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Glucose-lowering interventions and risk assessment in patients with coronary heart disease and disturbed glucose metabolism

by

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Cover
Connective tissue and spherical cells dotted with secretions, probably insulin droplets, within one of the Langerhans´ islets.
Photo: Lennart Nilsson Boehringer Ingelheim SCANPIX

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To my beloved family
Christer, Felicia and Ellinor

Det krävs ett helt annat sätt att tänka
för att lösa de problem vi skapat
med det gamla sättet att tänka.

Albert Einstein

Contents

ABSTRACT

Background
Abnormal glucose regulation (AGR) including impaired glucose tolerance (IGT) and diabetes mellitus type 2 (T2DM) are common in patients with stable and unstable coronary artery disease (CAD) and impair their prognosis. Available glucose lowering interventions have not fully succeeded in improving the detrimental prognosis and accurate screening methods and new treatment strategies are needed.
The aims of this thesis were to

1. decrease the restenosis rate after percutaneous coronary intervention (PCI) in patients with T2DM
2. validate the oral glucose tolerance test (OGTT) for the detection of glucose disturbances in patients with acute coronary syndromes (ACS)
3. evaluate the accuracy of a technique for continuous glucose monitoring in patients in the coronary care unit (CCU)
4. improve beta-cell function in patients with ACS and newly detected AGR

Restenosis in patients with T2DM (Study I)
The restenosis rate six months after PCI was investigated in 93 patients with T2DM randomised to either intensive glucose control by means of insulin (I group; n=44) or to continue ongoing glucose-lowering treatment (C group; n=49). At the end of the follow-up period restenosis rate was available in 82 patients. The glucose control did not differ between the two groups (change in HbA1c -0.2 *vs*.-0.1%; p=0.3 and in fasting blood glucose +0.2 *vs.* -0.3 mmol/L; p=0.3 in the I and C groups). The restenosis rate was 41% in the I and 44% in the C group (p=0.8). Independent predictors for restenosis were previous myocardial infarction (OR 8.0, 95% CI 2.5–25.7; p<0.001) and fasting blood glucose at baseline (OR 1.4, 95% CI 1.1–1.9; p=0.015).

Screening for glucose abnormalities in ACS (Studies II and III)
The value of an OGTT for screening of unknown AGR in patients with ACS was explored. Infarct size did not influence the result but patients with transmural myocardial infarctions (MI) (n=70) had higher glucose levels at admission and fasting during the next two days (7.0, 5.7, 5.4 mmol/L) compared to those with subendocardial MI (n=102; 6.0, 5.3, 5.0 mmol/L; p<0.001, p=0.01, p=0.004; **Study II**). More patients were classified as T2DM according to OGTT (n=27) compared to fasting plasma glucose (n=10) and HbA1c (n=2; **Study III**).

Continuous glucose monitoring in the coronary care unit (Study IV)
The accuracy of an intravenously inserted microdialysis catheter intended for continuous glucose monitoring was validated in 14 patients in a CCU setting. Although predominantly delivering correct values, the stability over time was insufficient. Thus the microdialysis technique requires further improvements to be useful in this setting.

Beta-cell function in ACS (Study V)
The effect of a DPP-IV inhibitor on beta-cell function, expressed as insulinogenic index (IGI) and acute insulin response to glucose (AIRg), in patients with ACS and newly discovered AGR was investigated by randomising 34 such patients to sitagliptin (S) and 37 to placebo (P). After 12 weeks of treatment the IGI improved in the sitagliptin group (S: 69.9 to 85.0 *vs.* P: 66.4 to 58.1 pmol/mmol; p=0.019) as did the AIRg (S:1394 to 1909 *vs*. P:1106 to 1043 pmol • l-1 • min-1; p<0.0001). Insulin resistance remained unaffected.

Conclusion
It is difficult to achieve glucose normalisation in patients with T2DM and CAD by means of available drugs. Early detection of AGR in patients with ACS is essential and an OGTT is a useful tool.
New technology in the form of equipment for continuous glucose monitoring and novel treatment strategies e.g. DPP-IV inhibitors deserves further attention in attempts to improve the management and thereby prognosis in this vulnerable patient category.

SAMMANFATTNING

Bakgrund
Störd glukosomsättning (AGR) som nedsatt glukostolerans (IGT) och diabetes mellitus typ 2 (T2DM) är vanligt förekommande hos patienter med stabil och instabil koronarsjukdom (CAD) och försämrar deras prognos. Tillgänglig glukossänkande behandling har inte förbättrat den dåliga prognosen och det finns ett behov av tillförlitliga screeningmetoder och nya behandlingsstrategier.

Syftet med denna avhandling är att

1. minska frekvensen av restenoser efter perkutan koronar intervention (PCI) vid T2DM.
2. validera oralt glukostoleranstest (OGTT) som metod att upptäcka glukosstörningar hos patienter med akut koronart syndrom (ACS).
3. utvärdera mätnoggrannheten hos en teknik avsedd för kontinuerlig glukosövervakning av patienter på hjärtintensivvårdavdelningar.
4. förbättra beta-cellfunktionen hos patienter med ACS och nyupptäckt AGR.

Restenos hos patienter med T2DM (Studie I)
Restenos-frekvensen sex månader efter PCI undersöktes hos 93 patienter med T2DM varav 44 randomiserats till insulinbaserad intensivbehandling (I-gruppen) och 49 till att fortsätta den pågående glukossänkande behandlingen (C-gruppen). Efter uppföljningstiden kunde förekomsten av restenos utvärderas hos 82 patienter. Det förelåg ingen skillnad i glukoskontroll mellan grupperna (förändring av HbA1c I: -0.2 jämfört med C: -0.1%; p=0.3 och i fastande blodglukos +0.2 jämfört med -0.3 mmol/L; p=0.3 i I- och C-grupperna). Frekvensen av restenoser var 41% i I-gruppen och 44% i C-gruppen (p=0.8). Oberoende prediktorer för restenos var tidigare hjärtinfarkt (OR 8.0, 95% CI 2.5–25.7; p<0.001) samt fastande blodglukos vid randomiseringen (OR 1.4, 95% CI 1.1–1.9; p=0.015).

Screening av glukosstörningar hos patienter med ACS (Studie II och III)
Värdet av ett OGTT för screening av okänd AGR hos patienter med ACS undersöktes. Resultaten påverkades inte av infarktstorlek men patienter med en transmural hjärtinfarkt (n=70) hade högre glukosnivåer vid ankomsten och de två följande dagarna (7.0, 5.7, 5.4 mmol/L) jämfört med patienter med en sub-endokardiell hjärtinfarkt (n=102; 6.0, 5.3, 5.0 mmol/L; p<0.001, p=0.01, p=0.004; **Studie II**). Fler patienter med T2DM klassificerades enligt OGTT (n=27) jämfört med fasteglukos (n=10) och HbA1c (n=2; **Studie III**).

Kontinuerlig glukosmonitorering på hjärtintensiven (Studie IV)
Mätnoggrannheten hos en intravenöst applicerad mikrodialyskateter avsedd för kontinuerlig glukosmonitorering utvärderades hos 14 hjärtintensivvårdade patienter. Även om flertalet mätpunkter var korrekta var stabiliteten över tid otillräcklig. Således behöver mikrodialystekniken förbättras för att vara användbar i detta sammanhang.

Beta-cell funktion hos patienter med ACS (Studie V)
En DPP-IV-hämmares effekt på beta-cellfunktionen, uttryckt som insulinogenic index (IGI) och akut insulinsvar på glukos (AIRg), undersöktes hos patienter med ACS och nyupptäckt AGR genom att randomisera 34 patienter till sitagliptin (S) och 37 till placebo (P). Efter 12 veckors behandling hade patienterna i sitagliptin gruppen förbättrat IGI (S: 69.9 till 85.0 jämfört med P: 66.4 till 58.1 pmol/mmol; p=0.019) och AIRg (S:1394 till 1909 jämfört med P:1106 till 1043 pmol • l-1 • min-1; p<0.0001). Insulinresistensen var oförändrad.

Sammanfattning
Det är svårt att åstadkomma en normalisering av glukosnivåerna hos patienter med T2DM och CAD med tillgängliga glukossänkande behandlingsalternativ. Tidig upptäckt av AGR hos patienter med ACS är angeläget och OGTT är för detta en användbar metod. Ny teknologi i form av kontinuerlig glukosmonitorering och nya behandlingsmetoder såsom DPP-IV-hämmare bör tas i beaktande i försöken att förbättra behandling och därmed prognos hos denna sårbara kategori av patienter.

List of Original Papers

This thesis is based on the following studies, which will be referred to by their Roman numerals.

I

Hage C, Norhammar A, Grip L, Malmberg K, Sarkar N M, Svane B, Rydén L
Fasting glucose predicts development of restenosis after PCI in patients with type 2 diabetes
Diabetes and Vascular Disease Research 2009;6:71-79

II

Hage C, Malmberg M, Rydén L, Wallander M
Infarction size and timing – important for the reliability of early oral glucose tolerance testing in patients with myocardial infarction
International Journal of Cardiology 2010;145:259-260

III

Hage C, Lundman P, Rydén L, Mellbin L
Fasting glucose, HbA1c or Oral Glucose Tolerance Testing for the Detection of Glucose Abnormalities in Patients with Acute Coronary Syndromes
European Journal of Preventive Cardiology 2012; In press

IV

Hage C, Mellbin L, Rydén L, Wernerman J
Glucose monitoring by means of an intravenous microdialysis catheter technique
Diabetes Technology & Therapeutics. 2010;12:291-295

V

Hage C, Brismar K, Efendic S, Lundman P, Rydén L, Mellbin L
Sitagliptin improves beta-cell function in patients with acute coronary syndromes and newly diagnosed glucose abnormalities
In manuscript

List of Abbreviations

ACS	acute coronary syndrome
ADA	American Diabetes Association
AGE	advanced glycation end products
AIRg	acute insulin response to glucose
AMI	acute myocardial infarction
BMS	bare metal stent
CABG	coronary bypass surgery
CAD	coronary artery disease
CCU	coronary care unit
CGMS	continuous glucose monitoring system
CHD	coronary heart disease
CVD	cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DES	drug eluting stent
DM	diabetes mellitus
DPP-IV	dipeptidyl peptidase-4
FBG	fasting blood glucose
FPG	fasting plasma glucose
FSIGT	frequently sampled intravenous glucose tolerance test
GIP	glucose-dependent insulinotropic polypeptide/gastric inhibitory polypeptide
GLP-1	glucagon-like peptide-1
HbA1c	glycated haemoglobin A1c
HOMA-IR	homeostatic model assessment insulin resistance
IFCC	International Federation of Clinical Chemistry
IFG	impaired fasting glucose
IGI	insulinogenic index
IGT	impaired glucose tolerance
MAPK	mitogen activated protein kinase
MI	myocardial infarction
NGT	normal glucose tolerance
NSTEMI	non ST-elevation myocardial infarction
OGTT	oral glucose tolerance test
PCI	percutaneous coronary intervention
PI3K	phosphatidylinostol-3 kinase
STEMI	ST-elevation myocardial infarction
T1DM	diabetes mellitus type 1
T2DM	diabetes mellitus type 2
VSMC	vascular smooth muscle cells
WHO	World Health Organisation
2hPG	plasma glucose two hours after start of OGTT

Introduction

Diabetes mellitus

History

Diabetes mellitus (DM) is an ancient disease. Its symptoms were described as early as 3,500 years ago in a compendium of medical diseases found in Luxor. Diabetes originates from the Greek word "diabaínein" (διαβαίνειν), "going through" or ''siphon'', introduced by Aretaeus of Cappadocia referring to the excessive amount of fluids passing through the body. He described diabetes as "a melting down of the flesh and limbs into the urine". One millennium ago, Avicenna in Baghdad noted the sweetish taste of the urine from patients with the disease and divided them into two groups: either young and thin or older and more obese (1, 2). In 1675, Thomas Willis added the Latin word "mellitus", which means sweet or honey, to diabetes and, the following century, Matthew Dobbins related the sweet taste to an excess of sugar in the urine and blood (3).

The pivotal function of the pancreas and its role in DM was unknown for a long time, even if the organ was described as early as 300 BC by the Greek surgeon Herophilus. The name "pancreas" was coined from *pan* (all) *kreas* (flesh) by the anatomist Ruphos 400 years later (4). Galen of Pergamon (AD 138-201), well known for his contribution to the understanding of anatomy, suggested that the pancreas served as a protective cushion for the large blood vessels and stomach (5). The first understanding of the causes of DM emerged with the development of experimental medicine by the end of the nineteenth century. In 1857, Claude Bernard described glycogen as a product of glucose metabolism in the liver, postulating that an alteration in this turnover caused DM (6). Etienne Lancereaux, a Parisian physician, comparing pancreatic histology in patients with and without glycosuria, was the first to report a connection between DM and the pancreas. He introduced the term "pancreatic diabetes" (7). In 1869, Paul Langerhans discovered the pancreatic islets (8) and others then observed pancreatic and islet abnormalities in deceased patients with DM. In 1893, Laguesse named them the islets of Langerhans after their discoverer and suggested that these islets produced a secretion involved in the regulation of digestion and the combustion of sugar (9). In 1889, Joseph von Mering and Oskar Minkowski made further progress. They noted that dogs deprived of the pancreas developed symptoms and eventually died of DM and concluded that an agent secreted in pancreas was involved in its aetiology (10).

Glucose-lowering drugs

Insulin

In 1910, Sir Edward Albert Sharpey-Schafer suggested that patients with DM lacked a substance, which he called insulin from the Latin word "insula" (*island*), normally produced in the islets of Langerhans (11). Insulin was first isolated in 1921 by the Romanian Professor Nicolae Paulescu, who named it pancreatine (12). It was, however, Sir Frederick Grant Banting and Charles Herbert Best who became world famous for their work on insulin, taking the experiment further by isolating and purifying insulin which, on 11 January 1922, they injected into a 14-year-old boy, Leonard Thompson, with DM (13). The following year, the Nobel Prize in Physiology or Medicine was awarded jointly to Banting and the head of the laboratory, John James Rickard Macleod, *"for the discovery of insulin"*. Initially,

Banting refused to accept the prize together with Macleod, claiming that the latter only contributed laboratory equipment and arguing that the laboratory assistant Best should be the co-recipient. However, Banting and Best experienced some difficulties in their initial experiments and were then advised by Macleod to replicate them with better equipment that he provided. Banting finally agreed to accept the prize together with Macleod, but he shared the money with Best.

Insulin secretagogues and sensitisers

For a long time, insulin was the only pharmacological treatment for DM and this still applies to diabetes mellitus type 1. The first alternative was discovered in France by Marcel Janon, who treated typhoid fever with sulfonamide and noted that it caused hypoglycaemia (14). In 1946, Auguste Loubatières concluded that the compound was an insulin secretagogue. Sulphonylureas were introduced in clinical practice during the 1950s. Insulin sensitisers in the form of biguanides, including metformin, were developed from the plant *Galega officinalis*. The glucose-lowering effects of metformin were first described in 1929 by Slotta and Tschesche but were then forgotten until 1950 when Eusebio Y. Garcia used the drug to treat influenza. He noted that the drug, which he called *Fluamine* "lowered the blood sugar to minimum physiological limit". This aroused the interest of the diabetologist Jean Sterne, who was the first to try metformin in patients with DM. He named it "glucophage" (glucose eater) and published his results in 1957 (15). Metformin became generally available in Europe back in 1958, but it was not approved in the USA until 1994 (16). A massive industrial screening of suitable molecules in the late 1990s resulted in another group of insulin sensitisers, the thiazolidinediones (or glitazones).

Figure 1. Historical persons of importance in the history of diabetes.
A. Aretaeus and a brief section of his description of diabetes.
B. Avicenna (Abu Ali Sina) of Baghdad wrote *The Canon of Medicine* (Al-Qanoon fi al-Tibb, The Laws of Medicine), including descriptions of the symptoms and complications of diabetes.
C. Frederick G. Banting and Charles H. Best. In the background is a page from Banting's research notebook, in which he plans his experimental approach to obtaining the internal secretion of the pancreas.

Incretins

In the 1960s, it was detected that orally consumed glucose triggers had a more pronounced insulin response than intravenous administration (17, 18). The explanation was that hormones, named incretins, that were released in the gut as a response to nutrients, especially glucose, stimulated as much as 50-70% of the postprandial beta-cell secretion of insulin (19). Several incretin hormones may be active and two of them are the most important: 1) the gastric inhibitory polypeptide (GIP) released from the entero-endocrine K cells in the proximal small bowel (20) and 2) the glucagon-like peptide-1 (GLP-1) which originates from the entero-endocrine L-cells in the distal ileum and colon (21-23). The action of GLP-1 and GIP is glucose dependent and rapidly vanishes with decreasing blood glucose. As a result, a search was initiated for pharmacological agents that lower glucose by interacting with the effects of incretins. One problem was the rapid degradation of GLP-1 and GIP by the enzyme dipeptidyl peptidase-4 (DPP-IV) (24, 25). The first agent to reach the market in 2005 was exenatide, a synthetic form of exendin-4, a peptide naturally occurring in the saliva of the lizard, the Gila monster *(Heloderma suspectum;* Figure 2), initially isolated by John Eng in 1992 (26). Exenadin-4 exhibits a 52% amino acid identity with human GLP-1 and displays similar functional properties but is resistant to DPP-IV degradation (27).

Figure 2. The Gila monster (*Heloderma suspectum*), native to the south-western United States and north-western Mexico. Copyright: Gary Bell/OceanwideImages.com

Definition and classification of diabetes mellitus

The World Health Organisation (WHO) and the American Diabetes Association (ADA) have defined DM as a group of metabolic diseases, characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both, associated with damage to and the dysfunction and failure of various organs (28, 29). There are several subgroups of DM and the classification is based on the pathogenetic process causing hyperglycaemia (30).

Diabetes mellitus type 1 (T1DM) is characterised by autoimmune beta-cell destruction resulting in insulin deficiency.
Diabetes mellitus type 2 (T2DM) is a more heterogeneous disease with variable degrees of impaired insulin secretion and insulin resistance.
Gestational diabetes develops during pregnancy and relates to an increased demand for insulin combined with insulin resistance. The majority of women with gestational diabetes revert to normal glucose metabolism after delivery, but the risk of subsequent T2DM is 30-60% (31).
Other aetiologies account for a relatively small percentage of DM. They include genetic defects in insulin secretion or action and pancreatic exocrine disease.

Glucose tolerance is classified into three categories: 1) normal (NGT); 2) impaired glucose metabolism, sometimes referred to as "pre-diabetes", comprising Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) as measured by an Oral Glucose Tolerance Test (OGTT), or increased risk of DM as expressed by HbA1c, and 3) T2DM. Since 1965, the WHO has published guidelines on the diagnosis of DM on several occasions, most recently in 2006 (32, 33). Similar guidelines have been issued by an expert group from the ADA (28). In 2003, the ADA modified its recommendations, by lowering the boundary for IFG from 6.1 to 5.6 mmol/L (34). In 2010, the ADA further revised its recommendations, adding HbA1c $\geq$ 6.5% as a diagnostic criterion for T2DM. This recommendation was approved by the WHO in 2011, provided that stringent quality tests and standardisation to the international reference values were in use and that no condition precluding the accuracy of the measurement was present (35). In the absence of symptoms of hyperglycaemia, the diagnosis should be based on glucose recordings above the limit on two separate occasions either in the fasting state (Fasting Plasma Glucose; FPG), following an OGTT, or based on elevated Haemoglobin A1c (HbA1c). The current diagnostic criteria are outlined in Table 1.

Table 1. Diagnostic criteria of glucose abnormalities according to WHO and ADA.

	Intermediate hyperglycaemia			DM*
Diagnostic method	**IFG**	**IGT**	**Increased risk of DM**	
Fasting glucose				
WHO	6.1-6.9 mmol/L	<7.0 mmol/L		≥7.0 mmol/L
ADA	5.6-6.9 mmol/L	<7.0 mmol/L		≥7.0 mmol/L
OGTT ** WHO and ADA		7.8-11.0 mmol/L		≥11.1 mmol/L
HbA1c				
WHO			Not recommended	6.5% (DCCT) 5.6% (Mono S) 48 mmol/mol (IFCC)
ADA			5.7% - 6.4% (DCCT) 4.7 - 5.5% (Mono S) 39 - 47 mmol/mol (IFCC)	6.5% (DCCT) 5.6% (Mono S) 48 mmol/mol (IFCC)
Random glucose and symptoms of hyperglycaemia				≥11.1 mmol/L

*Should be confirmed by repeat testing in the absence of unequivocal hyperglycaemia.
**Venous plasma glucose measured two hours after ingestion of 75 g of glucose in 200 ml of water.
DCCT=Diabetes Control and Complications trial standard; IFCC=International Federation of Clinical Chemistry standard; Mono S=Mono Sulphonic acid method

The diagnosis of T2DM is based on an arbitrary level of glucose, where microvascular complications, in particular retinopathy, start to develop in patients with T1DM. As outlined in Figure 3, there is usually a period of deteriorating glucose homeostasis lasting several years prior to the diagnosis during which symptoms of hyperglycaemia may or may not occur. Prerequisites for the development of atherosclerotic macrovascular disease are, however, already present during this period. Accordingly, the WHO report and guidelines on the management of diabetes, prediabetes and cardiovascular disease issued by the European Society of Cardiology and the European Association for the Study of Diabetes underline the importance of investigating intermediate hyperglycaemia and that glucose should be regarded as a continuous variable in the overall risk assessment of DM as well as cardiovascular disease (CVD) (33, 36).

Epidemiology

In 2011, approximately 366 million adults were affected by DM and the disease accounted for 4.6 million deaths worldwide. The global increase in DM is rapid and, by 2030, the prevalence is projected at 552 million (39). Between 2010 and 2030, there will be a 69% increase in the numbers of adults with DM in the developing countries and a 20% increase in the developed countries (40). The most substantial increase will be accounted for by T2DM, a disease that relates to a sedentary lifestyle and over-nutrition causing overweight and obesity. T2DM is responsible for 85-95% of all DM in high-income countries, a proportion that may be even higher in low- and middle-income countries (39). T2DM increases the risk of developing CVD (41) and serious cardiovascular events are two to four times more common in people with T2DM than among those without (42). Despite management improvements, CVD remains the leading cause of morbidity and mortality in people with DM (39, 43). Consequently, a considerable increase in CVD morbidity is to be expected in the future.

Pathogenesis of diabetes mellitus type 2

T2DM is caused by a combination of reduced insulin sensitivity and secretion. This impaired insulin sensitivity is most important in hepatic, muscular and adipose tissue. Insulin resistance

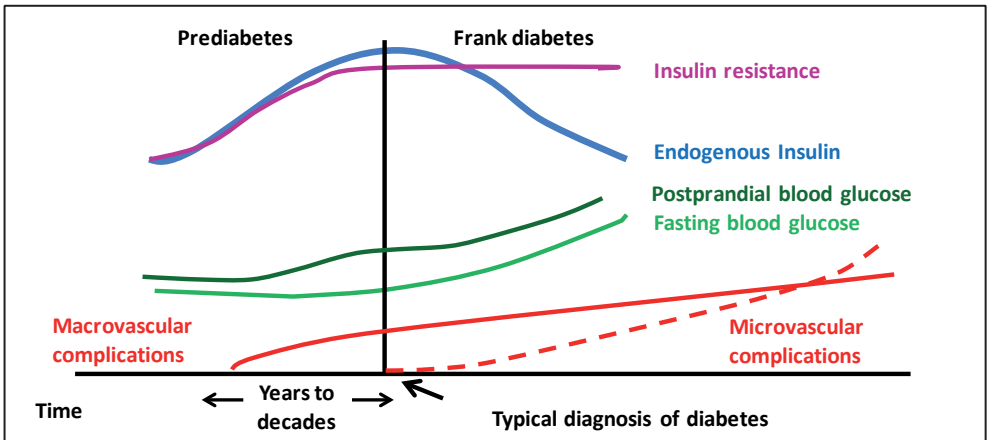

Figure 3. The natural history of impaired glucose tolerance and diabetes mellitus type 2 in relation to complications. Adapted and reprinted with permission from references (37, 38).

is reflected by the impaired suppression of gluconeogenesis in the liver, which increases glucagon secretion from the pancreatic alpha cells, adding to fasting hyperglycaemia in combination with reduced post-prandial glycogen storage (44-46). The development of T2DM is a long-term process initiated by an increase in insulin resistance, which, in combination with insufficient insulin secretion, causes post-prandial hyperglycaemia (Figure 3). Due to the progressive impairment of the pancreatic beta cells, insulin secretion over time becomes too low to keep blood glucose at a normal level even in the fasting state and the disease progresses to T2DM. The underlying causes of this process are complex interactions between genetic and environmental factors. Among the latter, lifestyle choices, including physical inactivity and weight gain, are important (44, 47).

The beta cell and insulin secretion

Insulin secretion

Insulin is produced by the pancreatic beta cells, with glucose as the key regulator. Increasing concentrations of glucose activate the secretion of insulin when transported into and metabolised within the beta cells. The subsequent increase in adenosine triphosphate (ATP) induces the closure of the ATP-sensitive K^+ channels, thereby depolarising the beta-cell membrane with a concomitant opening of the voltage-dependent Ca^{2+} channels. The resulting influx of Ca^{2+} triggers the beta cells to release insulin (48, 49).

The glucose-stimulated insulin response is biphasic (50). Experimental evidence suggests that secretory granules are located in two pools with different characteristics, a readily releasable pool containing ≤ 5% of all granules, and a reserve pool containing ≥ 95% (Figure 4). Granules in

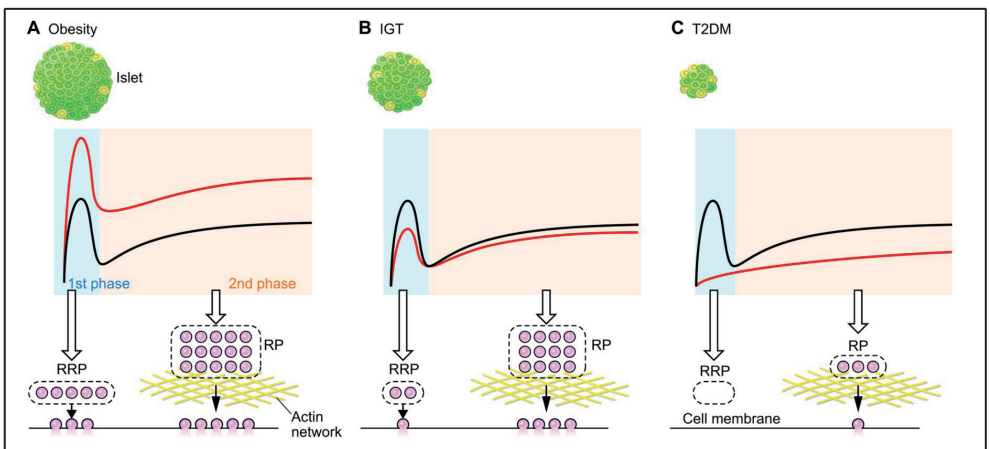

Figure 4. Dynamics of insulin secretion. The black lines indicate the dynamics of insulin secretion in the normal state and the red lines represent insulin secretion in disease.
A. Profile of glucose-induced insulin secretion, starting with a rapid first and a slower second phase. The first phase of insulin secretion reflects exocytosis of a readily releasable pool of granules (RRP). The second phase is due to the release of granules from the reserve pool (RP). The mobilisation of granules from the RP into the RRP involves ATP-dependent reactions.
B. In IGT, the first phase is slightly impaired because of a decrease in RRP size and/or a partial defect in the exocytotic process of granules in this pool, while the second phase is only moderately reduced.
C. In T2DM, the first phase is absent, since the RRP is lost and/or due to a completely defective exocytotic process. The second phase is also reduced, probably due to the decreased RP. Reprinted with permission from reference (48).
ATP=Adenosine TriPhosphate; IGT=Impaired Glucose Tolerance; T2DM=Diabetes Mellitus type 2

the readily releasable pool are immediately available, while those belonging to the reserve pool need to be mobilised before release. The first-phase insulin response involves the plasma-membrane fusion of granules from the readily releasable pool located at the membrane. It peaks within two to five minutes after intravenous glucose administration and lasts for approximately ten minutes. Patients with T2DM, as well as those within the IFG range, lack this first-phase response (51, 52). The second-phase response, which is evoked by nutrients, commences somewhat later and is maintained during the entire hyperglycaemic period. It is believed to represent the release of granules from the reserve pool mobilised within the beta cell and includes many granules with newly synthesised insulin (48, 49, 51).

Insulin signalling

As schematically depicted in Figure 5, the effect of insulin is mediated by receptors located on cellular surfaces. In healthy tissue, this leads to the activation of the phosphatidylinositol (PI)-3 kinase pathway (PI3K), which, via endothelial activation, enhances the uptake of glucose and nitric oxide (NO) production with concomitant anti-atherogenic effects (e.g. vasodilatory, anti-inflammatory and anti-thrombotic). Another important effect of insulin is the activation of the mitogen-activated protein kinase (MAPK) pathway, which is pro-atherogenic, by stimulating cellular growth, proliferation and differentiation. Data indicate that the PI3K pathway is down-regulated in the presence of insulin resistance, while the MAPK pathway appears to remain sensitive and thereby becomes excessively stimulated. This may contribute to cell proliferation, atherogenesis and the activation of inflammatory pathways, which may further increase insulin resistance (53, 54).

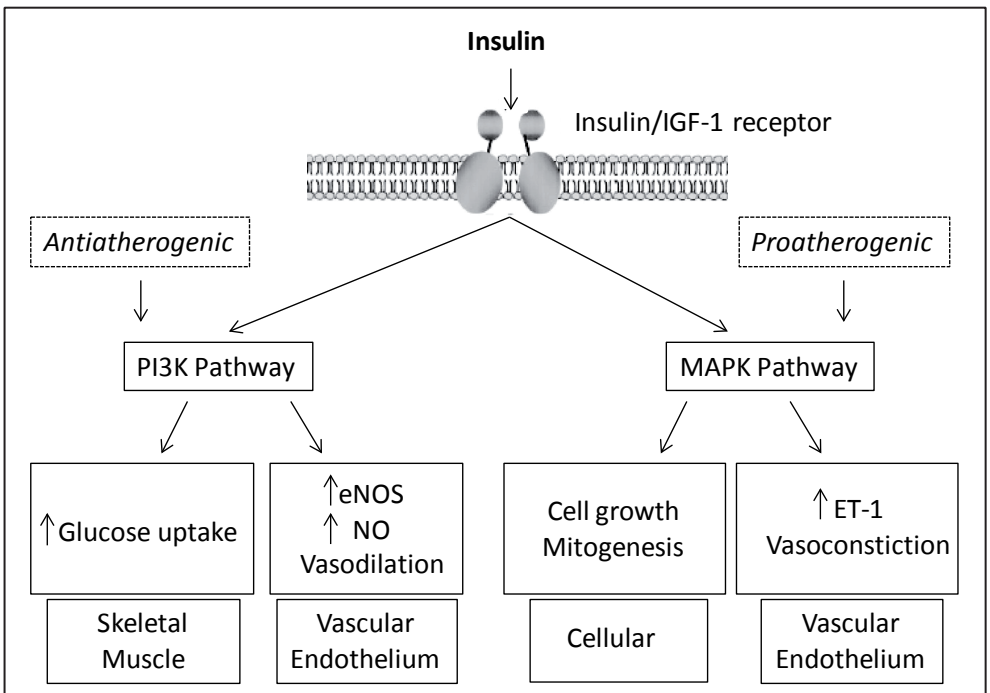

Figure 5. Insulin receptor signalling.
IGF=Insulin-like Growth Factor; eNOS=endothelial Nitric Oxide Synthase; ET-1=endothelin-1; MAPK=Mitogen-Activated Protein Kinase; NO=Nitric Oxide; PI3K=PhosphatIdylinositol 3-Kinase
Reprinted with permission from reference (54).

Beta-cell function and dysglycaemia

Both beta-cell function and beta-cell mass are impaired in patients with T2DM. To maintain normoglycaemia in the presence of insulin resistance, the beta cells up-regulate their insulin production and IGT and T2DM do not develop until they fail (44, 55). Autopsy studies reveal that subjects with IFG had lost about 40% of their beta-cell volume (56) and corresponding reports in T2DM vary between 0% and 63% (55, 57). The normal relation between insulin sensitivity and beta-cell function, referred to as the disposition index, is hyperbolic, implying that their product is constant (Figure 6). This index expresses the ability of the beta cells to compensate for insulin resistance (58, 59).

Dysfunctional beta cells, predicting the future development of T2DM, have been detected already in people with NGT (60, 61). In Pima Indians, who have a high prevalence of insulin resistance and T2DM, a failing beta-cell function predicts T2DM independently of obesity and insulin resistance (62). One distinctive characteristic in individuals who progress from NGT to T2DM is a markedly compromised beta-cell function in relation to insulin resistance (63). People in the upper tertile of NGT have a 50% decline in beta-cell function and subjects in the upper tertile of IGT a 70-80% decline (64) and the beta-cell function continues to deteriorate by approximately 4% a year in patients with T2DM, despite glucose-lowering treatment (65).

Hyperglycaemia has a stepwise-increasing, adverse effect on beta-cell function. The three steps have been categorised as follows: 1) *glucose desensitisation*, a rapid and reversible effect of exposure to high glucose concentrations; 2) *beta-cell exhaustion*, a depletion of the readily releasable pool of intracellular insulin without permanent defects; and 3) *glucotoxicity,* non-physiological and potentially irreversible cell damage caused by chronic exposure to pronounced hyperglycaemia (66). In addition, hyperglycaemia promotes the accumulation of free fatty acid (FFA) metabolites, which inhibits the glucose-induced insulin secretion and gene expression (67, 68). Finally, T2DM stimulates the development of amyloid polypeptide deposits within the islets of Langerhans. Its relevance for beta-cell function has been debated, but recent results from transplantation surgery involving the islets indicates a crucial role in the progressive decline in beta-cell function (69).

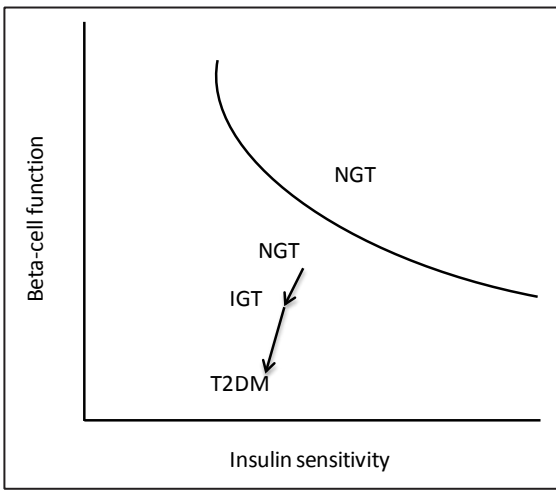

Figure 6. The hyperbolic relation between insulin sensitivity and beta-cell function. Changes in insulin secretion that fully balance changes in insulin resistance result in movement along the hyperbolic line and normoglycaemia is maintained. Deviation appears when beta-cell function is insufficient for the actual insulin sensitivity, as in patients with T2DM.
IGT = Impaired Glucose Tolerance
NGT = Normal Glucose Tolerance
T2DM = Diabetes Mellitus type 2
Adapted and reprinted with permission from reference (59).

Measures of insulin secretion and beta-cell function

Information on beta-cell function and insulin secretion and sensitivity is commonly obtained from mathematically derived expressions relating glucose and insulin concentrations to one another. Beta-cell function is often calculated as the homeostatic model assessment beta-cell function index (HOMA-β; (70) or the proinsulin to insulin (PI/I) ratio (71, 72). These simplified approaches do not provide information on the dynamic state of the relationship between insulin sensitivity and secretion and this is the reason why many studies include blood sampling during tests specially designed to study this question, some of which are described below. There is no consensus on a reference method, but, irrespective of the test that is chosen, the results should be interpreted in the context of insulin sensitivity and actual glucose concentrations.

The glucose clamp, originally described by DeFronzo et al. (73) for the estimation of insulin sensitivity, creates hyperinsulinemia by the administration of insulin at a constant rate. The amount of glucose infused to maintain plasma glucose at a given level serves as a measure of insulin sensitivity. Due to its high reproducibility, it has become the gold standard when measuring insulin sensitivity in peripheral tissues and for the evaluation of the second insulin secretory response. The disadvantages are that it is a non-physiological test since glucose consumption does not normally occur intravenously. Moreover, it is time consuming and requires special equipment together with considerable expertise.

The OGTT protocols usually include several blood sampling time points beyond the standard fasting and 120 minutes applied for diagnosing IGT and T2DM. The early insulin secretion/beta-cell function can be estimated as an insulinogenic index (IGI), i.e. the ratio between the delta values of insulin and glucose (44, 74). Several formulas have been suggested for the expression of insulin sensitivity based on an OGTT (75-77). The OGTT is easy to perform and frequently used, but it has been criticised due to low reproducibility.

The frequently sampled intravenous glucose tolerance test (FSIGT) is based on intravenous glucose administration and blood sampling during a period of two to four hours. This experimental procedure can be used for the estimation of both insulin sensitivity and secretion. One advantage is that the injected glucose stimulates the beta cells directly without any confounding effects from incretins, gastrointestinal hormones and possible problems related to gastric emptying. The disadvantages are that it is non-physiological and technically somewhat complicated. A simple measurement of beta-cell function based on an FSIGT is to calculate the area under the concentration curve of insulin ($AUC_{insulin}$) during the complete test or a specific interval. The $AUC_{insulin}$ represents the amount of insulin acting on the tissues, but it does not add any information on the dynamics of the hormone in terms of secretion and clearance. Beta-cell function or insulin secretion is often expressed as the acute insulin response to the intravenously administered glucose (AIRg), i.e. the mean concentration of insulin above the basal level during the peak between two and ten minutes (59).

Coronary artery disease

Epidemiology

Cardiovascular disease is the main cause of global mortality. In 2008, 17.3 million people died of these conditions, which represents one third of deaths worldwide, and the number is

expected to increase to 23.6 million people in 2030 (78). In Europe alone, 4.3 million people every year die of CVD, representing 48% of the mortality in this region and not only among elderly people. It is estimated that 12% of European men and 5% of European women will die of CVD before the age of 65 years (79). This burden is not expected to decrease in the foreseeable future due to a combination of increased longevity, exposing people to the risk of developing CVD, while improved survival in different disease manifestations will leave society with more individuals living with these conditions.

Pathogenesis and clinical manifestations

The most common reason for coronary artery disease (CAD) is atherosclerosis impairing the blood flow through narrowing of the coronary arteries in combination with a limited capacity to dilate in response to increased demands. Stable CAD may be asymptomatic or present itself as angina pectoris, i.e. chest pain due to myocardial ischaemia induced, for example, by physical or emotional stress. Acute manifestations of CAD relate to ruptured plaques triggering a thrombotic occlusion of the engaged coronary artery and causing an acute coronary syndrome (ACS) in the form of either an ST-elevation myocardial infarction (STEMI) indicating transmural myocardial damage or a non-ST-elevation myocardial infarction (NSTEMI) or unstable angina. The diagnosis of acute myocardial infarction (AMI) and unstable angina is confirmed by the rise and fall of cardiac biomarkers, particularly troponin, in combination with evidence of myocardial ischaemia in the form of symptoms, electrocardiogram (ECG) changes (new ST-T changes or left bundle branch block or the development of pathological Q-waves) or imaging signs of loss of viable myocardium or new regional wall abnormalities (80). A previous classification of myocardial infarction (MI) was based on whether or not there was any development of new pathological Q-waves (81). This ECG pattern indicated a *transmural infarction,* i.e an extensive myocardial cell death through the whole of the ventricular wall from endo- to epicardium. The absence of Q-waves indicated viable myocardial cells left in the ventricular wall, i.e. a *subendocardial infarction*.

Coronary revascularisation

Coronary revascularisation, restoring myocardial blood flow, may be performed as coronary bypass surgery (CABG) or through a percutaneous coronary intervention (PCI). In patients with stable, symptomatic CAD, revascularisation is mainly indicated for the relief of drug-refractory angina pectoris, but it may also increase longevity in patients with the engagement of the left main or proximal left anterior descending coronary arteries. In patients with ACS, immediate revascularisation will prevent or limit the extent of myocardial damage, thereby reducing mortality and future morbidity (82).

The dilatation of a coronary stenosis by means of a balloon catheter, PCI, was introduced as an alternative to CABG in 1977 (83). One common complication is restenosis, which is the reason this procedure is often supplemented by stenting of the dilatated artery. The bare metal stent (BMS) was introduced by the end of the 1980s, reducing the restenosis rate from 30-50% to 20-30%. Subsequently, the use of drug-eluting stents (DES) further reduced the rate to 5-15% (84, 85). The process of restenosis is initiated by the mechanical endothelial damage caused by balloon dilatation (Figure 7). The inflammatory response to the endothelial denudation and subintimal bleeding triggers a cascade of processes involving pro-inflammatory cytokines and the activation of leukocytes, platelets and smooth muscle

cells (VSMC) in the vessel wall. The activated VSMC stimulate growth factors, causing the surrounding cells to proliferate and migrate and to produce extracellular matrix provoking neointimal hyperplasia (86). In principle, this represents a physiological, healing reaction to injury, which may become overexpressed, resulting in a restenosed vessel usually within three to six months after the PCI (85).

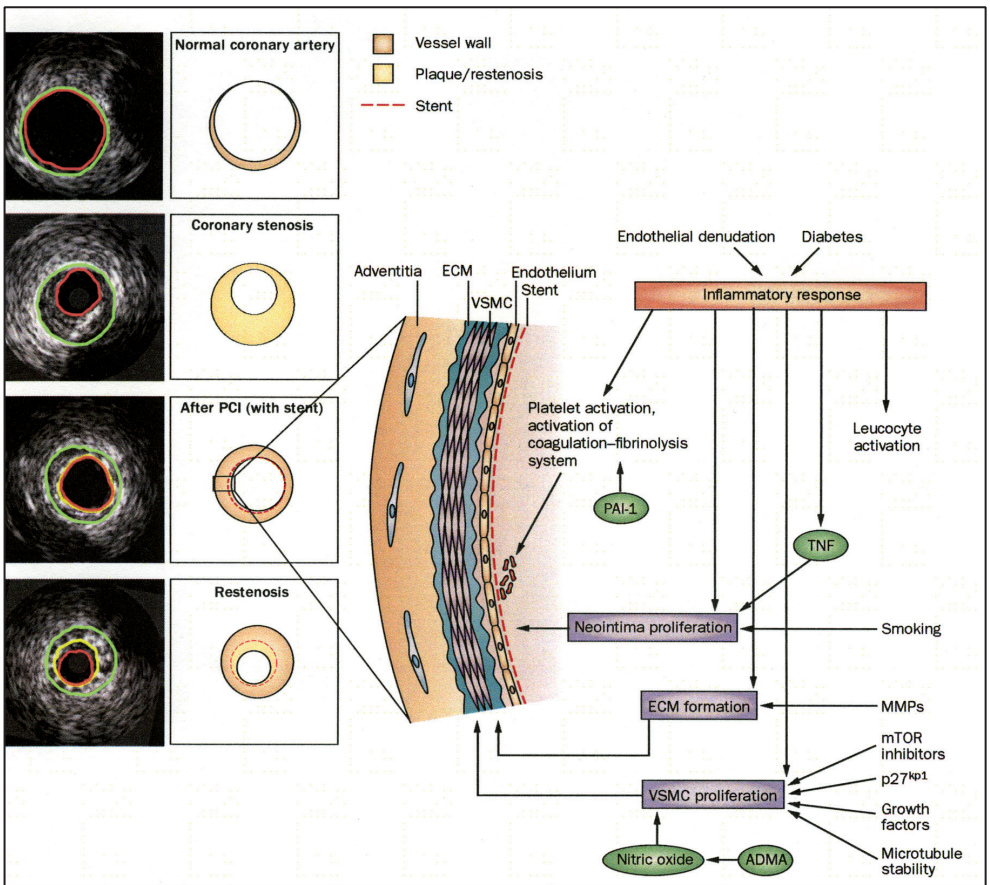

Figure 7. Mechanisms of restenosis after a PCI.
Intravascular ultrasound images of the various stages of CAD. The outer border of the vessel is indicated in green, the lumen in red and the coronary stent in yellow. A schematic presentation of the different processes is shown to the right of these images. ADMA=Asymmetric DiMethylArginine; ECM=Extracellular Matrix; MMPs=Matrix MetalloProteinases; mTOR=mammalian Target Of Rapamycin; PAI-1=Plasminogen Activator Inhibitor 1; PCI=Percutaneous Coronary Intervention; TNF=Tumour Necrosis Factor; VSMC=Vascular Smooth Muscle Cells
Reprinted with permission from reference (85).

Coronary artery disease and hyperglycaemia

The connection between DM and coronary heart disease (CHD) was first noted by Seegen, who, in 1864, drew attention to the occurrence of angina pectoris in patients with DM (87).

In 1888, Mayer complained that "… those who have occupied themselves with investigating the pathology of diabetes seem to have given their chief attention to the examination of those organs, which experimental physiology had shown to be more particularly involved in its production" (88). The short life span of people with DM and, for that reason, the low incidence of CHD may explain the low level of interest in the combination. The relationship between CHD and DM received more attention following the discovery of insulin, increasing longevity and the concomitant risk of developing cardiovascular complications (89-91).

In 1999, Norhammar et al. (92) were among the first to describe that hyperglycaemia at admission for an ACS worsens the prognosis, a finding subsequently confirmed in several other studies (93, 94). A systematic overview by Coutinho et al. (95) indicated that hyperglycaemia is a continuous risk factor from fasting or postload glucose levels below the diagnostic threshold for T2DM. Accordingly, hyperglycaemia is a marker of poor prognosis, but it may also be one of the underlying mechanisms contributing to the increased cardiovascular risk, making it of interest to study glucose metabolism in patients with CAD in more detail. The combination of AMI and established T2DM is common, with a prevalence of ≥ 20% (96). Moreover investigations of patients with acute and stable CAD without previously known glucose abnormalities by means of an OGTT revealed that about 30% had IGT and another third undiagnosed T2DM (97-99). The presence of glucose perturbations worsens the prognosis by increasing the risk of future cardiovascular morbidity and mortality in patients with both acute and stable CAD (100-103). Further investigations of patients with ACS revealed that their insulin resistance, expressed as the HOMA index, was somewhat high but not different from that of age- and gender-matched controls. The patients did, however, have a more pronounced beta-cell dysfunction, as demonstrated by a higher proinsulin/insulin ratio within each glucose category (NGT, IGT and T2DM) and a deteriorating IGI (104, 105).

Hyperglycaemia and vascular dysfunction

Hyperglycaemia impairs endothelium-dependent relaxation by reducing the bioavailability of NO and other vasodilators concomitant with the increased synthesis of vasoconstrictors such as endothelin-1(106). This process is presumed to be initiated by oxidative stress triggered by hyperglycaemia activating superoxide-producing enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH). The elevated superoxide levels stimulate the production of advanced glycation end products (AGEs) with an adverse impact on cellular function. The AGEs also contribute to the production of oxygen-derived free radicals. As depicted in Figure 8, four different pathways triggered by hyperglycaemia appear to be involved in the overproduction of superoxide anions by the mitochondrial electron-transport chain as the common denominator (107). Important implications of hyperglycaemia are inflammatory activation mediated by nuclear factor κ-B (NFκB), the oxidation of plasma low density lipoproteins (LDL) and the promotion of a prothrombotic state by platelet activation, thrombin generation in combination with impaired fibrinolysis due to the reduced synthesis of plasminogen activator inhibitor-1 (PAI-1) (106, 108, 109).

Revascularisation with PCI in diabetes mellitus type 2

Revascularisation by means of PCI is less rewarding in patients with T2DM than in those without (110). Even a modest increase in blood glucose at the time of the procedure is associated with increased long- and short-term mortality (111, 112). Further, the restenosis

rate is much higher in patients with T2DM (113, 114). After a PCI with BMS, ≥ 30% of patients with T2DM will develop a restenosis compared with ≤ 20% of their counterparts without DM (115). The subsequent high demand for repeat revascularisation exposes patients with T2DM to an increased risk of periprocedural complications (116). Although the introduction of DES reduced the need for target lesion revascularisation, the restenosis rate remains higher among patients with T2DM (117, 118).

The marked propensity for restenosis in T2DM may relate to processes induced by hyperglycaemia, further enhanced by inflammatory activation (119, 120). Interestingly, the increased production of the vasoconstrictor thromboxane A_2 correlates to fasting plasma glucose and can be restored to normal by strict glycaemic control. Moreover, abnormalities in the extracellular matrix relating to the reduced production of matrix-associated heparan sulphate, a potent inhibitor of VSMC proliferation and AGE accumulation in the vascular tissue, increases with exposure to glucose, stimulating VSMC proliferation and abnormal matrix production and thereby the propensity for restenosis (121).

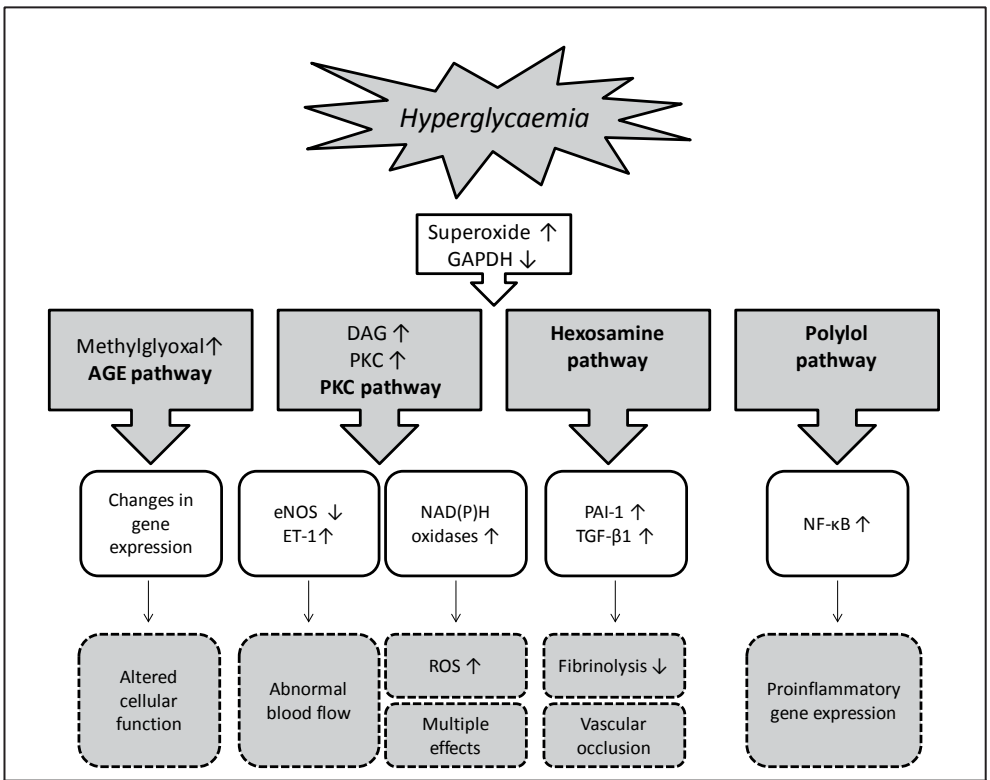

Figure 8. Potential mechanism by which hyperglycaemia-induced mitochondrial superoxide overproduction activates four pathways of hyperglycaemic damage.
AGE=Advanced Glycation End products; DAG=DiAcylGlycerol; ET-1=Endothelin-1; eNOS=endothelial Nitric Oxide Synthetase; GAPDH=GlycerAldehyde 3-Phosphate DeHydrogenase; NAD(P)H=NicotinAmide adenine Dinucleotide Phosphate-oxidase; NF-κB=Nuclear Factor κ-B; ROS=Reactive oxygen species; TGF-β1=Growth Factor-β1; PAI-1=Plasminogen Activator Inhibitor-1; PKC=Protein Kinase C

Glucose control

Short term

Attempts have been made to improve the prognosis of patients with T2DM and AMI by means of strict glucose control. In the Diabetes Glucose and Acute Myocardial Infarction study (DIGAMI), these patients were randomised to an acute insulin-glucose infusion instituted soon after the onset of symptoms and followed by multi-dose insulin therapy aiming at stable normoglycaemia or to conventional glucose-lowering therapy without any predefined glucose targets (122). After 3.4 years of follow-up, the insulin-based glycaemic control had reduced the mortality rate to 33% in the intensively treated group compared with 44% in patients belonging to the control group, a relative reduction of 28% (123). Subsequent studies were unable to confirm these findings. The DIGAMI 2 study was designed to resolve the question of whether the reduction in mortality was due to the acute administration of an insulin-glucose infusion or to improved metabolic control after hospital discharge. The prognosis among patients in the insulin infusion followed by insulin arm did not differ from that in patients randomised to the acute insulin infusion followed by conventional therapy or in patients in the control arm treated at the discretion of the attending physician (124). Likewise, the Australian Hyperglycemia: Intensive Insulin Infusion in Infarction (HI-5) study did not show a mortality reduction in the intensively treated arm (125). Interestingly, in HI-5, mortality was lower in a subgroup of patients with tight glycaemic control (mean glucose ≤ 8 mmol/L), regardless of whether the target was obtained by means of insulin or other glucose-lowering agents (125).

Apart from hyperglycaemia, the induction of hypoglycaemia is a concern in patients hospitalised with an ACS. Hypoglycaemia enhances catecholamine release which may cause arrhythmias (126) and aggravate myocardial ischaemia (127). Additionally fluctuations in glucose may also trigger the release of catecholamines further aggravating the negative effects of myocardial ischaemia (128) and studies in patients with T2DM have demonstrated that markers of oxidative stress are independently associated with the mean amplitude of glycaemic excursions (129). One possible cause could be that hyperglycaemic spike-evoked oxidative stress activates pathways involved in the pathogenesis of diabetes complications, particularly increased superoxide production at the mitochondrial level (130). There are, however, diverging results in this field (131). This underlines the importance of diminishing untoward variability while remaining within the narrow euglycaemic range (132, 133).

Glucose monitoring

Current insulin titration algorithms are based on isolated glucose measurements which, although frequent, provide a scattered picture of the overall glycaemic situation, including the possibility of missing rapid shifts in blood glucose. Patients in coronary care units (CCU) are not subjected to the same supervision as, for example, those admitted to intensive care units and fear of the induction of hypoglycaemia has been reported as an obstacle to the use of sufficient amounts of insulin to reach targeted glucose levels in patients with AMI (124, 125). Moreover, insulin algorithms are inconvenient for patients as they require frequent blood sampling (134), increasing the workload and thereby the costs (135). For this reason, reliable glucose monitoring equipment with an on line presentation of the results may be an important tool in future attempts finally to test the still unproven hypothesis that intense glucose control is prognostically beneficial for T2DM patients with AMI.

Long term

Improved long-term glucose control may have a beneficial effect on cardiovascular mortality and morbidity. In the UK Prospective Diabetes Study (UKPDS), intensive glucose control in patients with newly detected T2DM, based on either oral glucose-lowering drugs or insulin, reduced microvascular complications. Moreover, metformin was successful in reducing myocardial infarction by 33% (p=0.005) and mortality by 27% (p=0.002) (136). These findings are, however, not unchallenged and intensive glycaemic control has failed to show any significant reduction in cardiovascular outcomes or improve the prognosis in trials performed with different combinations of glucose-lowering agents in high-risk patients with T2DM established for several years (137-139). It may be that long-term, intensive glucose lowering is possibly more rewarding if instituted early and/or that the ideal agent for accomplishing glucose control is yet to be found (140, 141).

Incretins

The gut-derived hormones called incretins, GLP-1 and GIP, exert glucose-dependent insulinotropic actions on the beta cells, resulting in low secretion in the fasting state and a rapid post-prandial rise, which provides effective protection from hypoglycaemia (142-144). Both hormones exert their effects via the activation of their respective trans-membrane G protein-coupled receptors (GPCR). The GIP receptor is predominantly expressed on pancreatic beta cells and to a lesser extent in adipose tissue and the central nervous system. The GLP-1 receptors are found on a range of tissues including pancreatic alpha and beta cells, the kidneys, lungs, central and peripheral nervous system and the gastrointestinal tract (145-147). In the cardiovascular system, they have been further localised in cardiomyocytes, endothelial and endocardial cells and in aortic smooth muscle cells from mice (148). This ubiquitous presence of receptors suggests that incretins may perform other biological roles in addition to releasing insulin (145, 147). The active form of GLP-1 is only present in the circulation for one to two minutes because of rapid degradation by the DPP-IV enzyme which also degrades GIP (24, 25). Since the metabolites do not activate the GLP-1 receptor, they lack insulinotropic action, which limits the bioavailability of GLP-1 (149). In experimental studies, GLP-1 and GLP-1 analogues have been associated with the expansion of the beta-cell mass via the stimulation of beta-cell replication, the proliferation and promotion of islet cell neogenesis from precursor ductal cells and the inhibition of apoptosis (150-154).

Incretins in T2DM

In patients with T2DM, the incretin effect may be reduced or even lost (155) due to reduced incretin secretion or action. Until recently, the GLP-1 secretion was considered to be impaired, but recent data indicate that it is probably preserved (156, 157). The conflicting results may relate to the population being studied, as it seems as though the degree of insulin resistance and obesity is of importance for the secretion (156, 158). The diminished insulinotropic action of GIP, and to some extent also of GLP-1, has been demonstrated in patients with T2DM, although supraphysiological levels of the latter still increase insulin secretion (159). In addition to the insulinotropic effects, GLP-1 inhibits the secretion of glucagon, hepatic glucose production and gastrointestinal motility, in turn promoting satiety leading to reduced food intake and weight loss (Figure 9) (145, 160). In patients with T2DM, GLP-1 receptor agonists restore the first-phase and augment the second-phase insulin response (161), indicating improved beta-cell function when used alone (162) or in combination

with metformin and/or sulphonylurea (163, 164). Increasing the levels of incretins for the treatment of patients with IGT or T2DM seems logical, not the least when dealing with newly detected glucose perturbations in patients with ACS in whom beta-cell dysfunction appears to be an early and important pathophysiological aberration.

GLP-1 receptor agonists and DPP-IV-inhibitors

A great deal of effort has focused on amplifying the effects of GLP-1 for therapeutic applications and two concepts have been developed; the GLP-1 receptor agonists (incretin mimetics) and DPP-IV inhibitors (incretin enhancers) (165, 166). At present, there are two GLP-1 receptor agonists on the market, exenatide (Byetta®) and liraglutide (Victoza®), and several are under development (albiglutide, tasboglutide, dulaglutide, lixisenatide, exenatide long-acting release (LAR) and CJC-1134-PC). The GLP-1 receptor agonists, which require subcutaneous administration, reduce HbA1c by 0.7-1.7% (DCCT standard) in patients with T2DM compared with placebo (165). Several DPP-IV inhibitors, compounds that

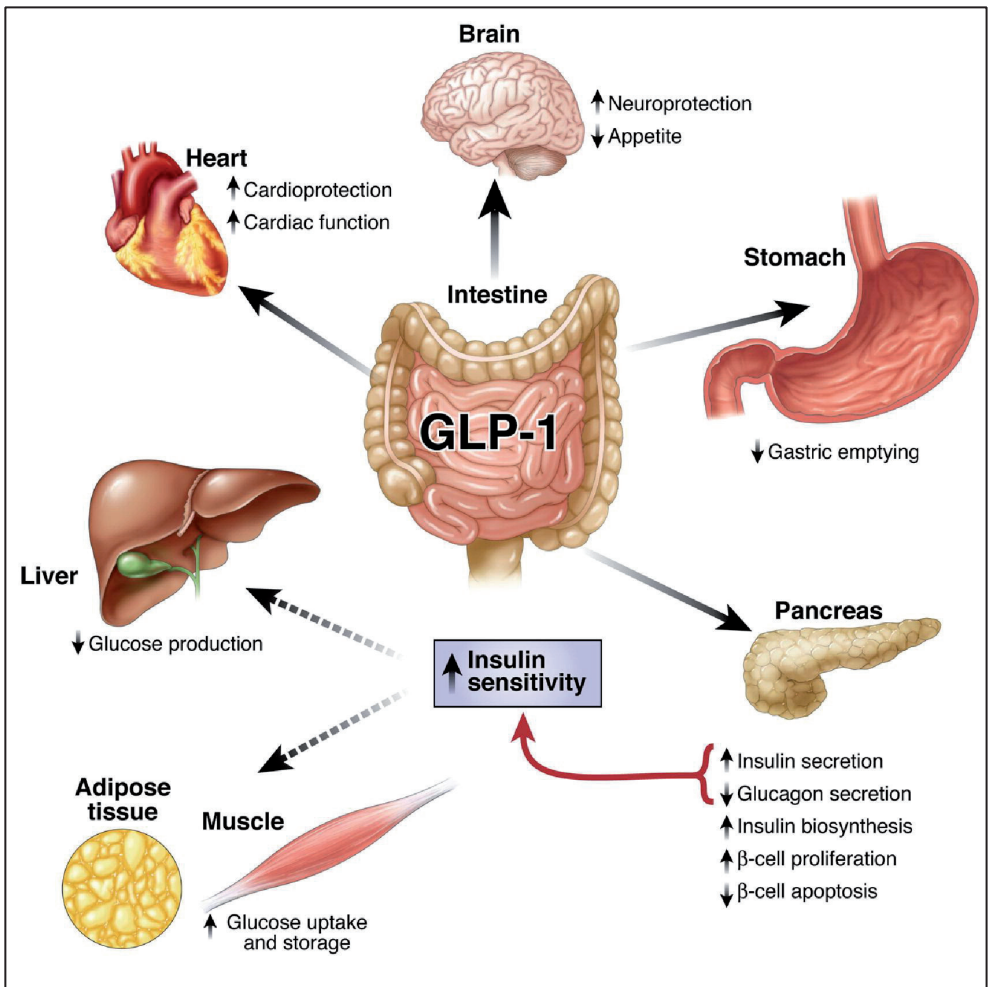

Figure 9. Effects of the GLP-1 system in T2DM. Reprinted with permission from reference (155).

reduce serum DPP-IV activity by ≥ 80% thereby delaying the degradation of GLP-1, are available; sitagliptin (Januvia®), vildagliptin (Galvus®), saxagliptin (Onglyza®), linagliptin (Tradjenta®) and alogliptin (Nesina®) or in the later stages of development (denagliptin, melogliptin, carmegliptin, dutogliptin, gosogliptin). They can be administered orally with some inhibition maintained for 24 hours after a single dose (167) and reduce HbA1c by 0.5-0.9% (DCCT standard) compared with placebo (165).

Cardiovascular effects

GLP-1 has wide-ranging cardiovascular actions including the modulation of heart rate, blood pressure, endothelial function and myocardial contractility (168-173). The GLP-1 agonist exenatide lowers blood pressure in patients with T2DM (174). GLP-1 infusion has been demonstrated to induce endothelial-dependent vasodilation in patients with CAD and T2DM (175) and it generated a trend towards improved systolic and diastolic myocardial function (176), an effect seen in patients both with and without T2DM (177). Ten patients with AMI and heart failure who were exposed to a GLP-1 infusion improved their left ventricular function after successful PCI (178) and GLP-1 infusion reduced the need for inotropic support after CABG (179). This ability to improve myocardial contractility and coronary blood flow is possibly independent of the activation of the GLP-1 receptor since it has been attributed to the gluco-metabolically inactive metabolite GLP-1 (9-36) amid (148, 180, 181). The GLP-1 agonist exenatide also reduces reperfusion injury and thereby potentially infarct size (182). In clinical trials recruiting patients with T2DM, GLP-1 agonists had positive effects on blood lipids, the lowering of total and LDL cholesterol, a finding that requires further verification since it may have been related to GLP-1-induced weight loss (173).

AIMS

1. To test the hypothesis that tight, insulin-based glucose control will reduce the rate of restenosis after a percutaneous coronary intervention in patients with diabetes mellitus type 2
   (Study I)

2. To validate the oral glucose tolerance test for the detection of glucose disturbances in patients with acute coronary syndromes
   (Studies II-III)

3. To evaluate the accuracy of microdialysis as a technique for continuous monitoring of glucose levels in patients in coronary care units
   (Study IV)

4. To test the hypothesis that the DPP-IV inhibitor sitagliptin improves beta-cell function in patients with acute coronary syndromes and newly detected glucose disturbances
   (Study V)

Patient Material and Methods

Patients and protocols

Studies I-V are based on four different sets of patient material; the IDA (*Insulin Diabetes Angioplasty*) Study (**I**), the GAMI (*Glucose metabolism in Acute Myocardial Infarction*) Study (**II**), the Microdialysis Study (**IV**) and the BEGAMI (*BEta-cell function in Glucose abnormalities and Acute Myocardial Infarction*) Study (**III** and **V**) recruiting patients between 1998 and 2010 (Table 2).

Table 2. Summary of essential characteristics in **Studies I – V**.

	Number of participants	Recruitment period	Glucose abnormality	Study design	Intervention	Endpoint
Study I IDA	82	March 1999-March 2003	T2DM	PROBE	Intensive, insulin-based glucose control	Restenosis after six months
Study II GAMI	172	Nov 1998-Dec 2000	NGT, IGT, T2DM	Prospective	-	Concordance of OGTT results
Study III BEGAMI	79	May 2008-Sept 2010	IGT, T2DM	Randomised, prospective	-	Classification of T2DM
Study IV Micro-dialysis	14	Feb-May 2009	NGT, IGT, T2DM	Prospective	-	Concordance of glucose measurements
Study V BEGAMI	71	May 2008-Sept 2010	IGT, T2DM	Randomised, prospective, controlled, double-blind	Sitagliptin/ placebo	Beta-cell function after 12 weeks

IGT=Impaired Glucose Tolerance; NGT=Normal Glucose Tolerance; PROBE= Prospective, Randomised with Open Blinded Evaluation; T2DM=Diabetes Mellitus type 2

Study I

Insulin Diabetes Angioplasty (IDA)

Hypothesis: **Study I** tested the hypothesis that intensified, insulin-based glucose control will reduce the propensity for restenosis.

Patients: A detailed description of patient recruitment is presented in Figure 10. Patients at Karolinska University Hospital in Stockholm and Sahlgrenska University Hospital in Gothenburg with established T2DM and who had been accepted for revascularisation with PCI were eligible for inclusion. The exclusion criteria were AMI within 48 hours before the intervention, inability to participate for physical or psychological reasons or residence outside the catchment area. Recruitment commenced in March 1999 and was prematurely stopped in March 2003 due to a slow inclusion rate and a change in treatment practice introducing drug-eluting stents.

Protocol: Following stratification according to the type of PCI (elective or acute), the 102 patients were randomised to either intensive insulin-based (I group) or standard (C group) glucose-lowering therapy. The final group in whom restenosis could be assessed consisted of 82 patients (I group=39; C group=43). Follow-up visits were scheduled two weeks and one and three months after the index PCI. A coronary angiogram was performed after six months. In the presence of symptoms indicating restenosis, an angiogram could be performed prior to this occasion. Patients without an angiographically demonstrable restenosis at that time were reinvestigated at six months, while an already verified restenosis was accepted without further angiography.

Glucose control: In elective patients randomised to the intensive group, efforts to optimise glycaemic control were initiated three weeks prior to the PCI to be continued during the complete study period. The aim was to achieve HbA1c of < 6.5% (Mono S), fasting blood glucose (FBG) of 5-7 mmol/L and blood glucose before meals of < 10 mmol/L. The protocol specified treatment with fast-acting meal insulin three times daily and long-acting insulin at bedtime.

Following randomisation, patients hospitalised with unstable angina were brought to the best possible glucose control as quickly as possible by means of a glucose-insulin infusion aiming at blood glucose of 4-9 mmol/L. The infusion continued for at least 12 hours after the PCI. After termination of the infusion, these patients followed the treatment outlined for elective patients during the six-month study period. Control patients continued ongoing glucose-lowering treatment (insulin, oral agents or combinations). Any change in their diabetes medication was at the discretion of the attending physician, as was the glucose target.

PCI: Balloon angioplasty and coronary stenting were performed according to standard at the time of the study. A ≥ 50% luminal diameter reduction was defined as significant. A BMS

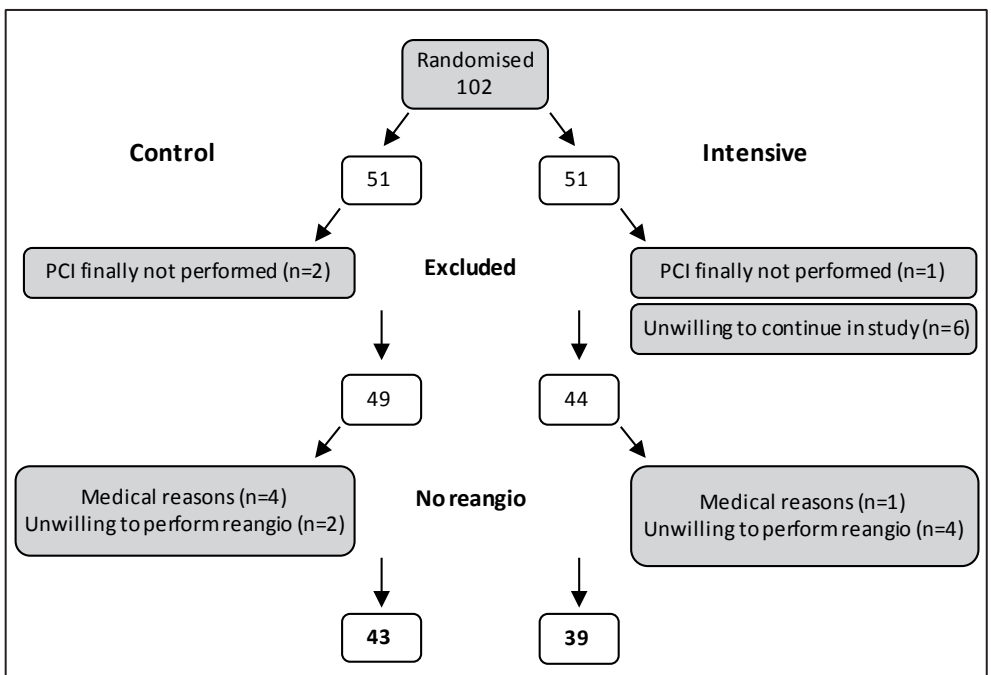

Figure 10. Patient flow in **Study I**. PCI=Percutaneous Coronary Intervention

was applied if the vessel diameter was ≥ 3 mm, if the balloon dilatation was suboptimal or if it caused dissection. After stent implantation, the goal for the intervention was a residual stenosis of < 20% of the reference diameter measured on line with Quantitative Coronary Angiography (QCA) in combination with TIMI 3 flow. If the lesion length was > 15 mm and/or the vessel diameter < 3 mm, a residual stenosis of < 50% at the dilatated site measured on line with QCA was accepted as successful in order to avoid multiple or long stents.

Endpoint: The primary endpoint was restenosis after six months analysed off line with the Cardiovascular Measurement System (Medis, Leiden, The Netherlands) by two interventionists blinded to the randomised intervention. Matched angiographic views were obtained for each patient before and after the procedure and at the six-month follow-up. Angiographic restenosis was defined as a ≥ 50% diameter stenosis.

Study II
Glucose metabolism in Acute Myocardial Infarction (GAMI)

Aim: To evaluate the accuracy of an early OGTT in relation to infarct type.

Patients: The material comprised the 172 patients with a recorded ECG from the GAMI trial (total n=181) that investigated the prevalence of previously unknown glucose abnormalities in patients with AMI admitted to Karolinska University Hospital in Stockholm and Västerås Hospital between November 1998 and December 2000 (97). The exclusion criteria were age > 80 years, serum creatinine of ≥ 200 μmol/L and living outside the catchment area.

Protocol: The patients were classified as having a transmural MI defined as the presence of a Q-wave in at least two contiguous leads or a subendocardial MI in the absence of such a pattern. During hospitalisation, FBG was measured on day one to four, a 75g standardised OGTT was performed on day four or five and at an outpatient visit three months later.

Endpoint: The primary endpoint was the concordance between the outcome of the OGTT performed prior to discharge and three months later.

Study IV
Continuous glucose monitoring in ACS

Aim: To evaluate the accuracy of a microdialysis catheter as a tool for safely and accurately monitoring of glucose levels.

Patients: Fourteen patients with ACS or congestive heart failure admitted to the CCU at Karolinska University Hospital in Stockholm between February and May 2009. The exclusion criteria were known allergy to dalteparin or difficulty obtaining venous access.

Protocol: A microdialysis catheter (CMA 64 IView, CMA Microdialysis AB, Solna, Sweden) was inserted in an arm vein for a maximum of three days. Microdialysis fluid consisting of saline and dalteparin (150 IU/mL) was transfused at 1 μL/min and collected in vials in eight-minute fractions. Reference plasma glucose was measured in blood obtained from a peripheral venous catheter in a contralateral arm vein in the middle of the eight-minute fraction. Eight pairs of samples were collected during one hour, a procedure that was repeated daily for three days.

Endpoint: The primary endpoint was the concordance between plasma glucose obtained via the microdialysis catheter and standard, laboratory-based measurements.

Studies III and V
BEta-cell function in Glucose abnormalities and Acute Myocardial Infarction (BEGAMI)

Hypothesis/aim: **Study III** aimed to compare an OGTT with FPG and HbA1c as methods for detecting glucose abnormalities in patients with ACS, while **Study V** tested the hypothesis that the DPP-IV inhibitor sitagliptin improves beta-cell function in patients with ACS and newly diagnosed glucose disturbances. In addition, the safety of early institution of sitagliptin in this patient category was studied.

Patients: BEGAMI recruited patients with ACS without previously known glucose perturbations at the CCU at Karolinska University Hospital and Danderyd Hospital in Stockholm. The exclusion criteria were age < 18 years, impaired renal function, BMI ≥ 34 kg/m^2, heart failure (NYHA III-IV), inability to follow the study protocol or a planned coronary revascularisation.

Patient recruitment started in May 2008 and follow-up was completed in December 2010. As shown in Figure 11, a total of 174 patients underwent the screening OGTT and 75 (43%) of them had a normal glucose tolerance, 63 (36%) IGT and 36 (21%) T2DM. Thirteen patients declined participation and seven were randomisation failures, leaving 79 patients with IGT or T2DM and a recorded HbA1c value for the analysis in **Study III**. Of these patients, 39 were randomised to sitagliptin and 40 to placebo. **Study V** is based on the 71 patients with complete follow-up data returning to the clinic after 12 weeks (Figure 11).

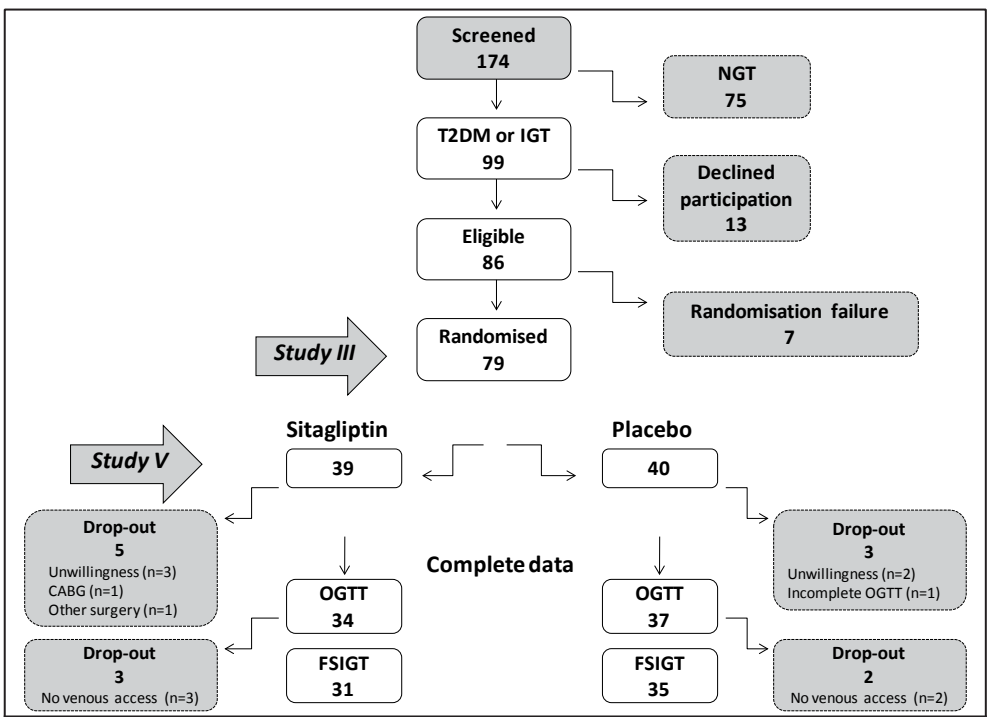

Figure 11. Patient flow in **Studies III** and **V**.
CABG=Coronary Artery Bypass Grafting; FSIGT=Frequently Sampled Intravenous Glucose Tolerance Test; IGT=Impaired Glucose Tolerance; NGT=Normal Glucose Tolerance; OGTT=Oral Glucose Tolerance Test; T2DM=Diabetes Mellitus type 2

Protocol: The protocol for **Studies III** and **V** is outlined in Figure 12. A screening OGTT was performed before hospital discharge four days after admission at the earliest or at a scheduled outpatient visit within three weeks. Patients with IGT or T2DM were eligible and returned to the clinic the following day for a FSIGT. When this was completed, they were randomised to receive 100 mg sitagliptin (Januvia™, Merck Sharp & Dohme AB) or a matching placebo once daily in double-blind fashion. All patients were given structured lifestyle advice. They were provided with a glucometer and instructed to check glucose regularly during the study period. The OGTT and FSIGT were repeated on two subsequent days after 12 weeks of follow-up.

Endpoints: The primary endpoint in **Study III** was the concordance between glucose characterisation with OGTT, FPG and HbA1c. The primary endpoint in **Study V** was an improvement in beta-cell function measured by means of the IGI (ΔI30/ΔG30) obtained from the OGTT. Secondary endpoints were 1. Improvement in glucose tolerance tested with an OGTT and 2. Improvement in GLP-1 independent beta-cell function measured as the ΔAIRg during the FSIGT.

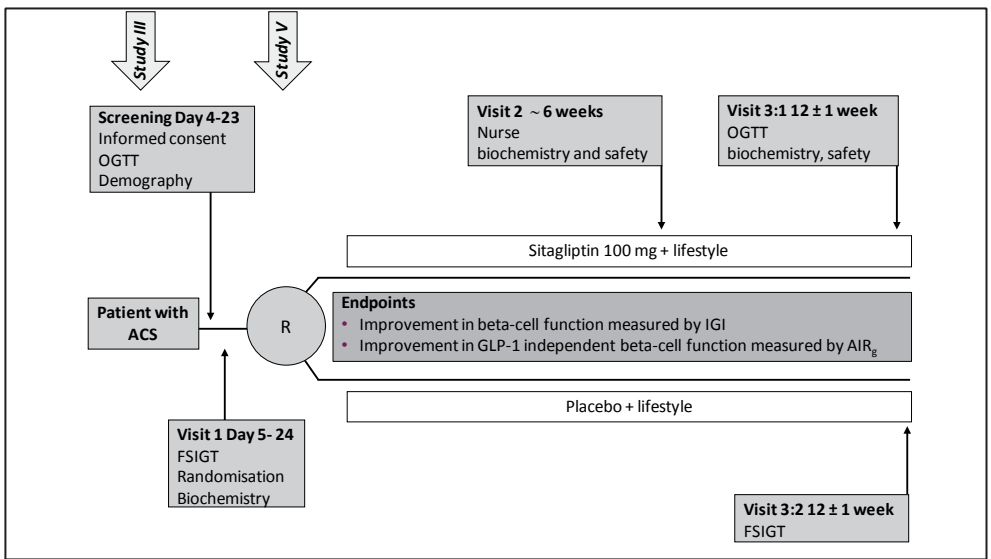

Figure 12. A schematic presentation of the study protocol in **Studies III** and **V.**
ACS=Acute Coronary Syndrome; AIRg=Acute Insulin Response to glucose; FSIGT=Frequently Sampled Intravenous Glucose Tolerance Test; GLP-1=Glucagon-like peptide-1 IGI=Insulinogenic Index; OGTT=Oral Glucose Tolerance Test; R=Randomisation

Study procedures

Microdialysis

In **Study II,** a microdialysis tube, inserted into an arm vein, was used as "an artificial blood capillary" in order to monitor blood glucose. The catheter had a double-space lumen (Figure 13). A perfusion fluid was infused at a constant rate through the catheter in the space between the inner tube and the outer dialysis membrane. Glucose in the blood diffused across the catheter membrane into the perfusion fluid, which then entered into the inner tube to be collected in micro vials for analysis.

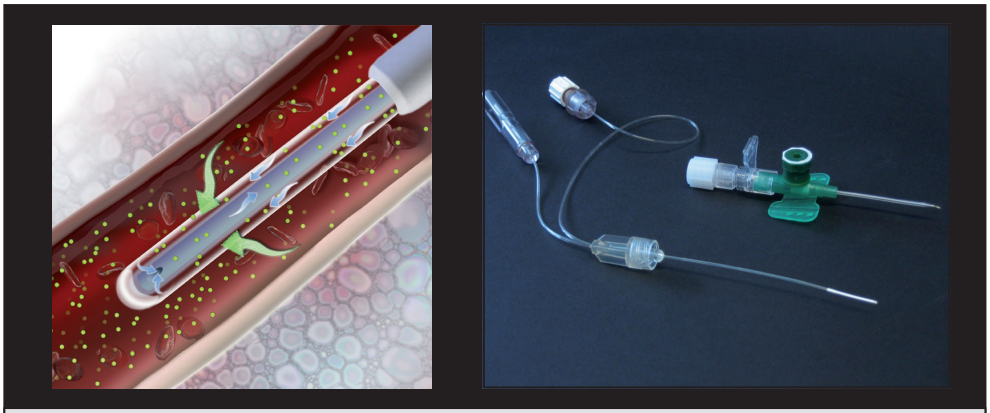

Figure 13. The microdialysis catheter, *IView.* See the text for further explanation.

OGTT

The OGTTs in **Studies II, III** and **V** were performed according to WHO recommendations (183). Following 12 hours of overnight fasting, 75 g of glucose dissolved in 200 ml of water was administered orally. A peripheral venous catheter was inserted into an antecubital vein for blood sampling. In **Study II,** venous blood samples and capillary blood glucose measurements were obtained prior to and 15, 30, 60 and 120 minutes after the glucose ingestion. In **Studies III** and **V,** venous blood samples for the analysis of glucose and insulin were obtained prior to and 30, 60 and 120 minutes after the glucose ingestion. Blood for the analysis of insulin was kept on wet ice until centrifugation, which was performed within one hour at 2,000g for 20 minutes.

FSIGT

The FSIGTs in **Study V** were performed after 12 hours of overnight fasting. Two peripheral venous catheters were inserted into contralateral antecubital veins; one for blood sampling and one for glucose injection. A basal sample was drawn at -5 min. At 0 min, glucose (300 mg/kg) was injected for two minutes. Blood samples were then collected at 2, 5, 7, 10, 20, 30, 60 and 120 minutes for the analysis of glucose and insulin. Blood for the analysis of insulin was kept on wet ice until centrifugation, which was performed within one hour at 2,000g for 20 minutes.

Laboratory analyses

Glucose was immediately analysed in capillary whole blood using a photometric technique (Hemocue® AB, Ängelholm, Sweden) in **Studies I-III** and **V**. The values in **Studies III** and **V** were recalculated to venous plasma glucose. In **Study IV**, venous plasma glucose was analysed at the end of the study on cool-handled blood stored as plasma at -70°C using a glucose oxidase-based method (Konelab 20 automated analyser, Thermo Clinical Labsystems Oy, Vantaa, Finland).

HbA1c in **Studies I** and **II** was analysed in a core laboratory by high-performance liquid chromatography (Department of Laboratory Medicine, Malmö University Hospital, Sweden) on capillary blood applied on filter paper with an upper normal limit of 5.3% (Mono S; Boehringer Mannheim Scandinavian AB, Bromma, Sweden) and, in **Studies III** (Mono S) and **V** (IFCC), with ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA).

Plasma insulin in **Studies II** and **V** was analysed after the termination of the study from plasma stored at -70°C and quantified with commercially available, specific immunoassays (DAKO Ltd, Cambridgeshire, UK).

Proinsulin in **Study V** was analysed using an ELISA kit (Mercodia AB, Uppsala, Sweden).

Calculations

In **Studies II** and **V**, insulin resistance was calculated as homeostatic model assessment insulin resistance (HOMA-IR) according to Matthews (70).

HOMA-IR=Fasting Glucose (mmol/L) x Fasting Insulin (mU/L)/22.5

In **Study V**, beta-cell function was calculated as the insulinogenic index (IGI) (74) as follows:

$IGI=\Delta insulin_{0-30}/\Delta glucose_{0-30}$

and Acute Insulin Response to glucose (AIRg) as the

incremental area under the curve by using the trapezoidal rule from 0 to 10 minutes.

The glucose disappearance constant (K_g) was calculated as the slope of the natural logarithm of the two glucose samples taken at 10-20 min

$K_g=[\Delta \ln \text{plasma glucose}/\Delta \text{min}] \times 100$

Statistics

Studies I-V

Descriptive statistics were presented as follows, unless otherwise stated: continuous variables were expressed as the median and lower and upper quartiles and Wilcoxon's random test was used for differences between groups. Categorical variables were expressed as numbers and percentages and analysed using Fisher's exact test.

The assumptions behind the power calculation in **Study I** were a restenosis rate of 30-40% and that a reduction to 25% would be clinically meaningful. Based on this, a number of 170 patients in each group was required for a significance level of 5% and a power of 80%.

Univariable predictors of restenosis were identified by logistic regression analysis. Multiple logistic regression analysis was performed with restenosis after six months as dependent variable, while candidate predictors defined as variables with a p-value of ≤ 0.2 were entered into a best subset selection.

A two-tailed p-value of < 0.05 was considered statistically significant.

Statistical analyses were performed using SAS software version 9.1.3 (**Studies I**, **II** and **IV**) or 9.2 (**Studies III** and **V**).

Study II

The concordance rates of glucose categories between repeated OGTTs were calculated as weighted Cohen's kappa.

Study IV

Congruence between glucose measurements was evaluated as the mean and median absolute difference and the mean and median absolute relative difference between each matched

pair of microdialysis and plasma glucose measurements. In addition, data were presented according to the ISO-15197 criteria, including the proportion of paired data points falling within ±0.8 mmol/L for a reference glucose level (in the present material plasma glucose) of < 4.2 mmol/L or within ±20% for reference glucose levels of ≥ 4.2 mmol/L (184).

To test clinical accuracy, all matched points from all patients were plotted on a Clarke error grid where the relevance of differences between reference and measured values is taken into account. The plasma glucose was used as the reference value and plotted on the *x*-axis against microdialysis values plotted on the *y*-axis (185).

Study V

The sample size calculation was based on a previous study (105), which estimated the mean and standard deviation of the IGI as 50 ± 35 (pmol/mmol). To detect an increase of 50% between the two treatment groups at a 5% level of significance with 80% power using a two-tailed t-test, a sample size of 64 patients was considered sufficient. An additional 5% was added to enable the use of non-parametric methods and cover for the potential loss of patients, resulting in a total sample size of 70 patients. The change in beta-cell function was calculated by an analysis of covariance (ANCOVA), using the linear model with terms for treatment (sitagliptin/placebo) and baseline IGI as covariates.

Ethical considerations

The studies were performed in accordance with good clinical practice guidelines (ICH-GCP) and followed the recommendations of the Helsinki Declaration. The separate protocols were approved by the Regional Ethical Review Board in Stockholm and all the patients provided written and oral informed consent prior to study participation.

Results

Study I

Restenosis in T2DM

As can be seen in Table 3, there were no major differences between patients in the I and C groups at randomisation.

Table 3. Clinical characteristics of the patients in **Study I.** Continuous variables are presented as the median and lower and upper quartiles (quartile 1; quartile 3) and categorical variables as numbers (n) and percentages (%), unless otherwise stated.

Variable	**I group** (n=39)		**C group** (n=43)	
Baseline characteristics				
Age (years)	66	59;72	62	59;68
Gender (male)	74		76	
Previous medical history				
Smoking	28	72	26	60
Hypertension	23	59	28	65
Hyperlipidemia	30	77	30	70
Angina pectoris	36	92	41	95
Myocardial infarction	19	49	17	40
Treatment at randomisation				
Aspirin	34	87	40	93
Beta blockers	33	85	38	88
ACE or ARB inhibitor	12	31	21	49
Diuretics	13	33	7	16
Lipid-lowering agent	32	82	28	65
Diabetes				
Duration (years)	6.4	4.3;12.1	6.5	2.7;12.7
Fasting blood glucose (mmol/L)	7.0	6.8;8.5	7.3	6.5;8.7
HbA1c (%; Mono S method)	6.5	5.8;7.7	6.5	5.8;7.6
Glucose-lowering treatment				
Non pharmacologic treatment	4	10	3	7
Acarbose	3	7	1	2
Sulphonylureas	19	49	24	56
Metformin	12	31	19	44
Insulin	17	44	15	35
Treatment at the index PCI				
Ticlopidine/clopidogrel	36	92	37	86
Stent (BMS)	35	90	34	81
GP IIb/IIIa inhibitor	16	41	14	33

ACE=Angiotensin Converting Enzyme; ARB=Angiotensin II Receptor Blocker, BMS=Bare Metal Stent; PCI=Percutaneous Coronary Intervention

At the follow-up six months after the initial PCI, there was no significant change in glucose control in either of the two groups. The FBG increased by 0.2 mmol/L in the I group and decreased by 0.3 mmol/L in the C group (p=0.3) and HbA1c decreased by 0.2% in the I group and 0.05% in the C group (p=0.2).

Descriptive variables from the index PCI and follow-up angiography are presented in Table 4. Regardless of randomised treatment, the percentage of target lesion restenosis was similar in both groups (I group 41% *vs*. C group 44%; p=0.8), as was the percentage of renewed target vessel intervention.

As the study groups did not differ in terms of achieved glucose control and restenosis rate, an analysis looking for predictors of restenosis within the complete patient cohort was performed. Relevant predictors are shown in Figure 14. Following adjustments in a multivariable analysis, the remaining two significant predictors of restenosis were FBG at randomisation (OR 1.4, 95% CI 1.1-1.9; p=0.015) and previous MI (OR 8.0, 95% CI 2.5-25.7; p<0.001).

Table 4. Angiographic findings at the time of follow-up in **Study I**.
Continuous variables are presented as the median and lower and upper quartiles (quartile 1; quartile 3) and categorical variables as numbers (n) and percentages (%), unless otherwise stated.

Angiographic findings	**I group** (n=39)		**C group** (n=43)		*p*-value
Before index PCI					
Reference diameter (mm)	2.9	2.3;3.3	2.9	2.4;3.3	0.7
Lesion length (mm)	27.8	21.7;36.3	30.1	20.1;40.0	0.6
Minimal lumen diameter (mm)	1.1	0.7;1.4	1.0	0.8;1.2	0.8
Percentage diameter stenosis	59.9	51.2;73.2	63.0	52.3;75.3	0.5
At index PCI					
Multiple stents in one lesion	6		6		1.0
Balloon-to-artery ratio	1.1	1.0;1.3	1.1	1.0;1.1	0.2
After index PCI					
Minimal lumen diameter (mm)	2.6	2.3;3.0	2.7	2.2;3.1	0.7
Percentage diameter stenosis	12.0	5.8;22.4	13.4	6.6;22.1	0.9
At 6 months					
Minimal lumen diameter (mm)	1.8	1.2;2.4	1.6	1.2;2.1	0.4
Percentage diameter stenosis	33.4	21.3;60.6	39.5	29.3;57.6	0.4
Restenosis	16	41	19	44	0.8
Target lesion revascularisation	6	15	5	12	0.7
Late loss (mm)	0.78	0.31;1.27	0.77	0.49;1.33	0.8

PCI=Percutaneous Coronary Intervention

In patients without a previous MI, the FBG at randomisation (median 7.5 (6.0;9.4) mmol/L) was somewhat higher compared with that in patients with a history of MI (FBG median 6.9 (6.5;7.6) mmol/L; p=0.07) and a univariable predictor of restenosis (OR 1.5, 95% CI 1.1-2.1; p=0.02), while this was not the case in patients with a history of MI (OR 1.2, 95% CI 0.6-2.2; p=0.6). Actual diabetes treatment at six months, irrespective of randomisation, did not influence the rate of restenosis (Figure 14).

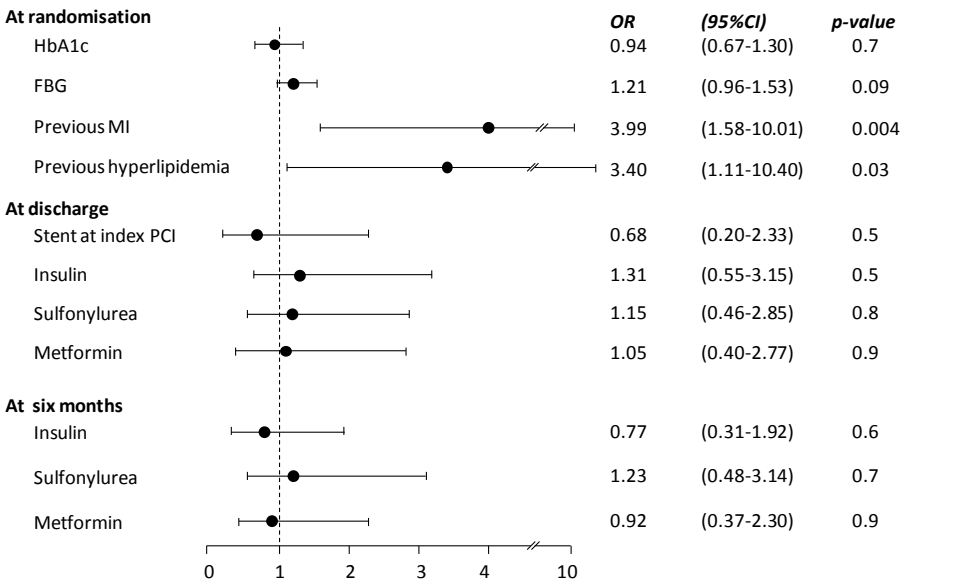

Figure 14. Univariable predictors of restenosis in **Study I** (n=82).
HbA1c=Hemoglobin A1c; FBG=Fasting Blood Glucose; MI=Myocardial Infarction; PCI= Percutaneous Coronary Intervention

Studies II and III

Detecting glucose perturbations in patients with ACS

The importance of time point and infarct size

The baseline characteristics of the 70 patients with a transmural MI and the 102 patients with a subendocardial MI in **Study II** are shown in Table 5, while glucose categorisation by means of OGTT is shown in Figure 15.

As outlined in Figure 15, a pre-discharge OGTT was performed in 67 (96%) of the patients with a transmural MI and it revealed that 31% were normal, while 33% had IGT and 36% T2DM. Three months later, these percentages were 35%, 39% and 26% in the 56 (80%) patients in whom the test was repeated, representing a reproducibility of 39% (κ=0.23, p=0.03). Among the 102 patients with a subendocardial MI, a pre-discharge OGTT was performed in 92 (90%) of them, classifying them as normal or with IGT or T2DM in 35%, 35% and 30% respectively. Three months later, these figures were 35%, 40% and 25% among the 78 (76%) patients who had a repeated OGTT, corresponding to a reproducibility of 55% (κ=0.40, p<0.001).

Admission glucose and FBG during the first two days were significantly higher in patients with transmural MI than in those with subendocardial MI (Figure 16). Insulin resistance measured as HOMA-IR on day two, pre-discharge and after three months did not differ between the two groups.

The diagnostic accuracy of fasting plasma glucose and HbA1c

Of the 79 patients in **Study III**, 52 (66%) were classified as having IGT and 27 (34%) as T2DM, according to the OGTT. As shown in Table 6, the baseline characteristics did not differ in patients with IGT compared with T2DM, except for FPG and two-hour post-load glucose (2hPG). HbA1c did not differ significantly between patients with IGT 39 (37;42) mmol/mol compared with T2DM 41 (38;44) mmol/mol (p=0.07). Diabetic status according to OGTT, FPG and HbA1c is shown in Figure 17. Two patients had T2DM according to HbA1c of whom one had IFG and IGT and one T2DM according to FPG and the 2hPG. Among the 52 patients defined by OGTT as having IGT, 14 had a normal FPG and 38 IFG, while four of the 27 T2DM patients had normal FPG and 13 IFG.

Table 5. Clinical characteristics of the patients by infarction type in **Study II**.
Continuous variables are presented as the median and lower and upper quartiles (quartile 1; quartile 3) and categorical variables as numbers (n) and percentages (%), unless otherwise stated.

Variable	**Transmural MI** (n=70)		**Subendocardial MI** (n=102)		*p*-value
Age (years)	61	55-70	66	58-73	0.03
Gender (male)	53	76	65	64	0.13
Previous medical history					
Smoking	53	76	64	62	0.10
Hypertension	18	26	35	34	0.25
Hyperlipidemia	8	11	16	17	0.51
Myocardial infarction	11	16	21	21	0.55
Biochemistry					
CK-MB > 10 during MI (n)	70	100	90	88	0.002
CRP day 2 (mg/L)	15.8	7.4;33.1	8.4	3.4;18.2	0.006
Blood glucose admission (mmol/L)	7.0	5.9;7.8	6.0	5.6;7.0	<0.001
Fasting day 2 (mmol/L)	5.7	5.2;6.2	5.3	4.8;6.0	0.01
Fasting day 3 (mmol/L)	5.4	4.9;6.1	5.0	4.7;5.5	0.004
Fasting day 4 (mmol/L)	5.3	4.9;5.7	5.2	4.6;5.6	0.14
HOMA-IR day 2	3.3	1.6;4.8	2.8	1.7;4.1	0.60
Discharge	2.5	1.5;3.7	2.2	1.4;3.5	0.42
3 months	2.2	1.7;4.3	2.4	1.6;3.6	0.67
Abnormal glucose regulation (IGT or T2DM)					
At discharge	46	69	60	65	0.73
At 3 months	37	65	52	65	1.00

CK-MB=Creatine Kinase–MB; CRP=C-Reactive Protein; HOMA-IR=HOmeostasis Model Assessment of Insulin Resistance; IGT=Impaired Glucose Tolerance; MI=Myocardial Infarction; T2DM=Diabetes Mellitus type 2

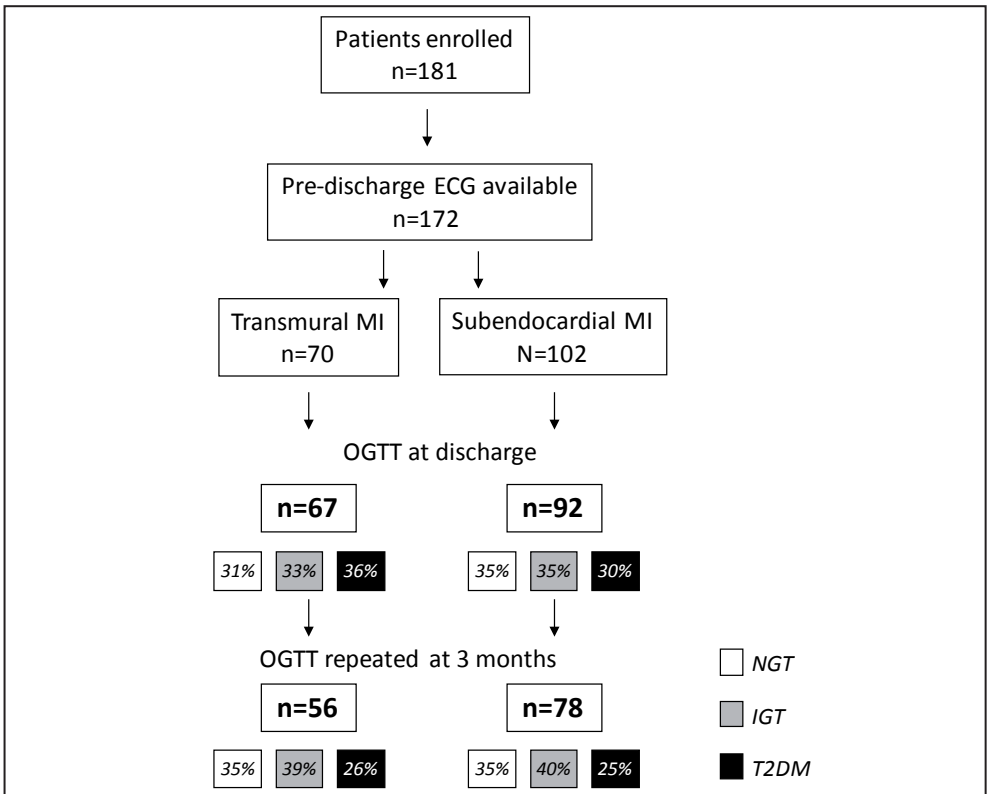

Figure 15. Patients divided according to glucose categories in **Study II**.
ECG=ElecroCardioGram; IGT=Impaired Glucose Tolerance; MI=Myocardial Infarction; NGT=Normal Glucose Tolerance; OGTT=Oral Glucose Tolerance Test; T2DM=Diabetes Mellitus type 2

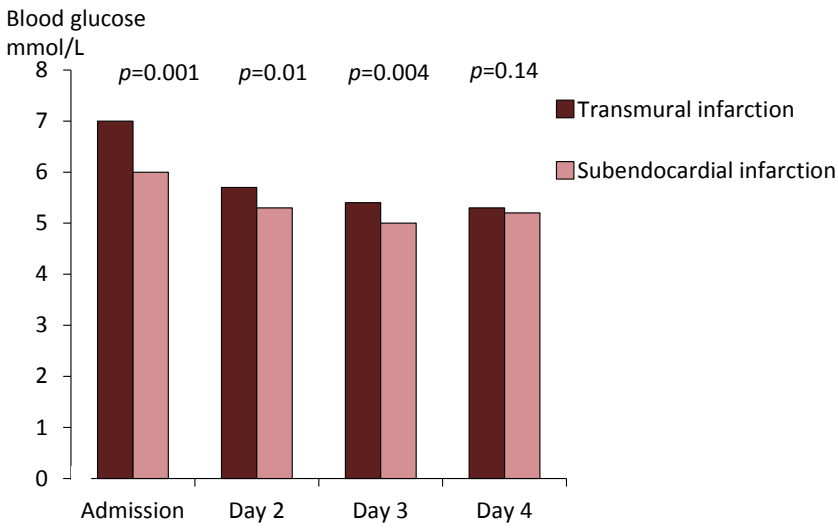

Figure 16. Glucose level divided by infarct size in **Study II**.

Table 6. Pertinent characteristics of patients in **Study III** divided according to glucose category defined by OGTT. Continuous variables are presented as the median and lower and upper quartiles (quartile 1; quartile 3) and categorical variables as numbers (n) and percentages (%), unless otherwise stated.

Variable	**IGT** (n=52)		**T2DM** (n=27)		*p*-value
Age (years)	66.5	61.8;73.7	69.3	62.0;73.4	1.00
Gender (males)	45	87	19	70	0.13
Previous medical history					
Smoking	35	67	20	74	0.61
Hypertension	29	56	14	52	0.81
Hyperlipidemia	17	33	8	30	1.00
Myocardial infarction	8	15	5	19	0.76
PCI/CABG	11	21	7	26	0.78
Diagnosis at discharge					
STEMI	21	40	16	59	0.15
NSTEMI	25	48	10	37	0.47
Unstable angina pectoris	6	12	1	4	0.71
Treatment at discharge					
Aspirin	50	96	26	96	1.00
Clopidogrel	51	98	27	100	1.00
Beta blocker	49	94	25	93	1.00
ACE or ARB inhibitor	47	90	21	78	0.17
Statin	50	96	27	100	0.54
Demographic and biochemical variables at randomisation					
BMI (kg/m^2)	27.7	25.0;29:0	26.9	24.0;28.6	0.37
Cholesterol (mmol/L)	4.8	4.2;5.8	4.5	3.8;4.9	0.13
LDL (mmol/L)	3.0	2.6;3.8	3.0	2.0;3.3	0.21
HDL (mmol/L)	1.0	0.9;1.3	1.0	0.8;1.2	0.10
Fasting plasma glucose (mmol/L)	6.0	5.5;6.4	6.3	5.7;7.2	0.03
Plasma glucose 2h (mmol/L)	9.0	8.3;9.7	11.8	11.1;13.7	<0.001
HbA1c (mmol/mol)	39	37;42	41	38;44	0.07

ACE=Angiotensin-Converting Enzyme-inhibitor; ARB=Angiotensin Receptor Blocker; BMI=Body Mass Index; CABG=Coronary Artery Bypass Grafting; HbA1C=Haemoglobin A1C; HDL=High Density Lipoprotein; IGT=Impaired Glucose Tolerance; LDL=Low Density Lipoprotein,; NSTEMI=Non ST-Elevation Myocardial Infarction; OGTT=Oral glucose Tolerance Test; PCI=Percutaneous Coronary Intervention; STEMI=ST-Elevation Myocardial Infarction; T2DM=Diabetes Mellitus type 2

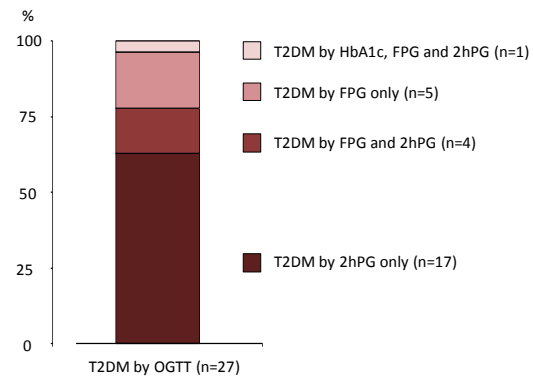

Figure 17. Diagnosis of T2DM in **Study III** by different methods.

FPG=Fasting Plasma Glucose
HbA1c= Hemoglobin A1c
2hPG=2 hour Post load Glucose OGTT
T2DM=diabetes mellitus type 2
OGTT=Oral Glucose Tolerance Test

Table 7. Clinical characteristics of the patients in **Study IV**.

Patient no	Diagnosis at discharge	Age* (years)	BMI* (kg/m²)	BP* (mm Hg)	Observation** (days)	Antithrombotic therapy**
1	Heart failure T2DM	71	29	95/55	3	Warfarin
2	NSTEMI T2DM	80	25	125/70	3	Fondaparinux Aspirin Clopidogrel
3	STEMI	64	36	160/80	3	Fondaparinux day 1 Aspirin Clopidogrel
4	Heart failure	41	34	100/55	3	Aspirin Clopidogrel
5	STEMI	55	30	110/55	3	Enoxaparin Aspirin Clopidogrel
6	Heart failure T2DM	73	40	120/70	2	Enoxaparin
7	STEMI	46	32	95/60	3	Aspirin Clopidogrel
8	Unstable angina pectoris	59	25	117/78	2	Fondaparinux day 1 Aspirin Clopidogrel
9	STEMI	74		113/77	2	Abciximab day 1 Aspirin Clopidogrel
10	STEMI	78	26	105/70	3	Fondaparinux Aspirin Clopidogrel
11	NSTEMI	66	30	128/74	2	Aspirin Clopidogrel
12	Unstable angina pectoris T2DM	75	32	130/70	3	Enoxaparin day 2, 3 Warfarin Aspirin Clopidogrel
13	NSTEMI T2DM	63	26	140/60	2	Fondaparinux Aspirin Clopidogrel
14	STEMI T2DM	59	30	125/80	2	Abciximab Aspirin Clopidogrel

*At study inclusion ; **During hospitalisation
BMI=Body Mass Index; BP=blood pressure; NSTEMI=Non ST-Elevation Myocardial Infarction; STEMI=ST-Elevation Myocardial Infarction; T2DM=Diabetes Mellitus type 2

Study IV

Continuous glucose monitoring in ACS

The 14 patients, all males, included in **Study IV** are presented in Table 7.

A total of 266 matched pairs of glucose measurements were collected. The lowest and highest measured plasma glucose were 4.5 mmol/L and 18.7 mmol/L while the corresponding values in microdialysis fluid were 2.8 mmol/L and 17.9 mmol/L respectively. Of the paired data points, 82% fell within ±20% of the reference glucose. The Clarke error grid of comparison is shown in Figure 18. All measurements fell within zone A or B, of which 81% were within zone A. In four of the 14 patients, the microdialysis measurements did not correspond to the measured plasma levels of glucose, as the values obtained by means of microdialysis were consistently too low. No relationship between the site of the catheter, body composition, blood pressure, diagnosis, or medical treatment and discrepant values could be detected.

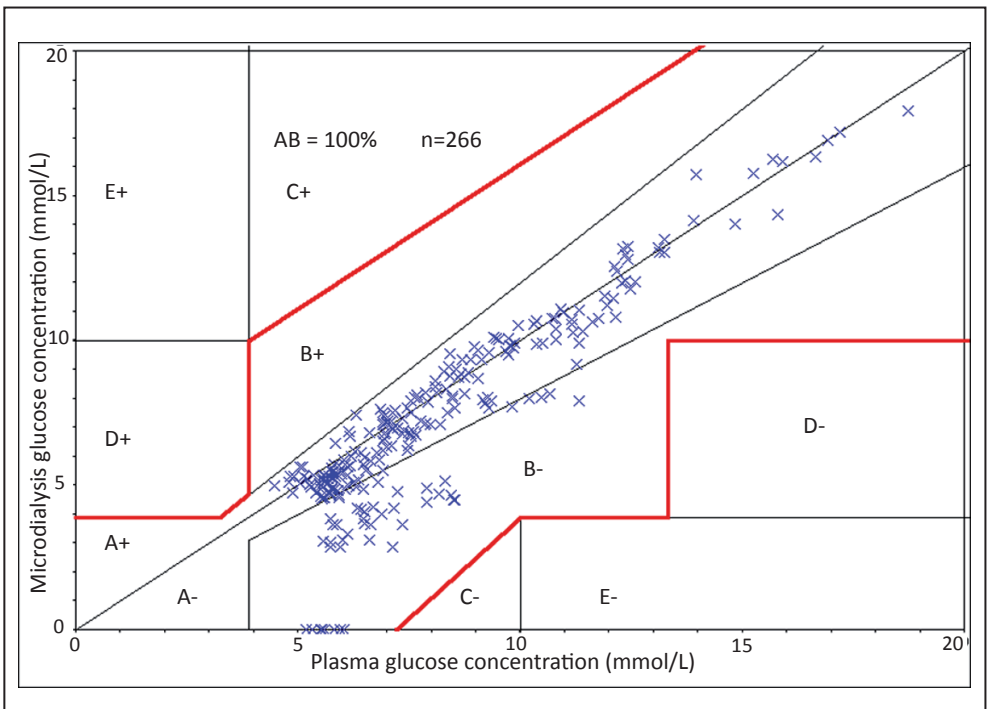

Figure 18. Plasma glucose in **Study IV** analysed by the Clark Error Grid Analysis. Values in Zone A; clinically accurate, Zone B; benign, Zone C; over corrections, Zone D; failure to detect and Zone E; erroneous.

Study V

Beta-cell function in ACS

The baseline characteristics of the 71 patients are presented in Table 8, while biochemical and clinical characteristics at randomisation and at the follow-up visit after 12 weeks on randomised treatment are presented in Table 9. HbA1c improved from 40 to 38 mmol/mol

in the sitagliptin group and remained unaltered in the placebo group (p=0.004). At the time of randomisation, 24 (71%) patients in the sitagliptin group had IGT and 10 (29%) had T2DM and the percentages of glucose abnormalities in the placebo group were 23 (62%) and 14 (38%) respectively. After 12 weeks, 26 (76%) patients in the sitagliptin group had normalised their glucose tolerance according to the OGTT, while the corresponding number for the placebo group was 15 (41%; Figure 19).

Table 8. Baseline characteristics of the patients in **Study V**. Continuous variables are presented as the median and lower and upper quartiles (quartile 1; quartile 3) and categorical variables as numbers (n) and percentages (%), unless otherwise stated.

Variable	**Sitagliptin** (n=34)		**Placebo** (n=37)	
Age (years)	69	61;77	66	61;72
Gender (male)	29	85	29	78
Previous medical history				
Family history				
Cardiovascular disease	14	44	16	43
Diabetes mellitus type 2	8	26	7	19
Smoking habits				
Present	7	21	6	16
Previous	15	44	22	60
Hypertension	20	59	18	49
Hyperlipidemia	10	29	11	30
Angina pectoris	5	15	3	8
Myocardial infarction	6	18	7	19
Procedures during hospitalisation				
Coronary angiogram	33	97	36	97
PCI	32	94	32	86
Diagnosis at discharge				
STEMI	16	47	16	43
NSTEMI	14	41	18	49
Unstable angina pectoris	4	12	3	8
Treatment at discharge				
Aspirin	33	98	36	97
Clopidogrel	34	100	36	97
Betablocker	31	91	35	95
ACE or ARB inhibitor	30	88	32	86
Statin	34	100	35	95

ACE=Angiotensin-Converting Enzyme-inhibitor; ARB=Angiotensin II receptor blocker ; NSTEMI=Non ST-Elevation Myocardial Infarction; PCI=Percutaneous Coronary Intervention; STEMI=ST-Elevation Myocardial Infarction

Beta-cell function expressed as IGI and AIRg did not differ between the sitagliptin group and the placebo group at baseline (69.9 *vs.* 66.4 pmol/mmol and 1394 *vs.* 1106 pmol · l^{-1} · min^{-1}). After the 12-week study period, the IGI was 85.0 in the sitagliptin group and 58.1 pmol/mmol in the placebo group (p=0.019), while AIRg was 1909 and 1043 pmol · l^{-1} · min^{-1} (p<0.0001) respectively (Figure 20 a-b). Fasting and 120-minute post-load levels of proinsulin decreased in both groups at 12 weeks, the latter somewhat more in the sitagliptin group (Table 9). Insulin resistance, estimated as HOMA-IR, and FPG did not change significantly in either of the two groups during follow-up (Figure 20 c-d).

Table 9. Biochemical and clinical characteristics presented at randomisation and after 12 weeks of treatment in **Study V**. Continuous data are presented as median (quartile 1; quartile 3) and categorical as numbers (%) unless otherwise stated. *P*-values represent comparison of delta values between groups at 12 weeks.

Parameter	Baseline				12 weeks				*p*
	Sitagliptin (n=34)		Placebo (n=37)		Sitagliptin (n=34)		Placebo (n=37)		
Physical examination									
Blood pressure									
Systolic (mmHg)	120	116;145	125	113;133	125	120;140	130	120;140	0.257
Diastolic (mmHg)	73	70;80	75	70;80	75	70;80	75	70;85	0.259
Heart rate (bpm)	59	51;64	56	52;61	55	49;61	52	48;58	0.935
Weight (kg)	84	76;90	82	75;93	83	74;89	80	74;93	0.115
Biochemistry									
Troponin I max (ng/L)	11.0	0.5;27.6	5.5	0.6;19.0	-	-	-	-	-
Plasma glucose at admission (mmol/L)	6.1	5.8;7.6	6.3	5.9;7.2	-	-	-	-	-
Glucose fasting (mmol/L)	6.1	5.5;6.6	6.0	5.8;6.7	5.8	5.5;6.0	5.9	5.7;6.5	0.429
HbA1c (IFCC mmol/mol)	40	37;42	40	37;43	38	36;42	40	37;43	0.004
Insulin fasting (pmol/L)	67.5	41.3;76.7	63.7	44.1;83.7	68.7	42.3;88.5	53.1	45.5;84.0	0.586
Proinsulin fasting (pmol/L)	12.9	9.1;19.5	12.9	9.6;16.1	10.2	7.3;15.6	11.0	7.7;19.3	0.101
Proinsulin 120 min OGTT (pmol/L)	109.2	69.9;177.2	115.6	76.0;156.2	64.6	43.9;90.1	76.9	60.1;111.0	0.094
Blood lipids									
Total cholesterol (mmol/l)	4.6	4.2;5.6	4.6	4.0;5.9	3.6	3.2;4.4	3.9	3.5;4.3	0.699
LDL (mmol/L)	3.0	2.3;3.7	3.0	2.3;3.9	2.1	1.7;2.5	2.2	1.9;2.6	0.948
HDL (mmol/L)	1.0	0.9;1.2	1.0	0.8;1.2	1.0	0.9;1.2	1.1	0.9;1.3	0.222
Triglycerides (mmol/L)	1.1	0.8;1.9	1.3	1.1;1.8	1.1	0.8;1.3	1.2	0.9;1.5	0.856

HbA1C=Haemoglobin A1C; HDL=High Density Lipoprotein; IFCC=International Federation of Clinical Chemistry standard; LDL=Low Density Lipoprotein; OGTT=Oral Glucose Tolerance test

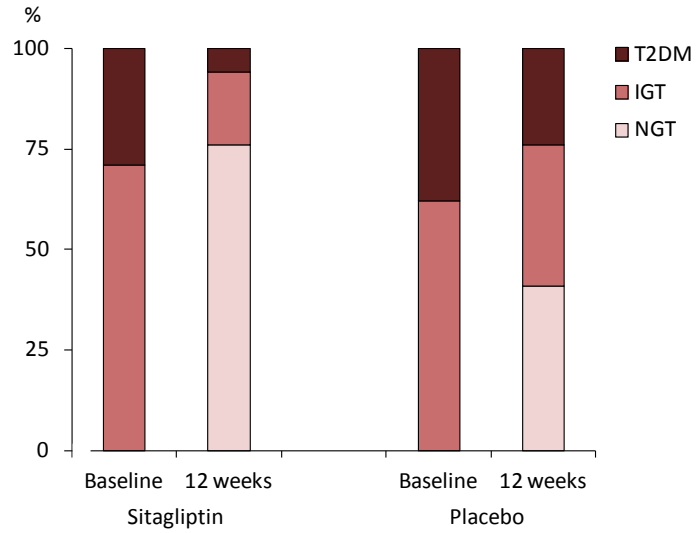

Figure 19. Glucose tolerance category according to OGTT at randomisation in **Study V** and after 12 weeks of treatment divided by treatment group.
IGT=Impaired Glucose Tolerance; NGT=Normal Glucose Tolerance; OGTT=Oral Glucose Tolerance Test; T2DM=Diabetes Mellitus type 2

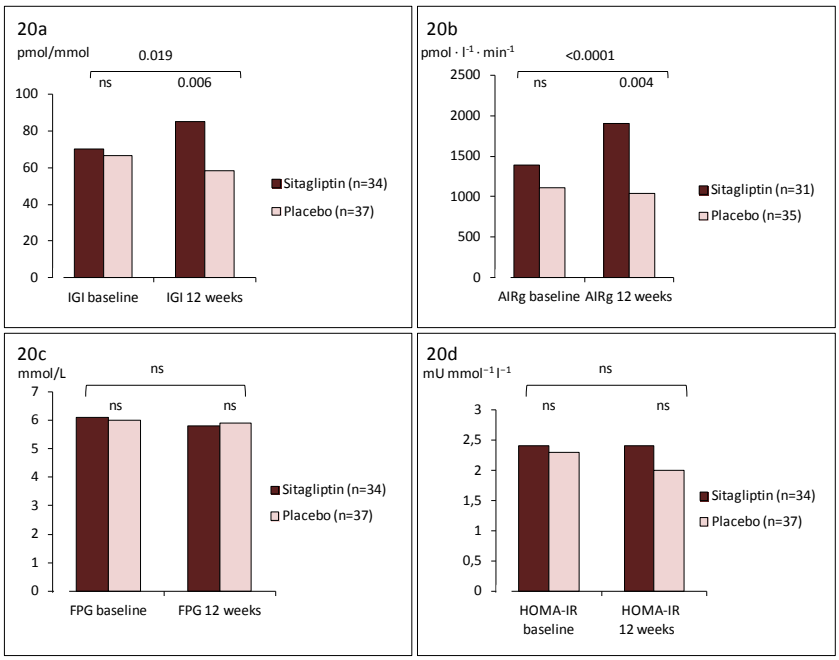

Figure 20 a-d. The primary endpoint beta-cell function in **Study V** expressed as the insulinogenic index **(20a)** and AIRg **(20b)**. Fasting plasma glucose **(20c)** and HOMA-IR **(20d)**. AIRg=Acute Insulin Response to glucose; FPG=Fasting Plasma Glucose; HOMA-IR= HOmeostatic Model Assessment Insulin Resistance; IGI=InsulinoGenic Index

General Discussion

Glucose control in patients with stable CAD

The fact that hyperglycaemia is related to increased CVD morbidity and mortality has been demonstrated in different clinical settings comprising general populations (186-188) and patients with stable CAD (103) or ACS (94, 101, 102, 189). The risk associated with increasing glucose levels is continuous and extends below the diagnostic threshold for T2DM (95, 190).

For many years, T2DM treatment focused on glucose lowering, based on the possibility to reduce microvascular complications (191, 192), and it is only during the last few decades that it has been acknowledged that glucose control may have an impact on the CVD prognosis. The insight into the negative impact of hyperglycaemia opened the door to attempts to improve outcome by reducing untoward glycaemia. The tool for managing hyperglycaemia was originally mainly insulin, subsequently supplemented by other pharmacological agents such as insulin secretagogues, biguanides and, more recently, glitazones and GLP-1-related drugs.

The impact of intensive insulin-based glucose control on the restenosis rate in patients with established T2DM was investigated in the IDA trial (**Study I)**. There are in fact observational data indicating that strict glycaemic control may reduce the need for target vessel revascularisation (193, 194). Insulin was chosen as a suitable agent as, in addition to its glucose-lowering capacity, it has anti-atherogenic properties inducing endothelial-dependent vasodilation by increasing NO production via the PI3K pathway, which also mediates VSMC quiescence. Further, insulin infusion inhibits platelet aggregation and reduces plasma markers of inflammation (195). Conversely, it has also been argued that insulin may have pro-atherogenic effects by enhancing the proliferative response after a PCI, especially in the presence of insulin resistance, possibly by potentiating the expression of mitogenic factors, e.g. insulin-like growth factor-l (IGF-1), and increasing VSMC proliferation via the MAPK pathway (121, 196). Disappointingly the IDA trial did not support the hypothesis that insulin-based, tight glycaemic control would lower the restenosis rate. These findings were recently confirmed by the STREAM (STent Restenosis And Metabolism) trial in which addition of insulin did not influence in stent restenosis in patients with T2DM (197). Among potential explanations, it is important to consider that the IDA patients had a history of T2DM for six years and that they all had symptomatic CAD. At this fairly late stage of their disease, when the T2DM has progressed for several years, it may be too late to influence the development of CVD complications. Similar findings have been reported from three large trials, ADVANCE; Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation, ACCORD; the Action to Control Cardiovascular Risk in Diabetes and VADT; Veterans Affairs Diabetes Trial, exploring the concept of reducing CVD events by means of intensive glucose lowering (137-139). Even if substantial glucose lowering was achieved in the intensively treated groups, the event rates were higher than in the control groups in which glucose levels were less well controlled. The patient populations in these trials had established T2DM since eight to ten years. In contrast to this disappointing outcome, it seems as if treatment aimed at optimising glucose levels soon after the identification of patients with T2DM is more rewarding. In the UKPDS, intensive glucose-lowering treatment with sulphonylurea/insulin or metformin was compared with lifestyle advice in patients with newly detected T2DM. During the initial 10 years of follow-

up, the groups differed in glucose control in favour of those given glucose-lowering drugs. Treatment with metformin resulted in a significant reduction in myocardial infarction and sulphonylurea/insulin therapy had a similar effect, however, only borderline significant. Continued follow-up of the UKPDS patients revealed a significant reduction in CVD morbidity and mortality after 17 years, although the difference in glucose control had diminished between the intensive and the routinely treated group (136). The beneficial effects of early institution of glucose-lowering treatment to prevent CVD are also supported by observational data from the Euro Heart Survey on Diabetes and the Heart (141) and the STOP-NIDDM trial (198). It therefore appears as if optimising glucose control in patients with advanced atherosclerosis and T2DM for several years is of little help. The potential benefits of glucose lowering may have been lost since the time window during which it may be possible to reverse the progress of CVD disease by tight glucose control has closed. This indicates that early detection and institution of effective glucose control may be of critical importance in improving the prognosis.

An alternative explanation to the negative results in **Study I** may have been the lack of a difference in glucose levels between the two groups. This demonstrates how difficult it may be to achieve glucose control in patients with T2DM even in the setting of a controlled trial. Surprisingly, patients in the intensively treated group, who were referred to a specialist in diabetology, had the same level of glucose as those in the control group after six months of follow-up. Moreover, other trials, attempting to achieve glucose normalisation by means of insulin in patients with T2DM and established CVD disease, failed in this respect (124, 199). In **Study I**, as well as in the DIGAMI 2 trial, it was hypothesised that insulin could have positive effects on the prognosis in patients with T2DM and established CAD, but this could not be confirmed. This indicates that insulin may not be the optimal drug in this category of patients, at least not when no difference in glucose control is achieved. A recent report from the DIGAMI 2 trial supports this notion, demonstrating that insulin, compared with sulphonylurea, metformin or no drugs, did not improve the prognosis in these patients; rather the contrary, as manifested by a higher proportion of non-fatal re-infarctions and stroke in the insulin-treated group (200).

The implications of **Study I** may be difficult to interpret in the era of modern PCI technology, when the introduction of DES has reduced the restenosis rate in all patients, including those with T2DM. But long-term studies are scarce and epidemiological studies consistently report that patients with DM have a considerably poorer prognosis after a PCI (110). Accordingly, attempts to improve the outcome remain important. The impact of glucose control continues to be of interest, not the least since **Study I** revealed a significant relationship between the pre-procedural FBG and the subsequent restenosis rate. The importance of the pre-procedural glucose level is also evident in other studies (194, 201, 202), indicating that targeting glucose normalisation during the time prior to, and maybe also, following a PCI may have a beneficial impact on the subsequent prognosis. However, the tools to achieve this need to be carefully considered when preparing these trials.

Glucose control in patients with ACS

Even if insulin appears to be less attractive for the long-term treatment of patients with T2DM and CAD (200), it remains the best option when treating patients admitted with ACS. Insulin has a rapid onset of action and can be titrated according to the prevailing situation. In

patients with ACS, the risk of mortality or non-fatal CVD events is amplified with increasing glucose levels. In this setting, it is reasonable to assume that lowering glucose will improve the prognosis. However, this concept may be challenged. As outlined in the introduction, the first DIGAMI study (122) demonstrated improved survival by means of insulin-based intensified glucose control in patients with DM and AMI, but subsequent trials attempting to confirm and extend these findings failed (124, 125). A meta-analysis comprising data from the three studies using a glucose-insulin infusion with the aim of achieving glucose control (DIGAMI 1, 2 and HI-5) showed that the treatment did not reduce mortality in the absence of glucose control (RR 1.07, 95% CI 0.85 to 1.36, p=0.547) (203). During the first 24 hours of hospitalisation, both HI-5 and DIGAMI 2 succeeded in reducing glucose levels, but, in the HI-5 study, there was no significant difference between the intervention and control groups and, in the DIGAMI 2 trial, the difference between the groups was small. These two studies must therefore be regarded as inconclusive, due to a lack of a significant improvement in the glucose levels of intensively treated patients, making the findings in DIGAMI 1 unopposed. Accordingly, it is still an open question whether the effective control of hyperglycaemia by means of insulin will improve the prognosis in T2DM patients with AMI.

One problem when it comes to treatment aiming at glucose normalisation during the acute phase of ACS is the fear of hypoglycaemia induction which has been related to an increased mortality risk in patients with ACS (204-206). These concerns were in fact discussed as a possible reason for the failure to reach the set glucose targets in DIGAMI 2 and HI-5. It has been claimed that hypoglycaemia, rather than being a cause in itself, is a marker of high risk due to other medical conditions (207, 208). Glucose variability, which may be a result of insulin treatment, is another factor that may explain the lack of consistent, positive results. In retrospective studies from surgical intensive care units, glycaemic variability is associated with an increased risk of mortality (209, 210). Similar findings have been reported in populations admitted for medical indications (211, 212), although glycaemic variability may be less important in patients with DM (213).

These concerns, in combination with the failed attempts to achieve tight glucose control in DIGAMI 2 and HI-5, illustrate the need for new tools for glucose monitoring and guiding the administration of insulin. One solution would be to use a reliable, continuous glucose monitoring system (CGMS). A pilot study among nurses at a CCU found these devices helpful, but only after a training period with this new technology (214). In a subsequent randomised trial, 20 STEMI patients were assigned to either 48 hours of strict glycaemic control with a subcutaneous insulin pump augmented by continuous glucose monitoring or standard management with glucose measured by blinded continuous glucose monitoring. Hyperglycaemia was effectively reduced by the sensor-augmented insulin pump, but at the cost of a small yet significant increase in hypoglycaemic episodes (215). The studies quoted used a subcutaneous CGMS developed for home glucose monitoring of patients with DM (MiniMed CGMS and The Paradigm Real-Time system; Medtronic MiniMed, Northridge, California, USA). When monitoring patients with ACS, it is, however, important that the device picks up changes in the glucose quickly, alerting the staff for dosage adjustment. In this perspective, intravenous glucose measurement rather than recordings from subcutaneous interstitial fluid seem preferable, as it is independent of tissue perfusion. This was the reason for the choice of the microdialysis system in **Study IV**. Further, when relying on measurements to steer insulin intervention, accuracy is crucial, especially in the lower glucose

ranges where a difference of one or two millimoles may distinguish good glucose control from hypoglycaemia. During extended observations, the microdialysis catheter tended to display measurements as too low. The study design did not allow a full analysis of the reasons for the discrepancy between the glucose concentrations measured at the laboratory and the microdialysis technique. Possible explanations may be the coating of the catheter or impaired blood flow in the vessel. Thus adjustments have to be made before this system can be regarded as reliable.

Detection of glucose abnormalities in patients with CAD

Since glucose abnormalities are common and prognostically important in patients with stable as well as unstable CAD, the actual gluco-metabolic state should be explored. In fact, the mere presence of CAD should lead to an investigation of glucose metabolism, according to European guidelines (36). Importantly, glucose perturbations are not always apparent unless they are actively screened for. When identifying risk subjects, the accuracy of the screening method is of importance, opening the door to the initiation of the correct preventive measures. When comparing different screening methods, it is important to take account of the fact that glucose measurements, due in part to sample collection and processing, have higher within-individual day-to-day variability than HbA1c, amounting to about 17% for the OGTT, 6-15% for FPG and < 2% for HbA1c (216, 217). The FPG is less sensitive and identifies different populations to some extent compared with the OGTT (186, 218, 219). HbA1c is the most specific but least sensitive of the three methods for detecting glucose abnormalities (220). Despite this shortcoming and due to its convenience, it has been suggested that HbA1c identifies more people with glucose perturbations in a general population than FPG (221) and probably also OGTT. This method bypasses the problem of the day-to-day variability of glucose values and avoids the need for the individual to fast.

In patients hospitalised due to ACS, the method for screening, as well as the optimal time point, are subject to debate. **Studies II** and **III** investigate diagnostic tools and what may influence the screening methods in a high-risk population of patients with a recent ACS. Glucose is often measured routinely at some point during hospitalisation for an ACS, at admission, fasting or at random. The diagnosis of glucose disturbances in patients with ACS does not differ from what is required in other groups, i.e. at least two pathological values are needed for the diagnosis of T2DM in the absence of symptoms of hyperglycaemia. Even if no formal diagnosis is made, elevated glucose levels per se should, however, warrant attention, as they indicate an increased risk. As demonstrated in **Study III,** the current HbA1c diagnostic cut-off of 48 mmol/mol only identified two patients, and the FPG only ten of the 27 patients classified as having T2DM by an OGTT. Due to their lower sensitivity, the use of FPG and HbA1c alone will result in ineffective screening, as many individuals with glucose aberrations will remain undetected. Other limitations are that HbA1c may be affected by a variety of genetic and haematological factors such as anaemia and haemoglobinopathies (35, 222). Moreover, depending on the method of analysis, increased levels of triglycerides, bilirubin and white blood cells may interfere with the result (217). HbA1c is a simple test to perform and, if elevated, it indicates an increased risk, but a normal HbA1c should prompt further investigations and preferably an OGTT.

The OGTT has been criticised for being inconvenient and for its poor reproducibility, as the results are influenced by previous dietary intake, factors affecting insulin sensitivity and

secretion, such as smoking and physical activity, as well as gastrointestinal hormones. As further outlined below, the influence of hyperglycaemia induced by stress is an additional confounder, not the least important during an ACS. The within-individual variability of an OGTT may result in a patient being classified as having T2DM on one occasion and IGT the next, especially in patients close to the cut-off levels (223).
However, as clearly demonstrated in **Study III**, the OGTT adds information compared with FPG or HbA1c and the majority of patients with glucose perturbations were indeed disclosed by an increased 2hPG. Information on postprandial glucose elevations, which increase prior to the FPG during the progression of glucose perturbations towards overt T2DM (see Figure 3), can only be derived from an OGTT. This is of special importance in risk prediction, as the 2hPG in this respect is superior to FPG (186). Moreover, patients in the range of IGT run an increased risk of CVD morbidity and mortality compared with those with isolated IFG (187, 224).
One limitation of **Study III** (and **II**) is the lack of repeated OGTTs, especially so if the purpose was to diagnose T2DM. However, in ACS patients, the primary aim is not to establish the diagnosis of T2DM but rather to assess glucose perturbations as a continuously increasing risk factor of importance to take into account when assessing a patient's total CVD risk. The sensitivity of the test that is chosen is especially important when dealing with high risk individuals, a strong reason to use an OGTT in such patients with normal HbA1c and FPG.

One important question is whether stress impairs the reliability of screening for glucose abnormalities in patients hospitalised with ACS. Hyperglycaemia, detected by glucose measurements during hospitalisation, may be related to a catecholamine-induced stress reaction, but it may also indicate an underlying glucose abnormality in need of further investigation. In this context, the HbA1c has an advantage as it reflects the mean glucose levels during the past four to eight weeks (225) uninfluenced by short-term glycaemic variations. However, it misses a substantial number of patients with IGT or T2DM, as already discussed.
The impact of timing on the results of an OGTT was explored in **Study II** and the topic has also been investigated in other studies where serial OGTTs have been performed during and after hospitalisation for an ACS (226-231). In these studies, the number of glucose abnormalities during hospitalisation varied between 46-79%, with a tendency to improve at follow-up, to a percentage of 25-42%, especially if the initial OGTT was performed close to admission, i.e. within 48 hours (228-230) and in younger patients with a first-time MI (228, 231). In **Study II**, two thirds of the patients still had an abnormal glucose regulation when the OGTT was repeated after three months, even if some patients close to the cut-off levels may have transferred up or down to another glucose category, thereby impairing the value of agreement. Compared with the studies by Knudsen and Bronisz, the patients in **Study II** were five to six years older, which may have contributed to more permanent beta-cell dysfunction, as this has a tendency to decline with age. **Study II** further suggests that a large infarct may aggravate stress, thereby influencing the glucose levels during the very first days after admission. This impact declines during the hospitalisation, making the results of an OGTT performed four to seven days after admission reliable. This notion is confirmed by other studies in which the impact of infarct size, determined as CK_{max} (227) or troponin T levels (230), did not relate to the OGTT result. Bronisz et al. performed an OGTT immediately prior to discharge and at three months in STEMI patients. They reported an improvement in glucose tolerance but did not reveal any relationship with the extent of myocardial necrosis (231).

When the GAMI study (**Study II**) was performed, more than ten years ago, a hospitalisation period of five days was not unusual, while many patients nowadays are discharged after just three days, which means that the OGTT needs be performed closer to the acute event if conducted during hospitalisation. To increase the accuracy of an OGTT in ACS patients the procedure is better scheduled at the first follow-up visit when the potential effects on glucose metabolism of acute stress have declined.

During the last decade, awareness of glucose disturbances in ACS patients has increased and clinical practice has changed. Rehabilitation programmes after an ACS focusing on improvements in lifestyle, including physical activity and food choices, have developed. It is to be hoped that this growing knowledge will contribute to a healthier lifestyle, resulting in recovered glucose tolerance after an ACS event. This may also be an explanation of the "spontaneous" improvement in glucose tolerance observed in **Study III** compared with **Study II** and perhaps also in some of the previously mentioned studies of OGTT in ACS. In fact, lifestyle interventions introduced during the early stages of glucose intolerance can delay further progress into T2DM and thereby potentially avoid CVD events (232, 233). There are however patients who do not recuperate in terms of glucose tolerance, despite lifestyle interventions. The early identification of patients with glucose abnormalities is important in order to optimise future treatment, which may include the initiation of glucose-lowering agents (141, 232, 234).

New options in glucose lowering - targeting beta-cell function

Although observational data indicate that an early pharmacological approach could improve the prognosis in patients with ACS and newly detected glucose perturbations, this has not yet been verified in randomised controlled trials (141). In general, glycaemic treatment has often focused more heavily on glucose lowering rather than targeting the pathogenesis of the perturbation, which may be a reason why even the use of insulin may eventually end in treatment failure, not the least in patients with reduced insulin sensitivity as a primary defect (235). Further, there are indications that the choice of glucose-lowering agent may have prognostic implications. As mentioned previously, a report from the DIGAMI 2 trial, as well as epidemiological data in patients with T2DM and AMI, imply potentially harmful effects of insulin or insulin secretagogues with an increased risk of CVD events and mortality compared with metformin, which appears to have a more desirable profile (200, 236-238). Thus metformin, which acts by increasing insulin sensitivity, is a logical choice as reflected by recent treatment recommendations (239). Other alternatives are the glitazones, which are strong insulin sensitisers with additional positive effects on beta-cell function (68), but their CVD safety is under debate, especially rosiglitazone (240, 241), which have been withdrawn from the European market.

As previous investigations by our group revealed high proinsulin levels reflecting beta-cell dysfunction in patients with ACS (104, 105), pharmacological agents targeting beta-cell function may be of special interest for further exploration in this setting. In the UKPDS, neither metformin nor sulphonylurea were able to prevent a 4% annual decline in beta-cell function (65), while rosiglitazone did somewhat better in the ADOPT study, with a 2% decrease compared with 6.1% in the glyburide group (p=<0.001) and 3.1% in the metformin group (p = 0.02) (242).

The GLP-1 analogues and DPP-IV inhibitors are attractive compounds in this respect. GLP-1 has been anticipated to have disease-modifying properties on beta cells via proliferative and anti-apoptotic actions primarily shown in T2DM rodent models, human cell lines and islets. Moreover, in reports from animal models, the DPP-IV inhibitor sitagliptin, used in **Study V,** has been demonstrated to restore islet morphology (243, 244) and reduce apoptosis in beta cells (245, 246). Exactly how these experimental findings translate into a clinical situation is still to be explored in more detail. In human studies, where the pancreatic beta-cell function is primarily estimated from glucose and insulin measurements, sitagliptin appears to improve beta-cell function in patients with established T2DM (247-254). Similar effects have also been reported for other DPP-IV inhibitors, suggesting a class effect rather than a specific effect for sitagliptin (255).

In **Study V**, the beta-cell function was improved by the DPP-IV inhibitor sitagliptin.
The clinical studies presented so far mainly recruited patients with T2DM, who had presumably lost a substantial amount of their beta-cell function. In these patients, there are still data indicating sustained effects of exenatide and sitagliptin after up to two to three years of treatment, especially if combined with metformin (256-258). Improvements in beta-cell function have also been demonstrated in patients with IFG using the DPP-IV inhibitor vildagliptin (259). Initiating treatment at an early stage offers an opportunity to have beta-cell function left to save. In **Study V,** all the patients had previously unknown glucose disturbances with modestly elevated glucose levels, which suggests that they are detected early in their diabetes career. The findings of increased proinsulin levels, as demonstrated in the GAMI study (104), were replicated in the present patient population in which these levels were in fact somewhat higher than in the GAMI study with a HOMA-IR index of the same magnitude, confirming the impaired beta-cell function in this group of patients. In the control group, as well as in the treatment group, the patients were given structured and reinforced lifestyle advice on food and physical activity, according to guidelines. This is a potential explanation for an average weight loss of two kg and the fact that 41% of the control patients had a normal glucose metabolism after three months. In contrast to the sitagliptin-treated group of patients, they did not improve their beta-cell function. The beneficial effects of a DPP-IV inhibitor on beta-cell function in the sitagliptin group should therefore be seen as a result achieved in addition to lifestyle-initiated improvements which, if permanent, may potentially delay the further development of hyperglycaemia and T2DM and hopefully also further CVD events.

Although DPP-IV inhibitors in general and sitagliptin in particular are well tolerated (260, 261), the safety profile has not been extensively studied in patients with CAD, especially not with a recent ACS, and there are reports of potential drug interactions between sitagliptin and CVD agents (262-266). One important aspect of **Study V** was therefore to evaluate the feasibility of the early prescription of sitagliptin to patients with ACS. While small in patient numbers, **Study V** demonstrates that the drug was well tolerated, a finding that requires confirmation in larger sets of patient material. Furthermore, there are indications that DPP-IV inhibitors such as sitagliptin have beneficial CVD effects apart from improvements in glucose control (169). In patients with T2DM, sitagliptin increased circulating endothelial progenitor cells, which may have potential favourable CVD implications (267). Moreover, the lipid profile is positively influenced by DPP-IV inhibitors (268, 269) and there are data in patients with CAD indicating that sitagliptin plays a cardioprotective role, showing enhanced

left ventricular response to stress, attenuated post-ischaemic stunning and improved global and regional left ventricular performance (270). When evaluating the impact of DPP-IV inhibitors on CVD events there seem to be a possible protective effect as indicated by a meta-analysis including 20,312 patients on DPP-IV inhibitors and 13,569 on comparator drug (271).

Future perspectives

Efforts to improve prognosis in dysglycaemic patients with CAD or at high risk for such conditions by a stringent glucose control has not been convincing, neither in the outpatient setting nor during hospitalisation. A possible explanation is inefficacy of available glucose lowering drugs, which although initially effective may provide insufficient glycemic control in the long term perspective, leaving insulin as the remaining option. However, even when given insulin, many patients do not reach recommended glucose targets and in addition, the treatment increases the risk for glucose swings, including hypo- and hyperglycemia. Lowering of glucose levels during the hospitalisation for an ACS may necessitate insulin administration. Such treatment may, however, be complicated to balance due to varying degree of stress, the possible impact of concomitant medications, variable food intake and a fear to induce hypoglycaemia. Development of new techniques as such as CGMSs may facilitate insulin based therapy by frequent, automatically delivered glucose values offering a potential to eliminate excessive blood sampling and to make the staff in coronary and intensive care units more confident in using adequate amounts of insulin and to enable titration towards tighter glucose targets. Reliable technology of that kind would open for the possibility to test the hypothesis of an improved prognosis by a stable tight glucose control guided by CGMS.

Many patients have their dysglycaemic condition discovered at admission for an acute coronary event. The high proportion of previously unknown glucose perturbations in patients with both stable and unstable CAD necessitates effective screening procedures within cardiac care. The HbA1c has recently been proposed as a diagnostic tool and may be useful as a complement but, an OGTT is considerably more sensitive for the detection or exclusion of glucose disturbances in such patients, as shown in this thesis. Thus an OGTT is advocated when FPG and HbA1c are normal to avoid that a large-scale proportion of patients with glucose perturbations remain undetected. In addition, an accurate identification of prediabetic states is important since the development of T2DM in patients with IGT may be prevented or retarded by means of lifestyle adjustments or, if needed pharmacological agents such as metformin or acarbose (232, 234). Since the cardiovascular prognosis can be improved in patients with glucose perturbations by an aggressive multifactorial approach, combining lifestyle advice with pharmacological treatment aiming at optimal blood pressure and lipid control, early detection is of importance (272, 273).

In the future, new treatment options should focus on the underlying pathophysiological mechanisms in patients with CAD and glucose perturbations. In ACS patients, in whom beta-cell dysfunction seems to be an important characteristic, this may be of particular value as indicated by the present findings. Such compounds could hopefully delay T2DM progression and retard the atherosclerotic process by improving insulin secretion and possibly also other mechanisms, especially if initiated soon after the detection of the abnormal glucose

metabolism. In this context pharmacological agents such as GLP-1 analogues or DPP-IV inhibitors seem promising. Along with these there are other alternatives under development e.g. balanced peroxisome proliferator-activated receptor (PPAR) alpha and gamma agonists both targeting insulin sensitivity and lipid profile. The BEGAMI study (**Study V**) was designed as a pilot study for subsequent larger outcome studies investigating DPP-IV inhibitors as a new treatment option in patients with disturbed glucose metabolism and CAD. Pilot trials offer opportunities to study the drug in a smaller population, providing an initial indication if the drug has potentially beneficial effects and an indication whether the drug is safe to study in large cohorts of patients. In addition, the smaller pilot trial usually offers better opportunities for detailed exploration of pathophysiological mechanisms than in multicenter, large-scale trials. The explorative studies have by necessity to be followed by outcome trials focusing on cardiovascular mortality and morbidity to be accepted in patient management. It is hoped that presently ongoing endpoint-driven trials in the area (274, 275) will provide such evidence.

Conclusions

1. Intensified insulin treatment does not reduce the rate of restenosis after a PCI in patients with T2DM. The glucose level prior to the procedure is a predictor of restenosis. To target glucose normalisation during the nearest time period prior to a PCI deserves further exploration.

2. The oral glucose tolerance test is a valid screening tool detecting more patients with glucose abnormalities than fasting plasma glucose and HbA1c in patients with ACS.

3. Continuous glucose monitoring by microdialysis technique appears promising in patients in a coronary care unit but further development of the technology is needed to improve the accuracy.

4. The DPP-IV inhibitor sitagliptin improves beta-cell function in patients with ACS and newly discovered glucose perturbations. The drug may safely be prescribed soon after an acute coronary event.

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References

1. Schneider T. Diabetes through the ages: a salute to insulin. *S Afr Med J* 1972;46:1394-400.
2. Bliss M. The discovery of Insulin. Chicago: University of Chicago Press; 1982.
3. Dobson M. Nature of the urine in diabetes. *Medical Observations and Inquiries* 1776;5:298–310.
4. Brunner JC. Experimenta nova circa pancreas, accedit diatribe de lympha et gemino pancreatis usu. *Amsterdam: Wetstein,* 1683.
5. Bernard C. Mémoire sur le pancréas et sur le rôle du suc pancréatique dans les phénomènes digestifs, particulièrement dans la digestion des matiéres grasses neutres. *Comptes rendus hebdomadaires de l'Académie des sciences* 1856;1:379–563.
6. Bernard C. De suc pancreatique et son role dans phenomenes de las digestion. *C R Soc Biol* 1850;1:99–101.
7. Lancereaux E. Note et re´flexions sur deux cas de diabe`te sucre avec alteration du pancre´as. *Bull Acad Me´d Paris* 1877;6 1215.
8. Langerhans P. Beitrage zur Mikroskopischen Anatomie de Bauchspeicheldrusse. Berlin Inaugural Thesis. 1869.
9. Laguesse E. Sur la formation des ilots de Langerhans dans le pancreas. *Comptes Rendus Hebdomadaires des Seances et Memoires de la Societe de Biologie* 1893;5:819–20.
10. Von Mering J. , Minkowski O. Nach Pancreas Extirpation. *Arch Exp Pathol Pharmakol* 1890;26:371–81.
11. Banting FG. The history of insulin. *Edinburgh Med J* 1929;36:1-18.
12. Paulesco N. *Comptes Rendus des Séances de la Société de Biologie Roumaine* 1921;85:555–9.
13. Banting FG, Best CH, Macleod JJR. The internal secretion of the pancreas (abstract). *Am J Physiol* 1922;59:479.
14. Janbon M, Chaptal J, Vedel A, et al. Accidents hypoglycémiques graves par un sulfamidothiodiazol (le VK 57 ou 2254 RP). *Montpellier Med* 1942;441:21–2.
15. Sterne J. Du Nouveau dans les antidiabétiques. *Maroc Med* 1957;36:1295-6.
16. Campbell IW. Metformin -- life begins at 50: A symposium held on the occasion of the 43rd Annual Meeting of the European Association for the Study of Diabetes, Amsterdam, The Netherlands, September 2007. *Br J Diabetes Vasc Dis* 2007;7:247-52.
17. McIntyre N, Holdsworth CD, Turner DS. NEW INTERPRETATION OF ORAL GLUCOSE TOLERANCE. *Lancet* 1964;2:20-1.
18. Elrick H, Stimmler L, Hlad CJ, Jr., et al. Plasma Insulin Response to Oral and Intravenous Glucose Administration. *J Clin Endocrinol Metab* 1964;24:1076-82.
19. Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986;63:492-8.
20. Dupre J, Ross SA, Watson D, et al. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 1973;37:826-8.

21. Bell GI, Santerre RF, Mullenbach GT. Hamster preproglucagon contains the sequence of glucagon and two related peptides. *Nature* 1983;302:716-8.

22. Heinrich G, Gros P, Lund PK, et al. Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. *Endocrinology* 1984;115:2176-81.

23. Holst JJ, Orskov C, Nielsen OV, et al. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett* 1987;211:169-74.

24. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993;214:829-35.

25. Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 1995;136:3585-96.

26. Exenatide: AC 2993, AC002993, AC2993A, exendin 4, LY2148568. *Drugs R D* 2004;5:35-40.

27. Kolterman OG, Kim DD, Shen L, et al. Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. *Am J Health Syst Pharm* 2005;62:173-81.

28. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997;20:1183-97.

29. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539-53.

30. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2005;28 Suppl 1:S37-42.

31. D.L. Kasper, E. Braunwald, S. Hauser, et al. Harrison's Principles of Internal Medicine, 16th Edition. 2004.

32. Report of a WHO Consultation, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1 Diagnosis and classification of diabetes mellitus. World Health Organization, Department of Noncommunicable Disease Surveillance, Geneva. 1999.

33. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva, Switzerland: World Health Organization. 2006.

34. Genuth S, Alberti KG, Bennett P, et al. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003;26:3160-7.

35. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus, Abbreviated Report of a WHO Consultation 2011.

36. Rydén L, Standl E, Bartnik M, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2007;28:88-136.

37. Laakso M. Understanding patient needs Diabetology for cardiologists. *Eur Heart J* 2003;5 (Suppl B):B5-B13.

38. Mazze RS, Strock ES, Bergenstal RM, et al. Introduction to Staged Diabetes Management. Staged Diabetes Management: Wiley-Blackwell; 2011. p. 166.
39. International Diabetes Federation. IDF Diabetes Atlas, 5th edn. Brussels, Belgium: International Diabetes Federation, 2011. *http://www.idf.org/diabetesatlas*
40. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010;87:4-14.
41. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *Jama* 1979;241:2035-8.
42. Stamler J, Vaccaro O, Neaton JD, et al. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 1993;16:434-44.
43. Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes Association; The National Heart, Lung, and Blood Institute; The Juvenile Diabetes Foundation International; The National Institute of Diabetes and Digestive and Kidney Diseases; and The American Heart Association. *Circulation* 1999;100:1132-3.
44. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009;58:773-95.
45. Dunning BE, Gerich JE. The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. *Endocr Rev* 2007;28:253-83.
46. D'Alessio D. The role of dysregulated glucagon secretion in type 2 diabetes. *Diabetes Obes Metab* 2011;13 Suppl 1:126-32.
47. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 2003;46:3-19.
48. Seino S, Shibasaki T, Minami K. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *J Clin Invest* 2011;121:2118-25.
49. Rorsman P, Eliasson L, Renstrom E, et al. The Cell Physiology of Biphasic Insulin Secretion. *News Physiol Sci* 2000;15:72-7.
50. Curry DL, Bennett LL, Grodsky GM. Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* 1968;83:572-84.
51. Kahn SE. Clinical review 135: The importance of beta-cell failure in the development and progression of type 2 diabetes. *J Clin Endocrinol Metab* 2001;86:4047-58.
52. Brunzell JD, Robertson RP, Lerner RL, et al. Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J Clin Endocrinol Metab* 1976;42:222-9.
53. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia* 2010;53:1270-87.
54. Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of atherosclerosis. *Am J Cardiol* 2007;99:6B-14B.
55. Kahn SE, Zraika S, Utzschneider KM, et al. The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. *Diabetologia* 2009;52:1003-12.
56. Butler AE, Janson J, Bonner-Weir S, et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003;52:102-10.

57. Matveyenko AV, Butler PC. Relationship between beta-cell mass and diabetes onset. *Diabetes Obes Metab* 2008;10 Suppl 4:23-31.

58. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 1989;38:1512-27.

59. Kahn SE, Prigeon RL, McCulloch DK, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 1993;42:1663-72.

60. Gastaldelli A, Ferrannini E, Miyazaki Y, et al. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia* 2004;47:31-9.

61. Godsland IF, Jeffs JA, Johnston DG. Loss of beta cell function as fasting glucose increases in the non-diabetic range. *Diabetologia* 2004;47:1157-66.

62. Knowler WC, Pettitt DJ, Saad MF, et al. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 1990;6:1-27.

63. Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. *Diabetologia* 2001;44:929-45.

64. Ferrannini E, Gastaldelli A, Miyazaki Y, et al. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab* 2005;90:493-500.

65. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes* 1995;44:1249-58.

66. Robertson RP, Harmon J, Tran PO, et al. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 2003;52:581-7.

67. Poitout V, Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes--a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 2002;143:339-42.

68. Wajchenberg BL. beta-cell failure in diabetes and preservation by clinical treatment. *Endocr Rev* 2007;28:187-218.

69. Westermark P, Andersson A, Westermark GT. Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. *Physiol rev* 2011;91:795-826.

70. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.

71. Ward WK, LaCava EC, Paquette TL, et al. Disproportionate elevation of immunoreactive proinsulin in type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. *Diabetologia* 1987;30:698-702.

72. Tura A, Pacini G, Kautzky-Willer A, et al. Basal and dynamic proinsulin-insulin relationship to assess beta-cell function during OGTT in metabolic disorders. *Am J Physiol Endocrinol Metabol* 2003;285:E155-62.

73. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979;237:E214-23.

74. Seltzer HS, Allen EW, Herron AL, Jr., et al. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Invest* 1967;46:323-35.

75. Mari A, Pacini G, Murphy E, et al. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 2001;24:539-48.
76. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-70.
77. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 2000;23:295-301.
78. Global atlas on cardiovascular disease prevention and control; World Health Organisation World Heart Federation; World Stroke Organization. 2011.
79. European cardiovascular disease statistics 2008; http://www.bhf.org.uk/publications/view-publication.aspx?ps=1001443.
80. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *J Am Coll Cardiol* 2007;50:2173-95.
81. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *Eur Heart J* 2000;21:1502-13.
82. Wijns W, Kolh P, Danchin N, et al. Guidelines on myocardial revascularization. *Eur Heart J* 2010;31:2501-55.
83. Gruntzig A. Transluminal dilatation of coronary-artery stenosis. *Lancet* 1978;1:263.
84. Scott NA. Restenosis following implantation of bare metal coronary stents: pathophysiology and pathways involved in the vascular response to injury. *Adv Drug Deliv Rev* 2006;58:358-76.
85. Jukema JW, Verschuren JJ, Ahmed TA, et al. Restenosis after PCI. Part 1: pathophysiology and risk factors. *Nat Rev Cardiol* 2012;9:53-62
86. Libby P, Schwartz D, Brogi E, et al. A cascade model for restenosis. A special case of atherosclerosis progression. *Circulation* 1992;86:III47-52.
87. Hegglin P. Über Kreislaufprobleme bei gestörtem Zuckerstoffwechsel, insbesondere im Coma diabeticum. *Basic Res Cardiol* 1940:1-53.
88. Mayer J. On Diabetes and its Connection with Heart Disease. *Br Med J* 1888;1:949-51.
89. Blotner H. Coronary disease in diabetes mellitus. *N Engl J Med* 1930:709
90. Millard EB, Root HF. Degenerative vascular lesions and diabetes mellitus. *Am J Dig Dis* 1948;15:41-51.
91. Smith KS. Cardiac Syndromes Complicating Diabetes and Their Treatment. *Br Heart J* 1943;5:1-7.
92. Norhammar AM, Rydén L, Malmberg K. Admission plasma glucose. Independent risk factor for long-term prognosis after myocardial infarction even in nondiabetic patients. *Diabetes Care* 1999;22:1827-31.
93. Angeli F, Verdecchia P, Karthikeyan G, et al. New-onset hyperglycemia and acute coronary syndrome: a systematic overview and meta-analysis. *Curr Diabetes Rev* 2010;6:102-10.
94. Sinnaeve PR, Steg PG, Fox KA, et al. Association of elevated fasting glucose with increased short-term and 6-month mortality in ST-segment elevation and non-ST-segment elevation acute coronary syndromes: the Global Registry of Acute Coronary Events. *Arch Intern Med* 2009;169:402-9.

95. Coutinho M, Gerstein HC, Wang Y, et al. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 1999;22:233-40.

96. Malmberg K, Rydén L. Myocardial infarction in patients with diabetes mellitus. *Eur Heart J* 1988;9:259-64.

97. Norhammar A, Tenerz A, Nilsson G, et al. Glucose metabolism in patients with acute myocardial infarction and no previous diagnosis of diabetes mellitus: a prospective study. *Lancet* 2002;359:2140-4.

98. Bartnik M, Rydén L, Ferrari R, et al. The prevalence of abnormal glucose regulation in patients with coronary artery disease across Europe. The Euro Heart Survey on diabetes and the heart. *Eur Heart J* 2004;25:1880-90.

99. Hu DY, Pan CY, Yu JM. The relationship between coronary artery disease and abnormal glucose regulation in China: the China Heart Survey. *Eur Heart J* 2006;27:2573-9.

100. Norhammar A, Lindback J, Rydén L, et al. Improved but still high short- and long-term mortality rates after myocardial infarction in patients with diabetes mellitus: a time-trend report from the Swedish Register of Information and Knowledge about Swedish Heart Intensive Care Admission. *Heart* 2007;93:1577-83.

101. Bartnik M, Malmberg K, Norhammar A, et al. Newly detected abnormal glucose tolerance: an important predictor of long-term outcome after myocardial infarction. *Eur Heart J* 2004;25:1990-7.

102. Tamita K, Katayama M, Takagi T, et al. Impact of newly diagnosed abnormal glucose tolerance on long-term prognosis in patients with acute myocardial infarction. *Circ J* 2007;71:834-41.

103. Lenzen M, Rydén L, Öhrvik J, et al. Diabetes known or newly detected, but not impaired glucose regulation, has a negative influence on 1-year outcome in patients with coronary artery disease: a report from the Euro Heart Survey on diabetes and the heart. *Eur Heart J* 2006;27:2969-74.

104. Bartnik M, Malmberg K, Hamsten A, et al. Abnormal glucose tolerance--a common risk factor in patients with acute myocardial infarction in comparison with population-based controls. *J Intern Med* 2004;256:288-97.

105. Wallander M, Bartnik M, Efendic S, et al. Beta cell dysfunction in patients with acute myocardial infarction but without previously known type 2 diabetes: a report from the GAMI study. *Diabetologia* 2005;48:2229-35.

106. Creager MA, Luscher TF, Cosentino F, et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 2003;108:1527-32.

107. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813-20.

108. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet* 2008;371:1800-9.

109. Milman S, Crandall JP. Mechanisms of vascular complications in prediabetes. *Med Clin North Am* 2011;95:309-25, vii.

110. Norhammar A, Lagerqvist B, Saleh N. Long-term mortality after PCI in patients with diabetes mellitus: results from the Swedish Coronary Angiography and Angioplasty Registry. *EuroIntervention* 2010;5:891-7.

111. Muhlestein JB, Anderson JL, Horne BD, et al. Effect of fasting glucose levels on mortality rate in patients with and without diabetes mellitus and coronary artery disease undergoing percutaneous coronary intervention. *Am Heart J* 2003;146:351-8.

112. Worthley MI, Shrive FM, Anderson TJ, et al. Prognostic implication of hyperglycemia in myocardial infarction and primary angioplasty. *Am J Med* 2007;120:643 e1-7.

113. Cutlip DE, Chauhan MS, Baim DS, et al. Clinical restenosis after coronary stenting: perspectives from multicenter clinical trials. *J Am Coll Cardiol* 2002;40:2082-9.

114. Aronson D, Edelman ER. Revascularization for coronary artery disease in diabetes mellitus: angioplasty, stents and coronary artery bypass grafting. *Rev Endocr Metab Disord* 2010;11:75-86.

115. West NE, Ruygrok PN, Disco CM, et al. Clinical and angiographic predictors of restenosis after stent deployment in diabetic patients. *Circulation* 2004;109:867-73.

116. Abizaid A, Costa MA, Centemero M, et al. Clinical and economic impact of diabetes mellitus on percutaneous and surgical treatment of multivessel coronary disease patients: insights from the Arterial Revascularization Therapy Study (ARTS) trial. *Circulation* 2001;104:533-8.

117. Frobert O, Lagerqvist B, Carlsson J, et al. Differences in restenosis rate with different drug-eluting stents in patients with and without diabetes mellitus: a report from the SCAAR (Swedish Angiography and Angioplasty Registry). *J Am Coll Cardiol* 2009;53:1660-7.

118. Ma S, Yang D, Zhang X, et al. Comparison of restenosis rate with sirolimus-eluting stent in STEMI patients with and without diabetes at 6-month angiographic follow-up. *Acta Cardiol* 2011;66:603-6.

119. Natarajan R, Nadler JL. Lipid inflammatory mediators in diabetic vascular disease. *Arterioscler Thromb Vasc Biol* 2004;24:1542-8.

120. King DE, Mainous AG, 3rd, Buchanan TA, et al. C-reactive protein and glycemic control in adults with diabetes. *Diabetes Care* 2003;26:1535-9.

121. Aronson D, Bloomgarden Z, Rayfield EJ. Potential mechanisms promoting restenosis in diabetic patients. *J Am Coll Cardiol* 1996;27:528-35.

122. Malmberg K, Rydén L, Efendic S, et al. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. *J Am Coll Cardiol* 1995;26:57-65.

123. Malmberg K. Prospective randomised study of intensive insulin treatment on long term survival after acute myocardial infarction in patients with diabetes mellitus. DIGAMI (Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction) Study Group. *BMJ* 1997;314:1512-5.

124. Malmberg K, Rydén L, Wedel H, et al. Intense metabolic control by means of insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2): effects on mortality and morbidity. *Eur Heart J* 2005;26:650-61.

125. Cheung NW, Wong VW, McLean M. The Hyperglycemia: Intensive Insulin Infusion in Infarction (HI-5) study: a randomized controlled trial of insulin infusion therapy for myocardial infarction. *Diabetes Care* 2006;29:765-70.

126. Heller SR. Cardiac arrhythmias in hypoglycaemia. *Diabetes Nutr Metab* 2002;15:461-5; discussion 465-7.

127. Desouza C, Salazar H, Cheong B, et al. Association of hypoglycemia and cardiac ischemia: a study based on continuous monitoring. *Diabetes Care* 2003;26:1485-9.

128. Vetter NJ, Strange RC, Adams W, et al. Initial metabolic and hormonal response to acute myocardial infarction. *Lancet* 1974;1:284-8.

129. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006;295:1681-7.

130. Ceriello A, Ihnat MA. 'Glycaemic variability': a new therapeutic challenge in diabetes and the critical care setting. *Diabet Med* 2010;27:862-7.

131. Choi SW, Benzie IF, Ma SW, et al. Acute hyperglycemia and oxidative stress: direct cause and effect? *Free Radic Biol Med* 2008;44:1217-31.

132. Wade AO, Cordingley JJ. Glycaemic control in critically ill patients with cardiovascular disease. *Curr Opin Crit Care* 2006;12:437-43.

133. De Block C, Manuel YKB, Van Gaal L, et al. Intensive insulin therapy in the intensive care unit: assessment by continuous glucose monitoring. *Diabetes Care* 2006;29:1750-6.

134. McMullin J, Brozek J, McDonald E, et al. Lowering of glucose in critical care: a randomized pilot trial. *J Crit Care* 2007;22:112-8; discussion 118-9.

135. Aragon D. Evaluation of nursing work effort and perceptions about blood glucose testing in tight glycemic control. *Am J Crit Care* 2006;15:370-7.

136. Holman RR, Paul SK, Bethel MA, et al. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008;359:1577-89.

137. Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358:2560-72.

138. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008;358:2545-59.

139. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009;360:129-39.

140. Mellbin L, Malmberg K, Rydén L. Effects of glucose-lowering drugs in patients with diabetes and myocardial infarction. *Diabetes Management* 2011;1:281-9.

141. Anselmino M, Ohrvik J, Malmberg K, et al. Glucose lowering treatment in patients with coronary artery disease is prognostically important not only in established but also in newly detected diabetes mellitus: a report from the Euro Heart Survey on Diabetes and the Heart. *Eur Heart J* 2008;29:177-84.

142. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987;79:616-9.

143. Kreymann B, Williams G, Ghatei MA, et al. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987;2:1300-4.

144. Leech CA, Dzhura I, Chepurny OG, et al. Molecular physiology of glucagon-like peptide-1 insulin secretagogue action in pancreatic beta cells. *Prog Biophys Mol Biol* 2011;107:236-47.

145. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006;368:1696-705.

146. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007;132:2131-57.

147. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007;87:1409-39.

148. Ban K, Noyan-Ashraf MH, Hoefer J, et al. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. *Circulation* 2008;117:2340-50.

149. Vahl TP. Effects of GLP-1-(7-36)NH2, GLP-1-(7-37), and GLP-1- (9-36)NH2 on Intravenous Glucose Tolerance and Glucose-Induced Insulin Secretion in Healthy Humans. *J Clin Endocrinol Metab* 2003;88:1772-9.

150. Xu G, Stoffers DA, Habener JF, et al. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 1999;48:2270-6.

151. Perfetti R, Zhou J, Doyle ME, et al. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* 2000;141:4600-5.

152. Farilla L. Glucagon-Like Peptide-1 Promotes Islet Cell Growth and Inhibits Apoptosis in Zucker Diabetic Rats. *Endocrinology* 2002;143:4397-408.

153. Farilla L, Bulotta A, Hirshberg B, et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003;144:5149-58.

154. Perfetti R, Hui H. The role of GLP-1 in the life and death of pancreatic beta cells. *Horm Metab Res* 2004;36:804-10.

155. Nauck M, Stockmann F, Ebert R, et al. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986;29:46-52.

156. Ahren B, Carr RD, Deacon CF. Incretin hormone secretion over the day. *Vitam Horm* 2010;84:203-20.

157. Nauck MA, Vardarli I, Deacon CF, et al. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 2011;54:10-8.

158. Holst JJ, Knop FK, Vilsboll T, et al. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. *Diabetes Care* 2011;34 Suppl 2:S251-7.

159. Nauck MA, Heimesaat MM, Orskov C, et al. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 1993;91:301-7.

160. Lotfy M, Singh J, Kalasz H, et al. Medicinal Chemistry and Applications of Incretins and DPP-4 Inhibitors in the Treatment of Type 2 Diabetes Mellitus. *Open Med Chem J* 2011;5:82-92.

161. Fehse F, Trautmann M, Holst JJ, et al. Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2005;90:5991-7.

162. Moretto TJ, Milton DR, Ridge TD, et al. Efficacy and tolerability of exenatide monotherapy over 24 weeks in antidiabetic drug-naive patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* 2008;30:1448-60.

163. Mari A, Nielsen LL, Nanayakkara N, et al. Mathematical modeling shows exenatide improved beta-cell function in patients with type 2 diabetes treated with metformin or metformin and a sulfonylurea. *Horm Metab Res* 2006;38:838-44.

164. Degn KB, Juhl CB, Sturis J, et al. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 2004;53:1187-94.

165. Cernea S, Raz I. Therapy in the early stage: incretins. *Diabetes Care* 2011;34 Suppl 2:S264-71.

166. Dicembrini I, Pala L, Rotella CM. From Theory to Clinical Practice in the Use of GLP-1 Receptor Agonists and DPP-4 Inhibitors Therapy. *Exp Diabetes Res* 2011;2011:898913.

167. Herman GA, Bergman A, Stevens C, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2006;91:4612-9.

168. Sjöholm A. Impact of glucagon-like peptide-1 on endothelial function. *Diabetes Obes Metab* 2009;11 Suppl 3:19-25.

169. Anagnostis P, Athyros VG, Adamidou F, et al. Glucagon-like peptide-1-based therapies and cardiovascular disease: looking beyond glycaemic control. *Diabetes Obes Metab* 2011;13:302-12.

170. Grieve DJ, Cassidy RS, Green BD. Emerging cardiovascular actions of the incretin hormone glucagon-like peptide-1: potential therapeutic benefits beyond glycaemic control? *Br J Pharmacol* 2009;157:1340-51.

171. Okerson T, Chilton RJ. The Cardiovascular Effects of GLP-1 Receptor Agonists. *Cardiovasc Ther* 2010 (Epub ahead of print) doi: 10.1111/j.1755-5922.2010.00256.x.

172. Treiman M, Elvekjaer M, Engstrom T, et al. Glucagon-like peptide 1--a cardiologic dimension. *Trends Cardiovasc Med* 2010;20:8-12.

173. Verges B, Bonnard C, Renard E. Beyond glucose lowering: Glucagon-like peptide-1 receptor agonists, body weight and the cardiovascular system. *Diabetes Metab* 2011 (Epub ahead of print) doi: 10.1016/j.diabet.2011.07.001

174. Okerson T, Yan P, Stonehouse A, et al. Effects of exenatide on systolic blood pressure in subjects with type 2 diabetes. *Am J Hypertens* 2010;23:334-9.

175. Nystrom T, Gutniak MK, Zhang Q, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004;287:E1209-15.

176. Thrainsdottir I, Malmberg K, Olsson A, et al. Initial experience with GLP-1 treatment on metabolic control and myocardial function in patients with type 2 diabetes mellitus and heart failure. *Diab Vasc Dis Res* 2004;1:40-3.

177. Sokos GG, Nikolaidis LA, Mankad S, et al. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J Card Fail* 2006;12:694-9.

178. Nikolaidis LA, Mankad S, Sokos GG, et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. *Circulation* 2004;109:962-5.

179. Sokos GG, Bolukoglu H, German J, et al. Effect of glucagon-like peptide-1 (GLP-1) on glycemic control and left ventricular function in patients undergoing coronary artery bypass grafting. *Am J Cardiol* 2007;100:824-9.

180. Sonne DP, Engstrom T, Treiman M. Protective effects of GLP-1 analogues exendin-4 and GLP-1(9-36) amide against ischemia-reperfusion injury in rat heart. *Regul pept* 2008;146:243-9.

181. Davidson MH. Cardiovascular effects of glucagonlike peptide-1 agonists. *Am J Cardiol* 2011;108:33B-41B.

182. Lonborg J, Vejlstrup N, Kelbaek H, et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 2011 (Epub ahead of print) doi: 10.1093/eurheartj/ehr309

183. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979;28:1039-57.

184. International Organization for Standardization; In vitro diagnostic test systems: Requirements fot blood-glucose monitoring systems for self-testing in managing diabetes mellitus. Geneva (ISO publ no 15197) 2003.

185. Clarke WL, Cox D, Gonder-Frederick LA, et al. Evaluating clinical accuracy of systems for self-monitoring of blood glucose. *Diabetes Care* 1987;10:622-8.

186. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. *Lancet* 1999;354:617-21.

187. Nakagami T. Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. *Diabetologia* 2004;47:385-94.

188. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007;116:151-7.

189. Capes SE, Hunt D, Malmberg K, et al. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet* 2000;355:773-8.

190. Sarwar N, Gao P, Seshasai SR, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010;375:2215-22.

191. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993;329:977-86.

192. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 2003;290:2159-67.

193. Corpus RA, George PB, House JA, et al. Optimal glycemic control is associated with a lower rate of target vessel revascularization in treated type II diabetic patients undergoing elective percutaneous coronary intervention. *J Am Coll Cardiol* 2004;43:8-14.

194. Nusca A, Patti G, Marino F, et al. Prognostic role of preprocedural glucose levels on short- and long-term outcome in patients undergoing percutaneous coronary revascularization. *Catheter Cardiovasc Interv* 2011 (Epub ahead of print) doi:10.1002/ccd.23185

195. Dandona P, Chaudhuri A, Ghanim H, et al. Proinflammatory effects of glucose and anti-inflammatory effect of insulin: relevance to cardiovascular disease. *Am J Cardiol* 2007;99:15B-26B.

196. Breen DM, Giacca A. Effects of insulin on the vasculature. *Curr Vasc Pharmacol* 2011;9:321-32.

197. Natarajan MK, Strauss BH, Rokoss M, et al. Randomized trial of insulin versus usual care in reducing restenosis after coronary intervention in patients with diabetes. the STent Restenosis And Metabolism (STREAM) study. *Cardiovasc Revasc Med* 2012 (Epub ahead of print) doi: 10.1016/j.carrev.2011.12.001

198. Chiasson JL, Josse RG, Gomis R, et al. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA* 2003;290:486-94.

199. Raz I, Wilson PW, Strojek K, et al. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. *Diabetes Care* 2009;32:381-6.

200. Mellbin LG, Malmberg K, Norhammar A, et al. Prognostic implications of glucose-lowering treatment in patients with acute myocardial infarction and diabetes: experiences from an extended follow-up of the Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) 2 Study. *Diabetologia* 2011;54:1308-17.

201. De Luca L, De Persio G, Minati M, et al. Effects of abciximab and preprocedural glycemic control in diabetic patients undergoing elective coronary stenting. *Am Heart J* 2005;149:1135.

202. Lindsay J, Sharma AK, Canos D, et al. Preprocedure hyperglycemia is more strongly associated with restenosis in diabetic patients after percutaneous coronary intervention than is hemoglobin A1C. *Cardiovasc Revasc Med* 2007;8:15-20.

203. Zhao YT, Weng CL, Chen ML, et al. Comparison of glucose-insulin-potassium and insulin-glucose as adjunctive therapy in acute myocardial infarction: a contemporary meta-analysis of randomised controlled trials. *Heart* 2010;96:1622-6.

204. Pinto DS, Kirtane AJ, Pride YB, et al. Association of blood glucose with angiographic and clinical outcomes among patients with ST-segment elevation myocardial infarction (from the CLARITY-TIMI-28 study). *Am J Cardiol* 2008;101:303-7.

205. Pinto DS, Skolnick AH, Kirtane AJ, et al. U-shaped relationship of blood glucose with adverse outcomes among patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 2005;46:178-80.

206. Svensson AM, McGuire DK, Abrahamsson P, et al. Association between hyper- and hypoglycaemia and 2 year all-cause mortality risk in diabetic patients with acute coronary events. *Eur Heart J* 2005;26:1255-61.

207. Mellbin LG, Malmberg K, Waldenstrom A, et al. Prognostic implications of hypoglycemic episodes during hospitalisation for myocardial infarction in patients with type 2 diabetes. A report from the DIGAMI 2 trial. *Heart* 2009;95:721-7.

208. Kosiborod M, Inzucchi SE, Goyal A, et al. Relationship between spontaneous and iatrogenic hypoglycemia and mortality in patients hospitalized with acute myocardial infarction. *JAMA* 2009;301:1556-64.

209. Meynaar IA, Eslami S, Abu-Hanna A, et al. Blood glucose amplitude variability as predictor for mortality in surgical and medical intensive care unit patients: a multicenter cohort study. *J Crit Care* 2012 (Epub ahead of print) doi: 10.1016/j.jcrc.2011.11.004

210. Dossett LA, Cao H, Mowery NT, et al. Blood glucose variability is associated with mortality in the surgical intensive care unit. *Am Surg* 2008;74:679-85; discussion 85.

211. Hermanides J, Vriesendorp TM, Bosman RJ, et al. Glucose variability is associated with intensive care unit mortality. *Crit Care Med* 2010;38:838-42.

212. Monteiro S, Goncalves F, Monteiro P, et al. The magnitude of the variation in glycemia: a new parameter for risk assessment in acute coronary syndrome? *Rev Esp Cardiol* 2009;62:1099-108.

213. Egi M, Bellomo R, Stachowski E, et al. Variability of blood glucose concentration and short-term mortality in critically ill patients. *Anesthesiology* 2006;105:244-52.

214. Rowen M, Schneider DJ, Pratley RE, et al. On rendering continuous glucose monitoring ready for prime time in the cardiac care unit. *Coron Artery Dis* 2007;18:405-9.

215. Hermanides J, Engstrom AE, Wentholt IM, et al. Sensor-augmented insulin pump therapy to treat hyperglycemia at the coronary care unit: a randomized clinical pilot trial. *Diabetes Technol Ther* 2010;12:537-42.

216. Selvin E, Crainiceanu CM, Brancati FL, et al. Short-term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med* 2007;167:1545-51.

217. Lapolla A, Mosca A, Fedele D. The general use of glycated haemoglobin for the diagnosis of diabetes and other categories of glucose intolerance: still a long way to go. *Nutr Metab Cardiovasc Dis* 2011;21:467-75.

218. Qiao Q, Nakagami T, Tuomilehto J, et al. Comparison of the fasting and the 2-h glucose criteria for diabetes in different Asian cohorts. *Diabetologia* 2000;43:1470-5.

219. Will new diagnostic criteria for diabetes mellitus change phenotype of patients with diabetes? Reanalysis of European epidemiological data. DECODE Study Group on behalf of the European Diabetes Epidemiology Study Group. *BMJ* 1998;317:371-5.

220. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. *Diabetes Care* 2010;33:562-8.

221. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010;33 Suppl 1:S62-9.

222. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 2009;32:1327-34.

223. Balion CM, Raina PS, Gerstein HC, et al. Reproducibility of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) classification: a systematic review. *Clin Chem Lab Med* 2007;45:1180-5.

224. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. *Arch Intern Med* 2001;161:397-405.

225. Berne C, Agardh C-D, editors. Diabetes. Stockholm: Liber AB; 2010.

226. Choi KM, Lee KW, Kim SG, et al. Inflammation, insulin resistance, and glucose intolerance in acute myocardial infarction patients without a previous diagnosis of diabetes mellitus. *J Clin Endocrinol Metab* 2005;90:175-80.

227. Lankisch M, Futh R, Gulker H, et al. Screening for undiagnosed diabetes in patients with acute myocardial infarction. *Clin Res Cardiol* 2008;97:753-9.

228. Knudsen EC, Seljeflot I, Abdelnoor M, et al. Abnormal glucose regulation in patients with acute ST- elevation myocardial infarction-a cohort study on 224 patients. *Cardiovasc Diabetol* 2009;8:6.

229. Jimenez-Navarro MF, Garcia-Pinilla JM, Garrido-Sanchez L, et al. Poor reproducibility of the oral glucose tolerance test in the diagnosis of diabetes during percutaneous coronary intervention. *Int J Cardiol* 2010;142:245-9.

230. Srinivas-Shankar U, Somauroo JD, Delduca AM, et al. Temporal change in glucose tolerance in non-ST-elevation myocardial infarction. *Diabetes Res Clin Pract* 2008;82:310-6.

231. Bronisz A, Kozinski M, Magielski P, et al. Value of oral glucose tolerance test in the acute phase of myocardial infarction. *Cardiovasc Diabetol* 2011;10:21.

232. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393-403.

233. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344:1343-50.

234. Chiasson JL, Josse RG, Gomis R, et al. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* 2002;359:2072-7.

235. DeFronzo RA, Abdul-Ghani MA. Preservation of beta-cell function: the key to diabetes prevention. *J Clin Endocrinol Metab* 2011;96:2354-66.

236. Mellbin LG, Malmberg K, Norhammar A, et al. The impact of glucose lowering treatment on long-term prognosis in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. *Eur Heart J* 2008;29:166-76.

237. Jorgensen CH, Gislason GH, Andersson C, et al. Effects of oral glucose-lowering drugs on long term outcomes in patients with diabetes mellitus following myocardial infarction not treated with emergent percutaneous coronary intervention--a retrospective nationwide cohort study. *Cardiovasc Diabetol* 2010;9:54.

238. Schramm TK, Gislason GH, Vaag A, et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *Eur Heart J* 2011;32:1900-8.

239. Standards of medical care in diabetes--2012. *Diabetes Care* 2012;35 Suppl 1:S11-63.

240. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007;356:2457-71.

241. Nissen SE, Wolski K. Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. *Arch Intern Med* 2010;170:1191-201.

242. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006;355:2427-43.

243. Mu J, Woods J, Zhou YP, et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. *Diabetes* 2006;55:1695-704.

244. Han SJ, Choi SE, Kang Y, et al. Effect of sitagliptin plus metformin on beta-cell function, islet integrity and islet gene expression in Zucker diabetic fatty rats. *Diabetes Res Clin Pract* 2011;92:213-22.

245. Maiztegui B, Borelli MI, Madrid VG, et al. Sitagliptin prevents the development of metabolic and hormonal disturbances, increased beta-cell apoptosis and liver steatosis induced by a fructose-rich diet in normal rats. *Clin Sci (Lond)* 2011;120:73-80.

246. Shirakawa J, Amo K, Ohminami H, et al. Protective effects of a dipeptidyl peptidase-4 (DPP-4) inhibitor against increased {beta} cell apoptosis induced by dietary sucrose and linoleic acid in mice with diabetes. *J Biol Chem* 2011;286:25467-76.

247. Charbonnel B, Karasik A, Liu J, et al. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care* 2006;29:2638-43.

248. Rosenstock J, Brazg R, Andryuk PJ, et al. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* 2006;28:1556-68.

249. Aschner P, Kipnes MS, Lunceford JK, et al. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care* 2006;29:2632-7.

250. Raz I, Hanefeld M, Xu L, et al. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. *Diabetologia* 2006;49:2564-71.

251. Aaboe K, Knop FK, Vilsboll T, et al. Twelve weeks treatment with the DPP-4 inhibitor, sitagliptin, prevents degradation of peptide YY and improves glucose and non-glucose induced insulin secretion in patients with type 2 diabetes mellitus. *Diabetes Obes Metab* 2010;12:323-33.

252. Mohan V, Yang W, Son HY, et al. Efficacy and safety of sitagliptin in the treatment of patients with type 2 diabetes in China, India, and Korea. *Diabetes Res Clin Pract* 2009;83:106-16.

253. Xu L, Man CD, Charbonnel B, et al. Effect of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on beta-cell function in patients with type 2 diabetes: a model-based approach. *Diabetes Obes Metab* 2008;10:1212-20.

254. Riche DM, East HE, Riche KD. Impact of sitagliptin on markers of beta-cell function: a meta-analysis. *Am J Med Sci* 2009;337:321-8.

255. van Genugten RE, van Raalte DH, Diamant M. Dipeptidyl peptidase-4 inhibitors and preservation of pancreatic islet-cell function: a critical appraisal of the evidence. *Diabetes Obes Metab* 2012;14:101-11.

256. Migoya EM, Bergeron R, Miller JL, et al. Dipeptidyl peptidase-4 inhibitors administered in combination with metformin result in an additive increase in the plasma concentration of active GLP-1. *Clin Pharmacol Ther* 2010;88:801-8.

257. Bunck MC, Corner A, Eliasson B, et al. Effects of exenatide on measures of beta-cell function after 3 years in metformin-treated patients with type 2 diabetes. *Diabetes care* 2011;34:2041-7.

258. Williams-Herman D, Xu L, Teng R, et al. Effect of initial combination therapy with sitagliptin and metformin on beta-cell function in patients with type 2 diabetes. *Diabetes Obes Metab* 2012;14:67-76.

259. Utzschneider KM, Tong J, Montgomery B, et al. The dipeptidyl peptidase-4 inhibitor vildagliptin improves beta-cell function and insulin sensitivity in subjects with impaired fasting glucose. *Diabetes Care* 2008;31:108-13.

260. Cox ME, Rowell J, Corsino L, et al. Dipeptidyl peptidase-4 inhibitors in the management of type 2 diabetes: safety, tolerability, and efficacy. *Drug Healthc Patient Saf* 2010;2:7-19.

261. Williams-Herman D, Engel SS, Round E, et al. Safety and tolerability of sitagliptin in clinical studies: a pooled analysis of data from 10,246 patients with type 2 diabetes. *BMC Endocr Disord* 2010;10:7.

262. Kao DP, Kohrt HE, Kugler J. Renal failure and rhabdomyolysis associated with sitagliptin and simvastatin use. *Diabet Med* 2008;25:1229-30.

263. Bergman AJ, Cote J, Maes A, et al. Effect of sitagliptin on the pharmacokinetics of simvastatin. *J Clin Pharmacol* 2009;49:483-8.

264. DiGregorio RV, Pasikhova Y. Rhabdomyolysis caused by a potential sitagliptin-lovastatin interaction. *Pharmacotherapy* 2009;29:352-6.

265. Boucher BJ. Renal failure and rhabdomyolysis associated with sitagliptin and simvastatin use. But what about the amiodarone? *Diabet Med* 2009;26:192-3.

266. Skalli S, Wion-Barbot N, Baudrant M, et al. Angio-oedema induced by dual dipeptidyl peptidase inhibitor and angiotensin II receptor blocker: a first case report. *Diabet Med* 2010;27:486-7.

267. Fadini GP, Boscaro E, Albiero M, et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. *Diabetes Care* 2010;33:1607-9.

268. Monami M, Lamanna C, Desideri CM, et al. DPP-4 Inhibitors and Lipids: Systematic Review and Meta-Analysis. *Adv Ther* 2012;29:14-25.

269. Tremblay AJ, Lamarche B, Deacon CF, et al. Effect of sitagliptin therapy on postprandial lipoprotein levels in patients with type 2 diabetes. *Diabetes Obes Metab* 2011;13:366-73.

270. Read PA, Khan FZ, Heck PM, et al. DPP-4 Inhibition by Sitagliptin Improves the Myocardial Response to Dobutamine Stress and Mitigates Stunning in a Pilot Study of Patients with Coronary Artery Disease. *Circ Cardiovasc Imaging* 2010;3:195-201.

271. Monami M, Dicembrini I, Martelli D, et al. Safety of dipeptidyl peptidase-4 inhibitors: a meta-analysis of randomized clinical trials. *Curr Med Res Opin* 2011;27 Suppl 3:57-64.

272. Anselmino M, Malmberg K, Ohrvik J, et al. Evidence-based medication and revascularization: powerful tools in the management of patients with diabetes and coronary artery disease: a report from the Euro Heart Survey on diabetes and the heart. *Eur J Cardiovasc Prev Rehabil* 2008;15:216-23.

273. Gaede P, Lund-Andersen H, Parving HH, et al. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008;358:580-91.

274. TECOS. *(Sitagliptin Cardiovascular Outcome Study)*:ClinicalTrials.gov Identifier: NCT00790205.

275. EXAMINE. *(EXamination of CArdiovascular OutcoMes: AlogliptIN vs Standard of CarE in Patients with Type 2 Diabetes Mellitus and Acute Coronary Syndrome)*:ClinicalTrials.gov Identifier: NCT00968708.