AT, Hu FB, Meigs JB, Pankow JS, Pedersen O, Wichmann HE, Barroso I, Florez JC, Frayling TM, Groop L, Sladek R, Thorsteinsdottir U, Wilson JF, Illig T, Froguel P, van Duijn CM, Stefansson K, Altshuler D, Boehnke M, McCarthy MI; MAGIC investigators; GIANT Consortium. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010; 42:579-589
79. Nielsen T, Sparso T, Grarup N, Jorgensen T, Pisinger C, Witte DR, Diabetes Genetics R, Meta-analysis C, Hansen T, Pedersen O. Type 2 diabetes risk allele near CENTD2 is associated with decreased glucose-stimulated insulin release. Diabetologia 2011; 54:1052-1056
80. Freathy RM, Mook-Kanamori DO, Sovio U, Prokopenko I, Timpson NJ, Berry DJ,Warrington NM, Widen E, Hottenga JJ, Kaakinen M, Lange LA, Bradfield JP, Kerkhof M, Marsh JA, Mägi R, Chen CM, Lyon HN, Kirin M, Adair LS, Aulchenko YS, Bennett AJ, Borja JB, Bouatia-Naji N, Charoen P, Coin LJ, Cousminer DL, de Geus EJ, Deloukas P, Elliott P, Evans DM, Froguel P; Genetic Investigation of ANthropometric Traits (GIANT) Consortium, Glaser B, Groves CJ, Hartikainen AL, Hassanali N, Hirschhorn JN, Hofman A, Holly JM, Hyppönen E, Kanoni S, Knight BA, Laitinen J, Lindgren CM; Meta-Analyses of Glucose and Insulin-related traits Consortium, McArdle WL, O'Reilly PF, Pennell CE, Postma DS, Pouta A, Ramasamy A, Rayner NW, Ring SM, Rivadeneira F, Shields BM, Strachan DP, Surakka I, Taanila A, Tiesler C, Uitterlinden AG, van Duijn CM; Wellcome Trust Case Control Consortium, Wijga AH, Willemsen G, Zhang H, Zhao J, Wilson JF, Steegers EA, Hattersley AT, Eriksson JG, Peltonen L, Mohlke KL, Grant SF, Hakonarson H, Koppelman GH, Dedoussis GV, Heinrich J, Gillman MW, Palmer LJ, Frayling TM, Boomsma DI, Davey Smith G, Power C, Jaddoe VW, Jarvelin MR; Early Growth Genetics (EGG) Consortium, McCarthy MI. Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. Nat Genet 2010; 42:430-435
81. Boesgaard TW, Grarup N, Jorgensen T, Borch-Johnsen K, Meta-Analysis of G, Insulin-Related Trait C, Hansen T, Pedersen O. Variants at DGKB/TMEM195, ADRA2A, GLIS3 and C2CD4B loci are associated with reduced glucose-stimulated beta cell function in middle-aged Danish people. Diabetologia 2010; 53:1647-1655
82. Simonis-Bik AM, Nijpels G, van Haeften TW, Houwing-Duistermaat JJ, Boomsma DI, Reiling E, van Hove EC, Diamant M, Kramer MH, Heine RJ, Maassen JA, Slagboom PE, Willemsen G, Dekker JM, Eekhoff EM, de Geus EJ, 't Hart LM. Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic beta-cell function. Diabetes 2010; 59:293-301
83. Kirchhoff K, Machicao F, Haupt A, Schafer SA, Tschritter O, Staiger H, Stefan N, Haring HU, Fritsche A. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. Diabetologia 2008; 51:597-601
84. Stancáková A, Pihlajamäki J, Kuusisto J, Stefan N, Fritsche A, Häring H, Andreozzi F, Succurro E, Sesti G, Boesgaard TW, Hansen T, Pedersen O, Jansson PA, Hammarstedt A, Smith U, Laakso M; EUGENE2 Consortium. Single-nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. J Clin Endocrinol Metab 2008; 93:1924-1930

85. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorradottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007; 39:770-775
86. Andersson EA, Pilgaard K, Pisinger C, Harder MN, Grarup N, Faerch K, Poulsen P, Witte DR, Jørgensen T, Vaag A, Hansen T, Pedersen O. Type 2 diabetes risk alleles near ADCY5, CDKAL1 and HHEX-IDE are associated with reduced birthweight. Diabetologia 2010; 53:1908-1916
87. Grarup N, Rose CS, Andersson EA, Andersen G, Nielsen AL, Albrechtsen A, Clausen JO, Rasmussen SS, Jørgensen T, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 2007; 56:3105-3111
88. Grarup N, Overvad M, Sparsø T, Witte DR, Pisinger C, Jørgensen T, Yamauchi T, Hara K, Maeda S, Kadowaki T, Hansen T, Pedersen O. The diabetogenic VPS13C/C2CD4A/C2CD4B rs7172432 variant impairs glucose-stimulated insulin response in 5,722 non-diabetic Danish individuals. Diabetologia 2011; 54:789-794
89. Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, Rafnar T, Gudbjartsson D, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Blondal T, Stacey SN, Helgason A, Gunnarsdottir S, Olafsdottir A, Kristinsson KT, Birgisdottir B, Ghosh S, Thorlacius S, Magnusdottir D, Stefansdottir G, Kristjansson K, Bagger Y, Wilensky RL, Reilly MP, Morris AD, Kimber CH, Adeyemo A, Chen Y, Zhou J, So WY, Tong PC, Ng MC, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Tres A, Fuertes F, Ruiz-Echarri M, Asin L, Saez B, van Boven E, Klaver S, Swinkels DW, Aben KK, Graif T, Cashy J, Suarez BK, van Vierssen Trip O, Frigge ML, Ober C, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Palmer CN, Rotimi C, Chan JC, Pedersen O, Sigurdsson G, Benediktsson R, Jonsson E, Einarsson GV, Mayordomo JI, Catalona WJ, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat Genet 2007; 39:977-983
90. Pivovarova O, Nikiforova VJ, Pfeiffer AF, Rudovich N. The influence of genetic variations in HHEX gene on insulin metabolism in the German MESYBEPO cohort. Diabetes Metab Res Rev 2009; 25:156-162
91. Groenewoud MJ, Dekker JM, Fritsche A, Reiling E, Nijpels G, Heine RJ, Maassen JA, Machicao F, Schäfer SA, Häring HU, 't Hart LM, van Haeften TW. Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. Diabetologia 2008; 51:1659-1663
92. Stancakova A, Kuulasmaa T, Paananen J, Jackson AU, Bonnycastle LL, Collins FS, Boehnke M, Kuusisto J, Laakso M. Association of 18 confirmed susceptibility loci for type 2 diabetes with indices of insulin release, proinsulin conversion, and insulin sensitivity in 5,327 nondiabetic Finnish men. Diabetes 2009; 58:2129-2136
93. Wu Y, Li H, Loos RJ, Yu Z, Ye X, Chen L, Pan A, Hu FB, Lin X. Common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, and HHEX/IDE genes are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. Diabetes 2008; 57:2834-2842
94. Grarup N, Andersen G, Krarup NT, Albrechtsen A, Schmitz O, Jorgensen T, Borch-Johnsen K, Hansen T, Pedersen O. Association testing of novel type 2 diabetes risk alleles in the JAZF1, CDC123/CAMK1D, TSPAN8, THADA, ADAMTS9, and NOTCH2 loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. Diabetes 2008; 57:2534-2540
95. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer C, Thorsteinsson B, Borch-Johnsen K, Hansen T, Pedersen O. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. Diabetes 2003; 52:573-577
96. Tschritter O, Stumvoll M, Machicao F, Holzwarth M, Weisser M, Maerker E, Teigeler A, Haring H, Fritsche A. The prevalent Glu23Lys polymorphism in the potassium inward rectifier 6.2 (KIR6.2) gene is associated with impaired glucagon suppression in response to hyperglycemia. Diabetes 2002; 51:2854-2860
97. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, Furuta H, Hirota Y, Mori H, Jonsson A, Sato Y, Yamagata K, Hinokio Y, Wang HY, Tanahashi T, Nakamura N, Oka Y, Iwasaki N, Iwamoto Y, Yamada Y, Seino Y, Maegawa H, Kashiwagi A, Takeda J, Maeda E, Shin HD, Cho YM, Park KS, Lee HK, Ng MC, Ma RC, So WY, Chan JC, Lyssenko V, Tuomi T, Nilsson P, Groop L, Kamatani N, Sekine A, Nakamura Y, Yamamoto K, Yoshida T, Tokunaga K, Itakura M, Makino H, Nanjo K, Kadowaki T, Kasuga M. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 2008; 40:1092-1097
98. Jonsson A, Isomaa B, Tuomi T, Taneera J, Salehi A, Nilsson P, Groop L, Lyssenko V. A variant in the KCNQ1 gene predicts future type 2 diabetes and mediates impaired insulin secretion. Diabetes 2009; 58:2409-2413
99. Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, Spégel P, Bugliani M, Saxena R, Fex M, Pulizzi N, Isomaa B, Tuomi T, Nilsson P, Kuusisto J, Tuomilehto J, Boehnke M, Altshuler D, Sundler F, Eriksson JG, Jackson AU, Laakso M, Marchetti P, Watanabe RM, Mulder H, Groop L. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 2009; 41:82-88
100. Wagner R, Dudziak K, Herzberg-Schafer SA, Machicao F, Stefan N, Staiger H, Haring HU, Fritsche A. Glucose-raising genetic variants in MADD and ADCY5 impair conversion of proinsulin to insulin. PLoS One 2011; 6:e23639
101. Boesgaard TW, Zilinskaite J, Vänttinen M, Laakso M, Jansson PA, Hammarstedt A, Smith U, Stefan N, Fritsche A, Häring H, Hribal M, Sesti G, Zobel DP, Pedersen O, Hansen T; EUGENE 2 Consortium. The common SLC30A8 Arg325Trp variant is associated with

reduced first-phase insulin release in 846 non-diabetic offspring of type 2 diabetes patients--the EUGENE2 study. Diabetologia 2008; 51:816-820

102. Ingelsson E, Langenberg C, Hivert MF, Prokopenko I, Lyssenko V, Dupuis J, Mägi R, Sharp S, Jackson AU, Assimes TL, Shrader P, Knowles JW, Zethelius B, Abbasi FA, Bergman RN, Bergmann A, Berne C, Boehnke M, Bonnycastle LL, Bornstein SR, Buchanan TA, Bumpstead SJ, Böttcher Y, Chines P, Collins FS, Cooper CC, Dennison EM, Erdos MR, Ferrannini E, Fox CS, Graessler J, Hao K, Isomaa B, Jameson KA, Kovacs P, Kuusisto J, Laakso M, Ladenvall C, Mohlke KL, Morken MA, Narisu N, Nathan DM, Pascoe L, Payne F, Petrie JR, Sayer AA, Schwarz PE, Scott LJ, Stringham HM, Stumvoll M, Swift AJ, Syvänen AC, Tuomi T, Tuomilehto J, Tönjes A, Valle TT, Williams GH, Lind L, Barroso I, Quertermous T, Walker M, Wareham NJ, Meigs JB, McCarthy MI, Groop L, Watanabe RM, Florez JC; MAGIC investigators. Detailed physiologic characterization reveals diverse mechanisms for novel genetic Loci regulating glucose and insulin metabolism in humans. Diabetes 2010; 59:1266-1275

103. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjögren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes 2006; 55:2890-2895

104. Pilgaard K, Jensen CB, Schou JH, Lyssenko V, Wegner L, Brøns C, Vilsbøll T, Hansen T, Madsbad S, Holst JJ, Vølund A, Poulsen P, Groop L, Pedersen O, Vaag AA. The T allele of rs7903146 TCF7L2 is associated with impaired insulinotropic action of incretin hormones, reduced 24 h profiles of plasma insulin and glucagon, and increased hepatic glucose production in young healthy men. Diabetologia 2009; 52:1298-1307

105. Rung J, Cauchi S, Albrechtsen A, Shen L, Rocheleau G, Cavalcanti-Proença C, Bacot F, Balkau B, Belisle A, Borch-Johnsen K, Charpentier G, Dina C, Durand E, Elliott P, Hadjadj S, Järvelin MR, Laitinen J, Lauritzen T, Marre M, Mazur A, Meyre D, Montpetit A, Pisinger C, Posner B, Poulsen P, Pouta A, Prentki M, Ribel-Madsen R, Ruokonen A, Sandbaek A, Serre D, Tichet J, Vaxillaire M, Wojtaszewski JF, Vaag A, Hansen T, Polychronakos C, Pedersen O, Froguel P, Sladek R. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet 2009; 41:1110-1115

106. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O. Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant caucasians. Diabetologia 2001; 44:1170-1176

107. Boesgaard TW, Gjesing AP, Grarup N, Rutanen J, Jansson PA, Hribal ML, Sesti G, Fritsche A, Stefan N, Staiger H, Häring H, Smith U, Laakso M, Pedersen O, Hansen T; EUGENE2 Consortium. Variant near ADAMTS9 known to associate with type 2 diabetes is related to insulin resistance in offspring of type 2 diabetes patients--EUGENE2 study. PLoS One 2009; 4:e7236

108. Harder MN, Ribel-Madsen R, Justesen JM, Sparso T, Andersson EA, Grarup N, Jorgensen T, Linneberg A, Hansen T, Pedersen O. Type 2 diabetes risk alleles near BCAR1 and in ANK1 associate with decreased beta-cell function whereas risk alleles near ANKRD55 and GRB14 associate with decreased insulin sensitivity in the Danish Inter99 cohort. J Clin Endocrinol Metab 2013; 98:E801-E806

109. Fukuda H, Imamura M, Tanaka Y, Iwata M, Hirose H, Kaku K, Maegawa H, Watada H, Tobe K, Kashiwagi A, Kawamori R, Maeda S. A single nucleotide polymorphism within DUSP9 is associated with susceptibility to type 2 diabetes in a Japanese population. PLoS One 2012; 7:e46263

110. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, Been LF, Chia KS, Dimas AS, Hassanali N, Jafar T, Jowett JB, Li X, Radha V, Rees SD, Takeuchi F, Young R, Aung T, Basit A, Chidambaram M, Das D, Grundberg E, Hedman AK, Hydrie ZI, Islam M, Khor CC, Kowlessur S, Kristensen MM, Liju S, Lim WY, Matthews DR, Liu J, Morris AP, Nica AC, Pinidiyapathirage JM, Prokopenko I, Rasheed A, Samuel M, Shah N, Shera AS, Small KS, Suo C, Wickremasinghe AR, Wong TY, Yang M, Zhang F; DIAGRAM; MuTHER, Abecasis GR, Barnett AH, Caulfield M, Deloukas P, Frayling TM, Froguel P, Kato N, Katulanda P, Kelly MA, Liang J, Mohan V, Sanghera DK, Scott J, Seielstad M, Zimmet PZ, Elliott P, Teo YY, McCarthy MI, Danesh J, Tai ES, Chambers JC. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet 2011; 43:984-989

111. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, Prokopenko I, Kang HM, Dina C, Esko T, Fraser RM, Kanoni S, Kumar A, Lagou V, Langenberg C, Luan J, Lindgren CM, Müller-Nurasyid M, Pechlivanis S, Rayner NW, Scott LJ, Wiltshire S, Yengo L, Kinnunen L, Rossin EJ, Raychaudhuri S, Johnson AD, Dimas AS, Loos RJ, Vedantam S, Chen H, Florez JC, Fox C, Liu CT, Rybin D, Couper DJ, Kao WH, Li M, Cornelis MC, Kraft P, Sun Q, van Dam RM, Stringham HM, Chines PS, Fischer K, Fontanillas P, Holmen OL, Hunt SE, Jackson AU, Kong A, Lawrence R, Meyer J, Perry JR, Platou CG, Potter S, Rehnberg E, Robertson N, Sivapalaratnam S, Stančáková A, Stirrups K, Thorleifsson G, Tikkanen E, Wood AR, Almgren P, Atalay M, Benediktsson R, Bonnycastle LL, Burtt N, Carey J, Charpentier G, Crenshaw AT, Doney AS, Dorkhan M, Edkins S, Emilsson V, Eury E, Forsen T, Gertow K, Gigante B, Grant GB, Groves CJ, Guiducci C, Herder C, Hreidarsson AB, Hui J, James A, Jonsson A, Rathmann W, Klopp N, Kravic J, Krjutškov K, Langford C, Leander K, Lindholm E, Lobbens S, Männistö S, Mirza G, Mühleisen TW, Musk B, Parkin M, Rallidis L, Saramies J, Sennblad B, Shah S, Sigurðsson G, Silveira A, Steinbach G, Thorand B, Trakalo J, Veglia F, Wennauer R, Winckler W, Zabaneh D, Campbell H, van Duijn C, Uitterlinden AG, Hofman A, Sijbrands E, Abecasis GR, Owen KR, Zeggini E, Trip MD, Forouhi NG, Syvänen AC, Eriksson JG, Peltonen L, Nöthen MM, Balkau B, Palmer CN, Lyssenko V, Tuomi T, Isomaa B, Hunter DJ, Qi L; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium, Shuldiner AR, Roden M, Barroso I, Wilsgaard T, Beilby J, Hovingh K, Price JF, Wilson JF, Rauramaa R, Lakka TA, Lind L, Dedoussis G, Njølstad I, Pedersen NL, Khaw KT, Wareham NJ, Keinanen-Kiukaanniemi SM, Saaristo TE, Korpi-Hyövälti E, Saltevo J, Laakso M, Kuusisto J, Metspalu A, Collins FS, Mohlke KL, Bergman RN, Tuomilehto J, Boehm BO, Gieger C, Hveem K, Cauchi S, Froguel P, Baldassarre D, Tremoli E, Humphries SE, Saleheen D, Danesh J, Ingelsson E, Ripatti S, Salomaa V, Erbel R, Jöckel KH,

Moebus S, Peters A, Illig T, de Faire U, Hamsten A, Morris AD, Donnelly PJ, Frayling TM, Hattersley AT, Boerwinkle E, Melander O, Kathiresan S, Nilsson PM, Deloukas P, Thorsteinsdottir U, Groop LC, Stefansson K, Hu F, Pankow JS, Dupuis J, Meigs JB, Altshuler D, Boehnke M, McCarthy MI; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 2012; 44:981-990

112. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, Takeuchi F, Wu Y, Go MJ, Yamauchi T, Chang YC, Kwak SH, Ma RC, Yamamoto K, Adair LS, Aung T, Cai Q, Chang LC, Chen YT, Gao Y, Hu FB, Kim HL, Kim S, Kim YJ, Lee JJ, Lee NR, Li Y, Liu JJ, Lu W, Nakamura J, Nakashima E, Ng DP, Tay WT, Tsai FJ, Wong TY, Yokota M, Zheng W, Zhang R, Wang C, So WY, Ohnaka K, Ikegami H, Hara K, Cho YM, Cho NH, Chang TJ, Bao Y, Hedman ÅK, Morris AP, McCarthy MI; DIAGRAM Consortium; MuTHER Consortium, Takayanagi R, Park KS, Jia W, Chuang LM, Chan JC, Maeda S, Kadowaki T, Lee JY, Wu JY, Teo YY, Tai ES, Shu XO, Mohlke KL, Kato N, Han BG, Seielstad M. Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 2012; 44:67-72
113. Gupta V, Vinay DG, Rafiq S, Kranthikumar MV, Janipalli CS, Giambartolomei C, Evans DM, Mani KR, Sandeep MN, Taylor AE, Kinra S, Sullivan RM, Bowen L, Timpson NJ, Smith GD, Dudbridge F, Prabhakaran D, Ben-Shlomo Y, Reddy KS, Ebrahim S, Chandak GR; Indian Migration Study Group. Association analysis of 31 common polymorphisms with type 2 diabetes and its related traits in Indian sib pairs. Diabetologia 2012; 55:349-357
114. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, Chang CT, Wang TY, Chen RH, Shiu CF, Liu YM, Chang CC, Chen P, Chen CH, Fann CS, Chen YT, Wu JY. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet 2010; 6:e1000847
115. Qi L, Cornelis MC, Kraft P, Stanya KJ, Linda Kao WH, Pankow JS, Dupuis J, Florez JC, Fox CS, Paré G, Sun Q, Girman CJ, Laurie CC, Mirel DB, Manolio TA, Chasman DI, Boerwinkle E, Ridker PM, Hunter DJ, Meigs JB, Lee CH, Hu FB, van Dam RM; Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. Hum Mol Genet 2010; 19:2706-2715
116. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, Tai ES, Li X, Lin X, Chow WH, Go MJ, Seielstad M, Bao W, Li H, Cornelis MC, Yu K, Wen W, Shi J, Han BG, Sim XL, Liu L, Qi Q, Kim HL, Ng DP, Lee JY, Kim YJ, Li C, Gao YT, Zheng W, Hu FB. Identification of new genetic risk variants for type 2 diabetes. PLoS Genet 2010; 6:e1001127
117. Lu S, Xie Y, Lin K, Li S, Zhou Y, Ma P, Lv Z, Zhou X. Genome-wide association studies-derived susceptibility loci in type 2 diabetes: confirmation in a Chinese population. Clin Invest Med 2012; 35:E327
118. Yamauchi T, Hara K, Maeda S, Yasuda K, Takahashi A, Horikoshi M, Nakamura M, Fujita H, Grarup N, Cauchi S, Ng DP, Ma RC, Tsunoda T, Kubo M, Watada H, Maegawa H, Okada-Iwabu M, Iwabu M, Shojima N, Shin HD, Andersen G, Witte DR, Jørgensen T, Lauritzen T, Sandbæk A, Hansen T, Ohshige T, Omori S, Saito I, Kaku K, Hirose H, So WY, Beury D, Chan JC, Park KS, Tai ES, Ito C, Tanaka Y, Kashiwagi A, Kawamori R, Kasuga M, Froguel P, Pedersen O, Kamatani N, Nakamura Y, Kadowaki T. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet 2010; 42:864-868
119. Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, Daly A, Lango H, Frayling TM, Neumann RJ, Sherva R, Blech I, Pharoah PD, Palmer CN, Kimber C, Tavendale R, Morris AD, McCarthy MI, Walker M, Hitman G, Glaser B, Permutt MA, Hattersley AT, Wareham NJ, Barroso I. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet 2007; 39:951-953
120. Schafer SA, Mussig K, Staiger H, Machicao F, Stefan N, Gallwitz B, Haring HU, Fritsche A. A common genetic variant in WFS1 determines impaired glucagon-like peptide-1-induced insulin secretion. Diabetologia 2009; 52:1075-1082
121. Wei FY, Suzuki T, Watanabe S, Kimura S, Kaitsuka T, Fujimura A, Matsui H, Atta M, Michiue H, Fontecave M, Yamagata K, Suzuki T, Tomizawa K. Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. J Clin Invest 2011; 121:3598-3608
122. Dehwah MA, Wang M, Huang QY. CDKAL1 and type 2 diabetes: a global meta-analysis. Genet Mol Res 2010; 9:1109-1120
123. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, Shulman GI. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 2002; 277:50230-50236
124. Bort R, Martinez-Barbera JP, Beddington RS, Zaret KS. Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. Development 2004; 131:797-806
125. Foley AC, Mercola M. Heart induction by Wnt antagonists depends on the homeodomain transcription factor Hex. Genes Dev 2005; 19:387-396
126. Christiansen J, Kolte AM, Hansen T, Nielsen FC. IGF2 mRNA-binding protein 2: biological function and putative role in type 2 diabetes. J Mol Endocrinol 2009; 43:187-195
127. Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 1999; 19:1262-1270
128. John SA, Weiss JN, Xie LH, Ribalet B. Molecular mechanism for ATP-dependent closure of the K+ channel Kir6.2. J Physiol 2003; 552:23-34
129. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, Ng DP, Holmkvist J, Borch-Johnsen K, Jørgensen T, Sandbaek A, Lauritzen T, Hansen T, Nurbaya S, Tsunoda T, Kubo M, Babazono T, Hirose H, Hayashi M, Iwamoto Y, Kashiwagi A, Kaku K, Kawamori R, Tai ES, Pedersen O, Kamatani N, Kadowaki T, Kikkawa R, Nakamura Y, Maeda S. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 2008; 40:1098-1102
130. van Vliet-Ostaptchouk JV, van Haeften TW, Landman GW, Reiling E, Kleefstra N, Bilo HJ, Klungel OH, de Boer A, van Diemen CC, Wijmenga C, Boezen HM, Dekker JM, van 't Riet E, Nijpels G, Welschen LM, Zavrelova H, Bruin EJ, Elbers CC, Bauer F, Onland-Moret NC, van der Schouw YT, Grobbee DE, Spijkerman AM, van der A DL, Simonis-Bik AM, Eekhoff EM, Diamant M, Kramer MH,

Boomsma DI, de Geus EJ, Willemsen G, Slagboom PE, Hofker MH, 't Hart LM. Common variants in the type 2 diabetes KCNQ1 gene are associated with impairments in insulin secretion during hyperglycaemic glucose clamp. PLoS One 2012; 7:e32148

131. Korkmaz A, Topal T, Tan DX, Reiter RJ. Role of melatonin in metabolic regulation. Rev Endocr Metab Disord 2009; 10:261-270
132. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proença C, Sparsø T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, De Graeve F, Chèvre JC, Borch-Johnsen K, Hartikainen AL, Ruokonen A, Tichet J, Marre M, Weill J, Heude B, Tauber M, Lemaire K, Schuit F, Elliott P, Jørgensen T, Charpentier G, Hadjadj S, Cauchi S, Vaxillaire M, Sladek R, Visvikis-Siest S, Balkau B, Lévy-Marchal C, Pattou F, Meyre D, Blakemore AI, Jarvelin MR, Walley AJ, Hansen T, Dina C, Pedersen O, Froguel P. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet 2009; 41:89-94
133. Bonnefond A, Clément N, Fawcett K, Yengo L, Vaillant E, Guillaume JL, Dechaume A, Payne F, Roussel R, Czernichow S, Hercberg S, Hadjadj S, Balkau B, Marre M, Lantieri O, Langenberg C, Bouatia-Naji N; Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC), Charpentier G, Vaxillaire M, Rocheleau G, Wareham NJ, Sladek R, McCarthy MI, Dina C, Barroso I, Jockers R, Froguel P. Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. Nat Genet 2012; 44:297-301
134. Chimienti F, Devergnas S, Favier A, Seve M. Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes 2004; 53:2330-2337
135. Pound LD, Sarkar SA, Benninger RK, Wang Y, Suwanichkul A, Shadoan MK, Printz RL, Oeser JK, Lee CE, Piston DW, McGuinness OP, Hutton JC, Powell DR, O'Brien RM. Deletion of the mouse Slc30a8 gene encoding zinc transporter-8 results in impaired insulin secretion. Biochem J 2009; 421:371-376
136. Lu M, Sarruf DA, Talukdar S, Sharma S, Li P, Bandyopadhyay G, Nalbandian S, Fan W, Gayen JR, Mahata SK, Webster NJ, Schwartz MW, Olefsky JM. Brain PPAR-gamma promotes obesity and is required for the insulin-sensitizing effect of thiazolidinediones. Nat Med 2011; 17:618-622
137. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci U S A 2003; 100:15712-15717
138. Hevener AL, He W, Barak Y, Le J, Bandyopadhyay G, Olson P, Wilkes J, Evans RM, Olefsky J. Muscle-specific Pparg deletion causes insulin resistance. Nat Med 2003; 9:1491-1497
139. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 2000; 26:76-80
140. Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. Am J Epidemiol 2010; 171:645-655
141. Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. Diabetes 1990; 39:647-652
142. Sparsø T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jørgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T, Pedersen O. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia 2008; 51:70-75
143. Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, Zeltser LM, Chung WK, Leibel RL. Regulation of Fto/Ftm gene expression in mice and humans. Amer J Physiol Regul Integr Comp Physiol 2008; 294:R1185-R1196
144. Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE, Mootha VK. Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. Mol Syst Biol 2008; 4:214
145. Zhao X, Peter A, Fritsche J, Elcnerova M, Fritsche A, Haring HU, Schleicher ED, Xu G, Lehmann R. Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at? Am J Physiol Endocrinol Metabol 2009; 296:E384-E393
146. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009; 9:311-326
147. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. Nat Med 2011; 17:448-453
148. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, Carr SA, Vasan RS, Florez JC, Clish CB, Wang TJ, Gerszten RE. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. J Clin Invest 2011; 121:1402-1411
149. Menni C, Fauman E, Erte I, Perry JR, Kastenmüller G, Shin SY, Petersen AK, Hyde C, Psatha M, Ward KJ, Yuan W, Milburn M, Palmer CN, Frayling TM, Trimmer J, Bell JT, Gieger C, Mohney R, Brosnan MJ, Suhre K, Soranzo N, Spector TD. Biomarkers for type 2 diabetes and impaired fasting glucose using a non-targeted metabolomics approach. Diabetes Jul 24, 2013 [*Epub ahead of print*]
150. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, Campillos M, Holzapfel C, Thorand B, Grallert H, Xu T, Bader E, Huth C, Mittelstrass K, Döring A, Meisinger C, Gieger C, Prehn C, Roemisch-Margl W, Carstensen M, Xie L, Yamanaka-Okumura H, Xing G, Ceglarek U, Thiery J, Giani G, Lickert H, Lin X, Li Y, Boeing H, Joost HG, de Angelis MH, Rathmann W, Suhre K, Prokisch H, Peters A, Meitinger T, Roden M, Wichmann HE, Pischon T, Adamski J, Illig T. Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 2012; 8:615

151. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, Fritsche A, Häring HU, Hrabě de Angelis M, Peters A, Roden M, Prehn C, Wang-Sattler R, Illig T, Schulze MB, Adamski J, Boeing H, Pischon T. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 2013; 62:639-648
152. Meisinger C, Lowel H, Heier M, Schneider A, Thorand B, Group KS. Serum gamma-glutamyltransferase and risk of type 2 diabetes mellitus in men and women from the general population. J Intern Med 2005; 258:527-535
153. Kolberg JA, Jørgensen T, Gerwien RW, Hamren S, McKenna MP, Moler E, Rowe MW, Urdea MS, Xu XM, Hansen T, Pedersen O, Borch-Johnsen K. Development of a type 2 diabetes risk model from a panel of serum biomarkers from the Inter99 cohort. Diabetes Care 2009; 32:1207-1212
154. Salomaa V, Havulinna A, Saarela O, Zeller T, Jousilahti P, Jula A, Muenzel T, Aromaa A, Evans A, Kuulasmaa K, Blankenberg S. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One 2010; 5:e10100
155. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008; 57:2762-2767
156. Thorand B, Zierer A, Baumert J, Meisinger C, Herder C, Koenig W. Associations between leptin and the leptin / adiponectin ratio and incident Type 2 diabetes in middle-aged men and women: results from the MONICA / KORA Augsburg study 1984-2002. Diabet Med 2010; 27:1004-1011
157. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. Diabetes 2005; 54:2932-2938
158. Festa A, D'Agostino R, Jr., Tracy RP, Haffner SM, Insulin Resistance Atherosclerosis S. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002; 51:1131-1137
159. Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, Hotamisligil GS, Ridker PM, Rodriguez BL, Margolis KL, Oberman A, Liu S. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. Diabetes 2007; 56:1898-1904
160. Wannamethee SG, Sattar N, Rumley A, Whincup PH, Lennon L, Lowe GD. Tissue plasminogen activator, von Willebrand factor, and risk of type 2 diabetes in older men. Diabetes Care 2008; 31:995-1000
161. Stancáková A, Civelek M, Saleem NK, Soininen P, Kangas AJ, Cederberg H, Paananen J, Pihlajamäki J, Bonnycastle LL, Morken MA, Boehnke M, Pajukanta P, Lusis AJ, Collins FS, Kuusisto J, Ala-Korpela M, Laakso M. Hyperglycemia and a common variant of GCKR are associated with the levels of eight amino acids in 9,369 Finnish men. Diabetes 2012; 61:1895-1902
162. Wurtz P, Soininen P, Kangas AJ, Ronnemaa T, Lehtimaki T, Kahonen M, Viikari JS, Raitakari OT, Ala-Korpela M. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. Diabetes Care 2013; 36:648-655
163. Würtz P, Tiainen M, Mäkinen VP, Kangas AJ, Soininen P, Saltevo J, Keinänen-Kiukaanniemi S, Mäntyselkä P, Lehtimäki T, Laakso M, Jula A, Kähönen M, Vanhala M, Ala-Korpela M. Circulating metabolite predictors of glycemia in middle-aged men and women. Diabetes Care 2012; 35:1749-1756
164. Gordon RS, Jr.. Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J Clin Invest 1957; 36:810-815
165. Franks PW, Wong MY, Luan J, Mitchell J, Hennings S, Wareham NJ. Non-esterified fatty acid levels and physical inactivity: the relative importance of low habitual energy expenditure and cardio-respiratory fitness. Br J Nutr 2002; 88:307-313
166. Gaudet D, Arsenault S, Pérusse L, Vohl MC, St-Pierre J, Bergeron J, Després JP, Dewar K, Daly MJ, Hudson T, Rioux JD. Glycerol as a correlate of impaired glucose tolerance: dissection of a complex system by use of a simple genetic trait. Am J Hum Genet 2000; 66:1558-1568
167. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2010; 375:181-183
168. Hagen JH, Moorhouse JA, Steinberg J. Effect of insulin on plasma glycerol in man. Metabolism 1963; 12:346-351
169. Funahashi T, Nagasawa A, Hibuse T, Maeda N. Impact of glycerol gateway molecule in adipocytes. Cell Mol Biol 2006; 52:40-45
170. Skowronski R, Hollenbeck CB, Varasteh BB, Chen YD, Reaven GM. Regulation of non-esterified fatty acid and glycerol concentration by insulin in normal individuals and patients with type 2 diabetes. Diabet Med 1991; 8:330-333
171. Pei D, Chen YD, Hollenbeck CB, Bhargava R, Reaven GM. Relationship between insulin-mediated glucose disposal by muscle and adipose tissue lipolysis in healthy volunteers. J Clin Endocrinol Metab 1995; 80:3368-3372
172. Salgin B, Ong KK, Thankamony A, Emmett P, Wareham NJ, Dunger DB. Higher fasting plasma free fatty acid levels are associated with lower insulin secretion in children and adults and a higher incidence of type 2 diabetes. J Clin Endocrinol Metab 2012; 97:3302-3309
173. Paolisso G, Tataranni PA, Foley JE, Bogardus C, Howard BV, Ravussin E. A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. Diabetologia 1995; 38:1213-1217
174. Pankow JS, Duncan BB, Schmidt MI, Ballantyne CM, Couper DJ, Hoogeveen RC, Golden SH, Atherosclerosis Risk in Communities S. Fasting plasma free fatty acids and risk of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes Care 2004; 27:77-82
175. Charles MA, Eschwege E, Thibult N, Claude JR, Warnet JM, Rosselin GE, Girard J, Balkau B. The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. Diabetologia 1997; 40:1101-1106
176. Hjellvik V, Sakshaug S, Strom H. Body mass index, triglycerides, glucose, and blood pressure as predictors of type 2 diabetes in a middle-aged Norwegian cohort of men and women. Clin Epidemiol 2012; 4:213-224
177. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of Fatty acids and insulin action. Ann N Y Acad Sci 2002; 967:183-195

178. Feskens EJ, van Dam RM. Dietary fat and the etiology of type 2 diabetes: an epidemiological perspective. Nutr Metab Cardiovasc Dis 1999; 9:87-95
179. Djousse L, Biggs ML, Lemaitre RN, King IB, Song X, Ix JH, Mukamal KJ, Siscovick DS, Mozaffarian D. Plasma omega-3 fatty acids and incident diabetes in older adults. Am J Clin Nutr 2011; 94:527-533
180. Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA, Giles GG. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. Am J Clin Nutr 2007; 86:189-197
181. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ, Forouhi NG. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. Am J Clin Nutr 2010; 92:1214-1222
182. Zheng JS, Huang T, Yang J, Fu YQ, Li D. Marine N-3 polyunsaturated fatty acids are inversely associated with risk of type 2 diabetes in Asians: a systematic review and meta-analysis. PLoS One 2012; 7:e44525
183. Huang T, Wahlqvist ML, Xu T, Xu A, Zhang A, Li D. Increased plasma n-3 polyunsaturated fatty acid is associated with improved insulin sensitivity in type 2 diabetes in China. Mol Nutr Food Res 2010; 54 Suppl 1:S112-S119
184. Haag M, Dippenaar NG. Dietary fats, fatty acids and insulin resistance: short review of a multifaceted connection. Med Sci Monit 2005; 11:RA359-RA367
185. Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH, Investigators AS. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr 2003; 78:91-98
186. Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes 1994; 43:1353-1357
187. Laaksonen DE, Lakka TA, Lakka HM, Nyyssonen K, Rissanen T, Niskanen LK, Salonen JT. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 2002; 19:456-464
188. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006; 136:565-569
189. Lemaitre RN, King IB, Sotoodehnia N, Knopp RH, Mozaffarian D, McKnight B, Rea TD, Rice K, Friedlander Y, Lumley TS, Raghunathan TE, Copass MK, Siscovick DS. Endogenous red blood cell membrane fatty acids and sudden cardiac arrest. Metabolism 2010; 59:1029-1034
190. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003; 133 Suppl 3:925S-932S
191. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007; 86:74-81
192. Fuhrman BJ, Barba M, Krogh V, Micheli A, Pala V, Lauria R, Chajes V, Riboli E, Sieri S, Berrino F, Muti P. Erythrocyte membrane phospholipid composition as a biomarker of dietary fat. Ann Nutr Metab 2006; 50:95-102
193. Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr 2004; 24:345-376
194. Kroger J, Schulze MB. Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. Curr Opin Lipidol 2012; 23:4-10
195. Taylor AJ, Jennings PE, Barnett AH, Pandov HI, Lawson N. An alternative explanation for the changes in erythrocyte fatty acids observed in diabetes mellitus. Clin Chem 1987; 33:2083-2085
196. Allen HG, Allen JC, Boyd LC, Alston-Mills BP, Fenner GP. Determination of membrane lipid differences in insulin resistant diabetes mellitus type 2 in whites and blacks. Nutrition 2006; 22:1096-1102
197. Bakan E, Yildirim A, Kurtul N, Polat MF, Dursun H, Cayir K. Effects of type 2 diabetes mellitus on plasma fatty acid composition and cholesterol content of erythrocyte and leukocyte membranes. Acta diabetol 2006; 43:109-113
198. Peterson DB, Fisher K, Carter RD, Mann J. Fatty acid composition of erythrocytes and plasma triglyceride and cardiovascular risk in Asian diabetic patients. Lancet 1994; 343:1528-1530
199. Pelikanova T, Kohout M, Valek J, Base J, Stefka Z. Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. Metabolism 1991; 40:175-180
200. Tilvis RS, Miettinen TA. Fatty acid compositions of serum lipids, erythrocytes, and platelets in insulin-dependent diabetic women. J Clin Endocrinol Metab 1985; 61:741-745
201. Baldini P, Incerpi S, Lambert-Gardini S, Spinedi A, Luly P. Membrane lipid alterations and Na+-pumping activity in erythrocytes from IDDM and NIDDM subjects. Diabetes 1989; 38:825-831
202. Kroger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H, Schulze MB. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Am J Clin Nutr 2011; 93:127-142
203. Krachler B, Norberg M, Eriksson JW, Hallmans G, Johansson I, Vessby B, Weinehall L, Lindahl B. Fatty acid profile of the erythrocyte membrane preceding development of Type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2008; 18:503-510
204. Reichard GA, Jr., Owen OE, Haff AC, Paul P, Bortz WM. Ketone-body production and oxidation in fasting obese humans. J Clin Invest 1974; 53:508-515
205. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids 2004; 70:243-251
206. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 1999; 15:412-426
207. Dreschfeld J. The Bradshawe Lecture on Diabetic Coma. Br Med J 1886; 2:358-363
208. Stephens JM, Sulway MJ, Watkins PJ: Relationship of blood acetoacetate and 3-hydroxybutyrate in diabetes. Diabetes 1971; 20:485-489

209. Singh BM, Krentz AJ, Nattrass M. Insulin resistance in the regulation of lipolysis and ketone body metabolism in non-insulin dependent diabetes is apparent at very low insulin concentrations. Diabetes Res Clin Pract 1993; 20:55-62
210. Würtz P, Mäkinen VP, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, Savolainen MJ, Tammelin T, Viikari JS, Rönnemaa T, Kähönen M, Lehtimäki T, Ripatti S, Raitakari OT, Järvelin MR, Ala-Korpela M. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes 2012; 61:1372-1380
211. Avogaro A, Crepaldi C, Miola M, Maran A, Pengo V, Tiengo A, Del Prato S: High blood ketone body concentration in type 2 non-insulin dependent diabetic patients. J Endocrinol Invest 1996; 19:99-105
212. Vice E, Privette JD, Hickner RC, Barakat HA. Ketone body metabolism in lean and obese women. Metabolism 2005; 54:1542-1545
213. Robertson DA, Singh BM, Baddeley RM, Nattrass M. Metabolic abnormalities in obese patients with impaired glucose tolerance. Diabetic Med 1990; 7:45-49
214. Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, Järvelin MR, Kähönen M, Lehtimäki T, Viikari J, Raitakari OT, Savolainen MJ, Ala-Korpela M. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009; 134:1781-1785
215. Sarkkinen ES, Agren JJ, Ahola I, Ovaskainen ML, Uusitupa MI. Fatty acid composition of serum cholesterol esters, and erythrocyte and platelet membranes as indicators of long-term adherence to fat-modified diets. Am J Clin Nutr 1994; 59:364-370
216. Takkunen M, Agren J, Kuusisto J, Laakso M, Uusitupa M, Schwab U. Dietary Fat in Relation to Erythrocyte Fatty Acid Composition in Men. Lipids 2013;48:1093-1102
217. Stancakova A, Javorsky M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M. Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 Finnish men. Diabetes 2009; 58:1212-1221
218. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22:1462-1470
219. McCarthy MI, Zeggini E. Genome-wide association studies in type 2 diabetes. Curr Diab Rep 2009; 9:164-171
220. Jansson PA, Larsson A, Smith U, Lonnroth P. Glycerol production in subcutaneous adipose tissue in lean and obese humans. J Clin Invest 1992; 89:1610-1617
221. Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis 2000; 150:227-243
222. Sundstrom J, Lind L, Vessby B, Andren B, Aro A, Lithell H. Dyslipidemia and an unfavorable fatty acid profile predict left ventricular hypertrophy 20 years later. Circulation 2001; 103:836-841
223. Lovejoy JC, Champagne CM, Smith SR, DeLany JP, Bray GA, Lefevre M, Denkins YM, Rood JC. Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. Metabolism 2001; 50:86-92
224. Kouki R, Schwab U, Hassinen M, Komulainen P, Heikkila H, Lakka TA, Rauramaa R. Food consumption, nutrient intake and the risk of having metabolic syndrome: the DR's EXTRA Study. Eur J Clin Nutr 2011; 65:368-377
225. Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 1994; 37:1044-1050
226. Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP, Denkins YM, Rood JC, Veldhuis J, Bray GA. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care 2002; 25:1283-1288
227. Ginsberg BH, Brown TJ, Simon I, Spector AA. Effect of the membrane lipid environment on the properties of insulin receptors. Diabetes 1981; 30:773-780
228. Wallin A, Di Giuseppe D, Orsini N, Patel PS, Forouhi NG, Wolk A. Fish consumption, dietary long-chain n-3 fatty acids, and risk of type 2 diabetes: systematic review and meta-analysis of prospective studies. Diabetes Care 2012; 35:918-929
229. Corpeleijn E, Feskens EJ, Jansen EH, Mensink M, Saris WH, de Bruin TW, Blaak EE. Improvements in glucose tolerance and insulin sensitivity after lifestyle intervention are related to changes in serum fatty acid profile and desaturase activities: the SLIM study. Diabetologia 2006; 49:2392-2401
230. Werk EE, Jr., Knowles HC, Jr.. The blood ketone and plasma free fatty acid concentration in diabetic and normal subjects. Diabetes 1961; 10:22-32
231. Jenkins DJ. Modern concepts of free-fatty-acid and blood-glucose homoeostasis in diseases involving altered lipid metabolism. Lancet 1967; 2:341-344
232. Kozian DH, Barthel A, Cousin E, Brunnhofer R, Anderka O, Marz W, Bohm B, Winkelmann B, Bornstein SR, Schmoll D. Glucokinase-activating GCKR polymorphisms increase plasma levels of triglycerides and free fatty acids, but do not elevate cardiovascular risk in the Ludwigshafen Risk and Cardiovascular Health Study. Horm Metab Res 2010; 42:502-506
233. Stančáková A, Paananen J, Soininen P, Kangas AJ, Bonnycastle LL, Morken MA, Collins FS, Jackson AU, Boehnke ML, Kuusisto J, Ala-Korpela M, Laakso M. Effects of 34 risk loci for type 2 diabetes or hyperglycemia on lipoprotein subclasses and their composition in 6,580 nondiabetic Finnish men. Diabetes 2011; 60:1608-1616.

Yuvaraj Mahendran

Identification of Biomarkers for Type 2 Diabetes

The early diagnosis of diabetes is important in order to avoid long-term micro- and macrovascular complications in individuals at high risk of type 2 diabetes. This thesis aims to investigate the association of various metabolic and genetic biomarkers with hyperglycemia and type 2 diabetes. Several novel biomarkers were identified in this study as predictors of hyperglycemia and incident type 2 diabetes.

UNIVERSITY OF
EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Health Sciences

ISBN 978-952-61-1368-5