Myocardial infarction and diabetes mellitus

Studies on glucose lowering therapies and novel risk markers based on observations from the DIGAMI 2 trial

by

Linda Garcia Mellbin

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To the memory of my grandparents

Birgit and Rolf
Sezaltina and Fransisco

“Mudam-se os tempos, mudam-se as vontades”
“Times change, wills change”

Luís Vaz de Camões (1524-1580)
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ABSTRACT

Background
Patients with myocardial infarction (MI) and type 2 diabetes (T2DM) and have a poor prognosis. Hyperglycemia is an independent risk predictor. The best tools for glucose control are debated. Important is identification of biomarkers to gain further pathophysiological insights and new therapeutic possibilities.

Aims
In patients with acute MI and T2DM
1. Explore the prognostic impact of hypoglycemia during hospitalization for MI
2. Study the prognostic impact of glucose lowering treatment
3. Investigate the relation between Copeptin and IGFBP-1 and their prognostic impact
4. Characterize MBL geno- and phenotypes and to investigate their prognostic importance

Study population
This thesis is based on epidemiological reports from the DIGAMI 2 trial comprising 1253 patients with T2DM and acute MI. DIGAMI 2 was a randomized trial with the primary aim to compare three glucose lowering strategies testing the hypothesis that insulin-based metabolic control reduces mortality.

Hypoglycemia during hospitalization for acute MI
Hypoglycemic episodes were recorded in 153 patients (symptomatic = 45). Patients with hypoglycemia were older, had a longer duration of T2DM, a lower body weight and more often a history of heart failure. The mortality and cardiovascular morbidity did not differ between patients with or without hypoglycemia besides that patients who were symptomatic were at increased risk of death. This higher risk disappeared after adjustment for confounding factors.

Glucose lowering treatment and prognosis
During the initial follow-up of 2.3 years the adjusted hazard ratio (HR) for non-fatal MI and stroke, in patients discharged alive (n=1181), was 1.73 (95% CI 1.26–2.37; p =0.0007) with insulin treatment, 0.81 (95% CI 0.57–1.14; p = 0.23) with sulphonylureas and 0.63 (95% CI 0.42–0.95; p = 0.03) with metformin. None of the glucose lowering treatments influenced mortality. The odds ratio for insulin on non-fatal cardiovascular events was 1.90 (95% CI 1.38-2.63; p=<0.0001) without influencing mortality after an extended follow-up period of 4.1 years. Metformin was associated with a lower mortality and a lower risk of death of malignancies. There were no difference in total or cardiovascular mortality between the randomized treatment groups but the risk of dying of malignancies was highest in patients randomized to long-term insulin.

Novel risk markers and prognosis
Copeptin, a surrogate marker for vasopressin, was associated with IGFBP-1 (r = 0.53; p<0.001) in 393 patients participating in the biochemical program of DIGAMI 2. Both biomarkers were predictors of events (cardiovascular death, MI and stroke) in univariate analyses. In the final statistical model, adjusting for age and renal function, copeptin was the only independent predictor (HR 1.35; 95% CI: 1.16-1.57; p<0.001).

Serum (S)-MBL, an activator of the complement system, was determined in 387 and MBL genotypes in 287 patients. Fifty four percent had high coding (median S-MBL=2658 µg/l; IQR 1715 – 3829) and 46% low coding MBL genotype (median S-MBL=373µg/l; IQR 100-765). S-MBL did not predict events. The risk of events was lower in patients with high genotype and S-MBL above median for their genotype (HR 0.49; 95%C=0.26-0.92; p= 0.026) than among patients with low genotype and S-MBL below median for their genotype. This relation did, however, only reach borderline significance in adjusted analyses.

Conclusions
Hypoglycemia during hospitalization is not an independent risk factor for mortality and cardiovascular morbidity in patients with T2DM and MI. It is more prevalent in patient at high risk for other reasons. Glucose lowering agents seem to impact cardiovascular morbidity, mortality and deaths from malignancies, a finding that deserves further evaluation. Copeptin may explain at least some of the prognostic impact of IGFBP-1 in these patients an observation that may open for new therapeutic attempts. MBL did not have a significant impact on prognosis.
**SAMMANFATTNING**

*Bakgrund*


**Syfte**

Att hos patienter med akut hjärtinfarkt och T2DM

1. Utforska den prognostiska betydelsen av hypoglykemi under sjukhuvudstillselens tidpunkt
2. Studiera den prognostiska betydelsen av glukosmätningar och läkemedel
3. Undersöka den prognostiska betydelsen och relationen mellan Copeptin och IGFBP-1
4. Karaktärisera MBL geno- och fenotyper samt studera dess prognostiska betydelse

**Studiepopulation**

Grunden till denna avhandling är epidemiologiska rapporter från DIGAMI 2 studien vilken omfattade 1253 patienter med T2DM och akut hjärtinfarkt. DIGAMI 2 var en randomiserad studie med syfte att jämföra tre glukosmätningar och läkemedel för att studera hypotesen att insulin-baserad glukoskontroll minskar dödshetens risk.

**Hypoglykemi under sjukhuvudstillselens tidpunkt**


**Glukosmätningar och behandling**

För patienter som skrevs ut levande (n=1181) var den justerade risk ration (hazard ratio; HR) för icke-dödlig hjärtinfarkt och stroke under den inledda uppföljningsperioden, 2,3 år, 1,73 (95% CI 1,26–2,37; p =0,0007) för de som behandlats med insulin, 0,81 (95% CI 0,57–1,14; p = 0,23) för sulfonylureabehandlade och 0,63 (95% CI 0,42–0,95; p = 0,03) för metforminbehandlade medan inget glukosmätningshävande läkemedel påverkade dödshetens risk. Insulin påverkade icke-fatale kardiovaskulära händelser under en förlängd uppföljning, mediantid 4,1 år, odds ratio 1,90; (95% CI 1,38–2,63; p=<0,0001) men inte dödshetens. Metformin relaterade till lägre dödshetens risk och lägre risk att dö av maligna sjukdomar. Det förelåg ingen skillnad i total eller kardiovaskulära dödsfall mellan de randomiserade grupperna men risken att dö av malign sjukdom var högst bland patienter randomiserade till insulin.

**Nya riskmarkörer och prognos**

Copeptin, en surrogat markör för vasopressin, var associerad med IGFBP-1 (r = 0,53; p<0,001) hos 393 patienter som deltog i DIGAMI 2:s biokemiska program. Båda biomarkörerna predikterade kardiovaskulära händelser (kardiovaskulär död, hjärtinfarkt och stroke) i univariata analyser. I den slutgiltiga statistiska modellen, justerad för ålder och njurfunktion, kvarstod copeptin som den enda oberoende prediktorn (HR 1,35; 95% CI: 1,16-1,57; p<0,001).

Serum (S)-MBL, som aktiverar komplement systemet, analyserades i prover från 387 patienter och MBL genotyper från 287 patienter. Femtiofyra procent hade högt (median S-MBL=2658 µg/l; IQR 1715 – 3829) och 46 % lågt kodande MBL genotyp (median S-MBL=373µg/l; IQR 100-765). S-MBL predikterade inte kardiovaskulära händelser. Risken var lägre för patienter med hög genotyp och S-MBL över medianen för dess genotyp (HR 0,49; 95% CI 0,26-0,92; p= 0,026) jämfört med patienter med låg genotyp och S-MBL lägre än medianen för dess genotyp. Denna relation var dock endast av marginell betydelse i justerade analyser.

**Sammanfattning**

LIST OF ORIGINAL PAPERS

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

I
L G Mellbin, K Malmberg, A Waldenström, H Wedel and L Rydén
for the DIGAMI 2 Investigators
Prognostic implications of hypoglycaemic episodes during hospitalisation for myocardial infarction in patients with type 2 diabetes: a report from the DIGAMI 2 trial
Heart 2009;95:721-727

II
L G Mellbin, K Malmberg, A Norhammar, H Wedel and L Rydén
for the DIGAMI 2 Investigators
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
European Heart Journal 2008;29:166-176

III
L G Mellbin, K Malmberg, A Norhammar, H Wedel and L Rydén
for the DIGAMI 2 Investigators
Insulin in patients with acute myocardial infarction and diabetes – friend or foe
A report from the DIGAMI 2 study
Submitted for publication

IV
L G Mellbin, L Rydén, K Brismar, N G Morgenthaler, J Öhrvik and S B Catrina
C-terminal-ProVasopressin (Copeptin), IGFBP-1 and cardiovascular prognosis in patients with type 2 diabetes and myocardial infarction.
A report from the DIGAMI 2 trial
Diabetes Care 2010; In press

V
L G Mellbin, A Hamsten, K Malmberg, R Steffensen, L Rydén, J Öhrvik and T K Hansen
Mannose-binding lectin genotype and phenotype and their impact on cardiovascular prognosis in patients with type 2 diabetes and myocardial infarction
A report from the DIGAMI 2 trial
Submitted for publication
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced glycation end-products</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated kinase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery by pass grafting</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>Copeptin</td>
<td>C-terminal-ProVasopressin</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>GIK</td>
<td>Glucose-Insulin-Potassium</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin A1c</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin growth factor-1</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Insulin growth factor binding protein-1</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range (IQR)</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose-binding lectin</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinostol-3 kinase</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PTCA/PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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</tbody>
</table>
**INTRODUCTION**

**Myocardial infarction**

*Prevalence and prognosis of cardiovascular disease*

Cardiovascular disease is the leading cause of mortality accounting for half of all deaths, over 4.35 million each year in the 53 states comprising the World Health Organization (WHO) region Europe and more than two million in the European Union (EU). The proportion is higher in women (54% of all deaths) than in men (43% of all deaths) and among people living in poor socio-economic conditions (1). A high rate of cardiovascular deaths is seen in all parts of the industrialized world, and cardiovascular disease as a cause of death increases rapidly in developing countries (2). Although most EU countries report on declining rates of age standardized cardiovascular mortality, an increasing number of persons are living with various manifestations of such disease (3). This is explained by an improved treatment of cardiovascular disease in particular myocardial infarction and an increased longevity in the population (1). Cardiovascular disease imposes a considerable economic burden on the EU member states. The estimated cost is € 192 billion/year representing an average annual per capita cost of € 391 (1).

The Interheart study (4), a case-control study of acute myocardial infarction in 52 countries comprising 15 152 cases and 14 820 controls, revealed that 90% of the population attributable risks in men and 94% in women were accounted for by nine modifiable risk factors; smoking, raised ApoB/ApoA1 ratio, hypertension, diabetes, abdominal obesity, psychosocial factors, daily consumption of fruits and vegetables, alcohol consumption and regular physical activity. According to the European guidelines on prevention of cardiovascular disease; control of risk factors, especially among high risk individuals such as patients with diabetes should have high priority (3).

The prevalence of glucose abnormalities is high in patients with coronary artery disease, acute as well as chronic (5-7). Similar proportions were identified in patients with cerebrovascular or peripheral artery disease (8). Importantly patients with coronary artery disease and glucose abnormalities have a more serious prognosis than those without glucose perturbations (9-11). A reason is the clustering of conventional risk factors such as hyperlipidemia and hypertension in patients with abnormal glucose regulation but hyperglycemia in itself is also an important risk factor increasing cardiovascular morbidity and mortality. In fact markers related to a sedentary lifestyle and metabolic disturbances such as dysglycemia and dyslipidemia seems to be representative for a new phenotype among patients with myocardial infarction replacing more traditional risk factors such as stress and smoking (12,13).

**Manifestations and prognosis of coronary artery disease**

Symptomatic coronary artery disease is classified as stable angina pectoris or acute coronary syndrome (ACS). An ACS may take the form of a ST-segment elevation myocardial infarction (STEMI) or a non-STE ACS (non-ST-elevation myocardial infarction (NSTEMI) or unstable angina). These are diagnosed by a combination of chest pain, a typical ECG pattern and/or the release of troponins (14). Hospital admissions for a NSTE-ACS are estimated to 3/1000 inhabitants and a relative increase of a NSTE-ACS compared with STEMI has been noted.
over time (14). This is partly explained by the use of highly sensitive biomarkers for the diagnosis of ACS but may also reflect changes in the management of patients with coronary artery disease. In-hospital mortality is higher in patients with STEMI than NSTE-ACS (7 vs. 5%, respectively). This difference, which decreases over time, has converged after six months (12 vs. 13%). Indeed mortality becomes higher in NSTE-ACS than STEMI during extended periods of follow-up with a two-fold difference after four years (14). This development may be explained both by differences in the extent of coronary artery disease and by higher age and more frequent co-morbidities, such as diabetes and renal failure, in patients with NSTE-ACS (14).

Pathophysiology of coronary artery disease

Coronary artery disease, the most common cause of ACS, involves two pathophysiological pathways: atherosclerosis and thrombosis. Atherosclerosis, a progressive disorder in the arterial walls gradually narrowing their lumen, is an inflammatory disease (14,15). It is caused by an activation of innate and adaptive immune systems leading to the recruitment of monocytes, subsequently maturing into macrophages, and lymphocytes (16). Their multiplication and secretion of cytokines, adhesion molecules and growth factors are important for the building and progression of atherosclerotic plaques, which is triggered by the accumulation of oxidized low density lipoprotein (LDL) in the subendothelial matrix (15,16). If a plaque ruptures tissue factors gets in contact with the blood causing platelet aggregation and thrombus formation occluding the artery (15).

Endothelial function and coronary artery disease

The endothelium acts as a selectively permeable barrier between the blood and vascular tissues. It is involved in the regulation of many factors of importance for the development of atherosclerosis among them inflammation, vascular tone, vascular remodeling and thrombosis (15). Endothelial cells are involved in vasomotor function primarily by the secretion of vasoactive mediators, most importantly nitric oxide (NO), prostacyclin, endothelin-1 (ET-1) and thromboxane. The intracellular production of NO from L-arginine is regulated by endothelial nitric oxide synthase (eNOS) and NO release causes relaxation of vascular smooth muscle cells and thereby vasodilatation (17). Endothelial dysfunction, which at least partially is triggered by the influence of oxidized LDL, is characterized by decreasing NO bioavailability and an increase in the production of reactive oxygen species (ROS) (15,18,19). This causes an imbalanced blood-flow and may affect smooth muscle cell proliferation, monocyte activation, platelet aggregation and decreased endogenous fibrinolysis (17,20). Impaired endothelial function is an early feature in atherogenesis, documented in several conditions associated with atherosclerosis for example smoking, dyslipidemia, diabetes mellitus and hypertension (17).

Diabetes mellitus

Prevalence and consequences

Diabetes mellitus, is rapidly becoming more common due to increasing longevity in combination with a sedentary lifestyle and the increasing prevalence of overweight (21). Recent estimates suggest that the global prevalence, which in 2010 is 285 million people or 6.4% of the population, will increase by 69% in developing and 20% in developed countries to a total of 439 million or 7.7% in 2030 (22).
Although traditional diabetes specific complications; retino-, nephro- and neuropathy still are important an increasing attention has been directed to macrovascular complications i.e. coronary, cerebral and peripheral vascular disorders. The risk of developing various manifestations of macrovascular disease is several times higher for patients with than among those without diabetes (4,23-25) and following a cardiovascular event patients with diabetes have a considerably more serious prognosis (26,27). This is indeed apparent already in patients with newly detected glucose disturbances (10,11,28). The excess mortality in patients with diabetes is to a very high extent, 52-80%, due to cardiovascular complications (21,29). In addition to vascular complications there is a link between diabetes and fatal and non-fatal cancer (30). The excess risk for a patient with diabetes is about 30% for colon-, 50% for pancreas - and 20% for breast cancer (31-33).

**Definition and classification**

WHO has from 1965 issued guidelines for the diagnosis and classification of diabetes with the most recent update in 2006 (34). It is recommended that the diagnosis should be based on at least two fasting venous glucose values ≥ 7.0 mmol/l (35) or the outcome of an oral glucose tolerance test (OGTT) according to criteria presented in Table 1 (34). Although the diagnostic boundaries are dichotomized WHO emphasized that an overall evaluation of diabetes related cardiovascular risk should include an assessment of glucose as a continuous variable. The American Diabetes Association (ADA) recently added Glycated hemoglobin A1c (HbA1c) ≥ 6.5% as a diagnostic criterium (36). The 2006 WHO guidelines did not accept HbA1c as a diagnostic tool because of a lack of general availability and standardization of different laboratory methods in combination with the potential influence of such factors as anemia and pregnancy on the outcome. Despite the substantial impact of macrovascular complications on the prognosis in patients with diabetes both the WHO and ADA diagnostic criteria are based on glucose levels associated with an increased risk of the microvascular complication retinopathy. Numerous studies has reported on a continuous relationship between hyperglycemia and increasing macrovascular morbidity and mortality, even starting at levels far below those presently used (37-39). It has, however, been difficult to establish a definite threshold above which macrovascular complications start to develop. The present guidelines from the European Society of Cardiology (ESC) and European Association for the Study of Diabetes (EASD) underlines that the definition and diagnostic...

<table>
<thead>
<tr>
<th>Table 1. Diagnostic criteria for diabetes and intermediate hyperglycemia. Adapted after the 2006 WHO recommendations.</th>
</tr>
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<tr>
<td><strong>Diabetes</strong></td>
</tr>
<tr>
<td>Fasting plasma glucose or 2–h plasma glucose*</td>
</tr>
<tr>
<td>≥7.0mmol/l (126mg/dl) or ≥11.1mmol/l (200mg/dl)</td>
</tr>
<tr>
<td><strong>Impaired Glucose Tolerance (IGT)</strong></td>
</tr>
<tr>
<td>Fasting plasma glucose and 2–h plasma glucose*</td>
</tr>
<tr>
<td>&lt;7.0mmol/l (126mg/dl) and ≥7.8 and &lt;11.1mmol/l (140mg/dl and 200mg/dl)</td>
</tr>
<tr>
<td><strong>Impaired Fasting Glucose (IFG)</strong></td>
</tr>
<tr>
<td>Fasting plasma glucose and 2–h plasma glucose*</td>
</tr>
<tr>
<td>6.1 to 6.9mmol/l (110mg/dl to 125mg/dl) and &lt;7.8mmol/l (140mg/dl) (if measured)</td>
</tr>
</tbody>
</table>

* Venous plasma glucose 2–h after the ingestion of 75 g dissolved glucose in 200 ml water
classification of patients with diabetes and its prestates should be based on the level of subsequent risk of cardiovascular complications (40). As outlined in Table 2 diabetes may be classified into four types, all characterized by hyperglycemia (35). Type 2 diabetes, the by far most common form, affects 90% of adult patients.

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Pancreatic β-cell destruction usually leading to absolute insulin deficiency</th>
</tr>
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<tbody>
<tr>
<td>Type 2</td>
<td>Progressive insulin secretory defect on the background of insulin resistance</td>
</tr>
<tr>
<td>Other specific types of diabetes</td>
<td>Genetic defects in β-cell function or insulin action</td>
</tr>
<tr>
<td></td>
<td>Diseases of the exocrine pancreas (e.g. cystic fibrosis and pancreatitis)</td>
</tr>
<tr>
<td></td>
<td>Drug or chemically induced diabetes (e.g. treatment of AIDS or after organ transplantation)</td>
</tr>
<tr>
<td>Gestational diabetes mellitus (GDM)</td>
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</tbody>
</table>

**Table 2.** Etiological classification of disorders of glycemia.

The pathogenesis of type 2 diabetes

The key hormone for regulation of glucose homeostasis is insulin, which is secreted from the pancreatic β-cells in response to the actual glucose concentration and insulin actions. The relation between β-cell function and insulin sensitivity is hyperbolic, i.e. when insulin action decreases, for example in insulin resistant states such as obesity, the β-cells compensate by increasing insulin secretion. The typical background to type 2 diabetes is when the β-cell function for some reason is compromised and insulin secretion becomes too low for a specific level of insulin sensitivity. The progress towards overt type 2 diabetes is a long-lasting process over several years as depicted in Figure 1. In particular macrovascular complications starts to develop already during the prediabetic period i.e. well before the time when diabetes is diagnosed. Unfortunately as many as 50% of the patients already have one or more diabetes related complications at the time of diagnosis (21).

![Figure 1. Development of insulin resistance and glucose abnormalities in relation to complications. Reprinted with permission from reference (41).](image-url)
As shown in Figure 2 glucose homeostasis is dependent on a close interaction between the pancreatic β-cells and the function of hepatic, skeletal muscle and adipose tissue mediated by insulin, which decreases glucose production by inhibiting the hepatic glycogenolysis and gluconeogenesis at the same time stimulating glycogen synthesis in the liver. Moreover insulin enhances glucose uptake in the skeletal muscles and acts a potent anabolic hormone and regulator of lipid and protein metabolism (42).

A decreased net effect of insulin on the target tissues causes a combination of increased endogenous glucose production and decreased uptake of glucose in peripheral tissues and a subsequent hyperglycemia. The resulting glucotoxicity may further deteriorate β-cell function (43). The inability to decrease lipolysis increases the levels of circulating free fatty acids (FFA) from adipose tissue. This is, besides aggravating insulin resistance in the skeletal muscles and liver, harmful for the β-cells (lipotoxicity). It has been assumed that the β-cell toxicity caused by hyperglycemia and FFA accumulation relates to the production of ROS leading to apoptosis and interaction with insulin gene transcription or other genes such as PPARγ (42,43).

With insulin resistance as a key factor behind the development of type 2 diabetes the understanding of the insulin receptor and its function becomes of great importance. This receptor is a trans-membrane tyrosine kinase receptor composed of two α- and two β-subunits linked by disulfide bonds. The extracellular α-subunits contain insulin binding domains, while the linked β-chains penetrate through the plasma membrane (44). Binding of insulin to the α-subunits causes phosphorylation of the β-subunits (autophosphorylation).
thereby activating the catalytic activity of the receptor. The activated receptor phosphorylates a number of intracellular proteins, which alters their activity and generates a biological response. Activation of phosphatidylinositol-3 kinase (PI3K) plays a central role in insulin stimulated glucose uptake by the protein glucose transporter type 4 (GLUT 4) (44). In the endothelial cells insulin acts as a vasodilator by increasing the eNOS expression via the PI3K pathway thereby enhancing NO production (44,45). Moreover the PI3K pathway exerts anti-atherogenic effects on vascular smooth muscle and inflammatory cells by preventing apoptosis. In addition, insulin activates the mitogen-activated protein kinase (MAPK) pathway. Although not directly involved in glucose metabolism this pathway is related to cell survival and atherogenesis.

The exact reason and mechanism that causes insulin resistance in type 2 diabetes is not known. It may relate to up- or downstream defects in the signaling system or to defects in the insulin receptor. The latter may for instance be modulated by an increase in FFA or hyperglycemia (44). A fat-rich meal or an oral glucose load decrease flow-mediated vasodilatation in healthy individuals and in patients with diabetes (46). During insulin resistant states the PI3K pathway is down-regulated in metabolically active tissues leading to decreased glucose uptake and hyperglycemia and it appears as the anti-atherosclerotic PI3K pathway is impaired in both endothelial and vascular smooth muscle cells (20,44). At the same time the mitogenic MAPK pathway seems to remain intact or even enhanced, which may contribute to the progression of atherosclerosis, Figure 3 (20,44).

**Figure 3.** Insulin receptor signaling and proposed vascular effects in health (A) and insulin resistance (B). For explanation of abbreviations see page 9, in addition: NADPH oxidase (NOX), superoxide (O2-), peroxynitrite (ONOO-), phosphorylated amino acid residue (P). Adapted with permission after reference (20).
Linking hyperglycemia to vascular injury and cardiovascular disease

As previously described several population based studies have shown a relation between increasing glucose levels and macrovascular complications. Since some time four hypotheses have been brought forward on how hyperglycemia may initiate processes that end with vascular complications (47).

1) Increased polyol pathway flux which increases the susceptibility to intracellular oxidative stress.

2) Increased formation of advanced glycation end-products (AGE) modifies intracellular proteins involved in gene transcription and the extracellular matrix causing cellular dysfunction. In addition AGE accumulation promotes the production of inflammatory cytokines, growth factors and adhesion factors involved in the atherogenetic process.

3) Activation of protein kinase C (PKC) isoforms causes endothelial dysfunction due to a decreased production of NO and an increase in the vasoconstrictor endothelin-1, decreased fibrinolysis, inflammation and an increased production of ROS (Figure 4).

4) Increased hexosamine pathway flux increases the expression of transforming growth factor-1 (TGF-β) and plasminogen activator inhibitor-1 (PAI-1).

Recent findings suggest that these four pathways may have a common denominator. High glucose is believed to cause overproduction of superoxide via the mitochondrial electron-transport chain (47). The ROS induced DNA damage activates a DNA repair enzyme, poly(ADPribose) polymerase (PARP) that subsequently modifies and reduces the activity of a key glycolytic enzyme called glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Finally GADPH activates the four pathways (48).

![Figure 4](image.png)

**Figure 4.** Vascular effects of hyperglycemia induced PKC activation.

For explanation of abbreviation see page 9, in addition: Atrial natriuretic peptide (ANP), Brain natriuretic peptide (BNP), DiAcylGlycerol (DAG), NADPH oxidase (NOX), Nuclear factor-κB (NF-κB), Plasminogen activator inhibitor-1 (PAI-1), Transforming growth factors (TGF-β) and Vascular endothelial growth factor (VEGF). Adapted with permission after reference (47).
Other factors contributing to cardiovascular disease in diabetes

Endothelial dysfunction which, as already discussed, in several ways paves the way for the development of atherosclerosis is common among patients with diabetes and is indeed compromised already prior to the onset of type 2 diabetes possibly even before the onset of hyperglycemia (17). Endothelial dysfunction is related to insulin resistance (49) and impacts the PI3K pathway. This is enhanced by hyperglycemia, a phenomenon that has been observed even after relatively short periods (hours) of high glucose concentrations in healthy people (50). In addition other factors that may compromise endothelial function are common in patients with type 2 diabetes among them hypercholesterolemia and hypertension (17). Interestingly an increase in ROS, induced by an enhanced FFA flux from adipose tissue to endothelial cells during insulin-resistant states, seems to activate the same damaging pathways as hyperglycemia. This may obviously be of importance for the development of macrovascular disease (45,48).

Inflammatory activation may be another important contributor. Diabetes is associated with an increased susceptibility to inflammation which, as outlined, is an important pathophysiological component in the development of atherosclerosis. Both increased accumulation of AGE and activation of PKC promotes production of inflammatory cytokines and impairs the immune response due to phagocyte dysfunctions (15,51).

A prothrombotic state, combining an enhanced propensity to platelet aggregation with decreased endogenous fibrinolysis, prevalent in patients with diabetes, increases the risk for thrombotic occlusion of arteries not the least following plaque rupture (40,52). Furthermore the risk for plaque rupture is increased in patients with diabetes due to increased smooth muscle cell apoptosis and decreased production of collagen in affected vessels (44).

Myocardial energy production is another factor that is disturbed. Under normal conditions 60% of the energy rich adenosine triphosphate (ATP) is produced by beta oxidation of free fatty acids while 40% is derived from glucose oxidation. The latter, less oxygen consuming pathway, increases during stressful conditions (40). Glucose oxidation is reduced to a small proportion, 10%, in patients with diabetes forcing the ATP production towards the more oxygen demanding beta oxidation. During stress with corresponding catecholamine release the heart becomes exposed to even higher levels of FFA and thereby oxygen demand, which due to vascular injury and dysfunction, not always can be satisfied (53). Such mechanisms may indeed be behind the increased propensity to develop diabetes related myocardial dysfunction. Moreover, the regulation of coronary blood flow may be impaired due to autonomic nervous dysfunction with a decreased vagal and proportionately increased sympathetic tone resulting in the increased resting heart rate and decreased rate variability often seen in patients with diabetes.

Hyperglycemia and metabolic disturbances in the acute setting

Hyperglycemia in the setting of a cardiovascular event is associated with a worse outcome. In 1988 Malmberg and Rydén reported on the very poor prognosis in patients with diabetes and myocardial infarction (27). This knowledge was further expanded when Capes et al. performed a meta-analysis on trials from 1966 to 1998, studying in-hospital mortality or rates of congestive heart failure after a myocardial infarction in relation to glucose concentration at admission. It revealed that hyperglycemia in this setting related to an impaired prognosis both in patients with and without diabetes (54). The impact of hyperglycemia during hospitalization
on long and short-term prognosis has subsequently been confirmed in a large number of studies (55-62). Several reports suggest that there is a J- or U shaped relation between blood glucose and prognosis with the implication that not only high but also low levels are related to increased mortality in patients with myocardial infarction (57,58,63). If this reflects a dismal effect of hypoglycemia in itself or if it is related to other factors remains to be studied.

In the setting of an ACS stress induced catecholamine release (64) leads to increased mobilization of FFA from the adipose tissue and to decreased insulin release in combination with increased insulin resistance and glycogenolysis (52,53). These are all factors contributing to hyperglycemia, which becomes even more pronounced in the presence of any existing glucometabolic perturbation whether previously known or undetected. Hyperglycemia in such situations is therefore not only a reflection of acute stress but should raise a suspicion of the presence of hidden impaired glucose tolerance (IGT) or diabetes. This assumption gains support by the results of persisting abnormal glucose tolerance when an in-hospital OGTT is repeated up to 12 months after an acute coronary event (65).

Acute hyperglycemia may trigger several mechanisms of importance for the more dismal outcome seen after an ACS in patients with than those without diabetes (Figure 5) (66). It may reduce ischemic preconditioning and enhance the development of ischemia/reperfusion injury, decrease collateral circulation, cause lower rates of spontaneous reperfusion and be behind a no-reflow phenomenon due to microvascular dysfunction. High free fatty acid concentrations may further aggravate myocardial ischemia and trigger malignant arrhythmias.

**Figure 5.** Metabolic disturbances and subsequent consequences in the acute setting. Modified after reference (66).
Glucose lowering treatment

**Acute setting**

Efforts have been made to improve prognosis for patients with myocardial infarction, with and without diabetes by essentially two strategies: metabolic modulation and metabolic control. Studies on metabolic modulation focus on the potential beneficial effects of insulin and potassium during acute stress without any particular attention to blood glucose. This strategy, based on an infusion of fixed doses of Glucose-Insulin-Potassium (GIK), was first reported on by Sodi-Pallares in 1962 (67). The belief was that an increase of the intracellular level of potassium would stabilize the cardiomyocyte and facilitate glucose transportation into the cells and thereby reducing the risk of arrhythmias. Further theories on the potential benefits of GIK was that the infusion would improve glucose oxidation and decrease beta oxidation of FFA at the same time improving endothelial function and fibrinolysis (53). Unfortunately randomized trials in this field failed to show any beneficial mortality or morbidity effects (68,69). The concept of metabolic control includes the use of insulin in order to lower glucose to a prespecified level with the intention to reduce the negative effects of hyperglycemia and, in addition, to take advantage of the beneficial effects of insulin. The first study in this field was the Diabetes and Insulin–Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study (70). In this trial 306 patients with diabetes and acute myocardial infarction were randomly assigned to a ≥24-hour insulin–glucose infusion followed by multidose subcutaneous insulin with the aim to rapidly and consistently lower glucose to preset targets while 314 patients were randomized to routine glucose-lowering therapy. Metabolic control improved significantly as reflected by a 1% lower HbA1c in patients on intensive insulin treatment compared to those in the control group. During an average follow-up of 3.4 years, 33% of the patients in the intensive insulin group and 44% in the control group died ($p=0.011$) (71). A second DIGAMI trial was conducted to determine if the benefit was linked to the initial insulin–glucose infusion or to the continued insulin based glucose control. In DIGAMI 2 (72), as will be outlined in detail later on, there was no difference in glucose control between the different modalities for glucose control and no difference in total mortality or non-fatal cardiovascular events. It was speculated that the lack of a difference in glucose control accounted for this negative result, but effects related to the different glucose lowering management strategies (insulin versus oral drugs or lifestyle) cannot be excluded.

A potential problem with insulin treatment during the acute phase of a myocardial infarction is the risk of induction of hypoglycemia. A compensatory mechanism to hypoglycemia is enhanced catecholamine release. This may aggravate myocardial ischemia. The fear of inducing hypoglycemia has indeed been reported as an obstacle to the use of sufficient amount of insulin to reach targeted glucose levels (73). The impact of insulin induced hypoglycemia is however not fully understood and it may be different in comparison with spontaneously occurring episodes of hypoglycemia.

**Long-term treatment**

Considering the increased risk for macrovascular complications with hyperglycemia the assumption that lowering glucose would be beneficial seems reasonable.

In patients with newly established diabetes intensive glucose lowering therapy appears to be beneficial for both patients with type 1 and 2 diabetes (74-76). The impact on macrovascular and mortality and morbidity does, however, not appear instantly. It may indeed take up to a decade for glucose normalization to translate into improved prognosis (75,76). For patients with a more
advanced disease and already established complications the benefit of intensive glucose control has been debated and most recently even considered potentially harmful (77,78). How glucose lowering should be accomplished is not well understood. Most studies on glucose lowering drugs only focused on the glucose lowering capacity and it is only during the last years that the impact on cardiovascular morbidity and mortality has attracted interest.

Presently available oral drugs lower HbA1c by 0.5-1.5% while insulin is more effective lowering HbA1c by 1-2%. The main target tissues of the different drugs are outlined in Figure 6. Their effects on the cardiovascular system however remain uncertain to a large extent. Metformin appears to have beneficial cardiovascular effects, at least in overweight patients with newly detected diabetes (79). The impact of sulphonylureas has been questioned and related to increased cardiovascular risk and mortality by harmful effects on the myocardium such as impaired preconditioning (80,81). The use of thiazolidinediones is associated with an established increased risk of heart failure. Moreover it has been argued that at least some of the drugs in this class may provoke myocardial infarction (82,83). The recent class of drugs acting on the incretin system (Glucagone-like peptide (GLP-1) analogues and Dipeptidyl-peptidase IV (DPP-IV) inhibitors) still lack sufficient information in regards of vascular effects. Finally, insulin, that at least in the short term perspective seems to exert beneficial effects on glucose metabolism and endothelium may have different effects during long-term exposure. In registry and post-hoc trials the use of insulin has been associated with increased cardiovascular risk, but it is difficult to interpret these data due to the possibility of confounding by indication (84-87). Concerns have also been raised that insulin may enhance the risk of cancer in patients with diabetes although available registry based data are conflicting (88).

**Figure 6.** Target tissues and mechanisms of action of different glucose lowering drugs. Gastrointestinal tract (GI).
Risk markers

Traditional risk factors such as hyperglycemia, hypertension and dyslipidemia do not fully explain the unfavourable outcome for patients with diabetes after a myocardial infarction (10). Thus there is a need to identify novel prognostic biomarkers that may generate further insights in pathophysiological mechanisms, hopefully opening for therapeutic interventions. Three biomarkers have been studied, within this thesis, in relation to their prognostic impact in patients with myocardial infarction and diabetes.

*C-terminal-provasopressin (copeptin) and Insulin growth factor binding protein-1 (IGFBP-1)*

The hypothalamic arginine vasopressin (AVP) system plays an essential role in osmoregulation and the control of vascular tone (89,90). The AVP system is mainly regulated by serum osmolality even though other factors influence the hypothalamic release of vasopressin. They include cardiac filling pressure, adrenergic stimuli, angiotensin-2 and nausea (91). In addition, vasopressin is a traditional stress hormone, which is the most probable mechanism for its release in patients with myocardial infarction.

A connection between vasopressin and the prognosis in patients with myocardial infarction has been suggested (92). Its pathogenic role and use as a biomarker has, however, been hard to study since it is difficult to measure vasopressin due to its short half-life and binding to platelets. However, copeptin, the c-terminal degradation part of the vasopressin pre-hormone, is a stable peptide secreted in equimolar concentrations to vasopressin, making it more suitable to be measured as a marker for the AVP system (93,94). Recently, high levels of copeptin were linked to an impaired prognosis in patients with acute myocardial infarction and congestive heart failure (95-98). The impact of AVP activation, measured as the surrogate marker copeptin, in patients with diabetes is not known.

Another endocrine system that has been linked to cardiovascular disease is the insulin growth factor-1 (IGF-1) axis. Insulin growth factor binding protein-1 (IGFBP-1), one of 6 binding proteins, is involved in short-term IGF-1 bioavailability (99). IGFBP-1 is related to cardiovascular mortality and morbidity in patients with myocardial infarction (100,101) or with critical illness (102) but the mechanisms behind this is not fully understood. Recent observations indicate that there may be a link between the AVP and IGF-1 systems since an infusion of desmopressin, a vasopressin agonist, in patients with central diabetes insipidus, lacking endogenous vasopressin, has a direct impact on IGFBP-1 levels (103).

*Mannose-Binding Lectin (MBL)*

Inflammation and immune system activation, key features of atherosclerosis, are, as mirrored by markers such as CRP and IL-6, associated with an increased risk for cardiovascular events (16,104). Both the innate and adaptive immune systems plays a crucial role in this perspective (15,16) and the complement system, a complex system involving more than 30 soluble and membrane bound proteins, acts as a link them in-between.

MBL is a pattern recognition molecule of the innate immune system that has attracted interest in the context of cardiovascular disease because of its key role in the inflammatory process (105). MBL activates the lectin pathway, Figure 7, one of three arms of the complement system, which in an evolutionary perspective is one of the most ancient (about 600 – 700 million years old) defence system against pathogens (106).
The hepatic production of MBL is highly influenced by polymorphisms in the promoter region of the MBL2 gene on chromosome 10 and by three different single-base substitutions within exon 1 (107,108). Although MBL acts as an acute-phase reactant, the circulating levels remain quite stable over time, only increasing two to three times during stress (109).

MBL (initially called mannan binding protein) was discovered in rabbits 1978 by Kawasaki et al (110). In 1989 a group of children with recurrent infections were found to have defect opsonisation related to MBL deficiency (111). Thereafter MBL deficiency has been shown to be the most common, known, immunodeficiency, affecting ca 10 -25% of the population (112). MBL deficiency has been related to autoimmune and inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) (112,113). The exact role of MBL is somewhat conflicting. Activation of this pathway and subsequently the complement cascade seems to relate to atherosclerosis and evidence from diabetic mice indicates that MBL complement activation increases myocardial ischemia-reperfusion injury (114,115). MBL levels were higher among patients in a recent case-control study of patients with acute coronary syndromes (116). In patients with diabetes high MBL levels have furthermore been linked to increased risk for microvascular complications (117,118).

On the other hand in addition to MBL-deficiency being related to infections and autoimmune diseases, an increased risk for cardiovascular disease is revealed in some studies (113,119-122).
AIMS

1. To explore the prognostic impact of hypoglycemia during hospitalization for acute myocardial infarction in patients with type 2 diabetes (Study I)

2. To study the prognostic impact of glucose lowering treatment in patients with type 2 diabetes surviving an acute myocardial infarction (Studies II-III)

3. To investigate the prognostic impact and the relation between the two novel risk markers copeptin and IGFBP-1 in patients with acute myocardial infarction and type 2 diabetes (Study IV)

4. To characterize MBL geno- and phenotypes in patients with acute myocardial infarction and type 2 diabetes and to test if this information contains prognostic importance (Study V)
MATERIAL AND METHODS

The DIGAMI 2 trial

This thesis is based on the 1253 patients with type 2 diabetes and suspect acute myocardial infarction, who participated in the second Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction Trial (DIGAMI 2) (72). DIGAMI 2 was a prospective, randomized trial, conducted at 44 centres in Sweden, Finland, Norway, Denmark, UK and The Netherlands. The primary aim was to compare three different glucose lowering strategies testing the hypothesis that early instituted and continued insulin-based metabolic control would reduce mortality.

The inclusion criteria were type 2 diabetes, defined as previously established or as an admission blood glucose >11.0 mmol/l, and hospitalization due to suspect acute myocardial infarction. The criteria used for the latter diagnosis were chest pain >15 min during the preceding 24 hours and/or recent ECG signs indicating myocardial injury or ischemia i.e. new Q-waves and/or ST-segment deviations in two or more leads. Exclusion criteria were inability to cope with insulin treatment or to receive information on the trial, residence outside the hospital catchment area, participation in other studies or previous participation in DIGAMI 2. Eighty-four percent of the patients fulfilled the diagnosis of myocardial infarction and almost all remaining patients had coronary artery disease mostly presenting as unstable angina pectoris.

Patient recruitment started in January 1998 and ended in May 2003. The original time of follow up, ending December 2003, varied from a minimum of six months to a maximum of three years (median 2.1 and interquartile range 1.03 – 3.00 years). During this period treatment were to be continued according to the study protocol. In an extended follow up all centers were asked for information on the vital status, including cause and date of death, and of the occurrence of a myocardial infarction or stroke until December 2005.

Glucose-lowering treatment

The patients were randomized to one of three study groups: Group 1 (n= 474) received an insulin-glucose infusion (500 ml 5% glucose and 80 IU of soluble insulin, initial infusion rate 30 ml/h to be adjusted in relation to subsequent glucose levels) (70). The infusion was started as soon as possible after hospital admission lasting until stable normoglycemia and at least for 24 hours with the aim to reach a blood glucose level of 7–10 mmol/l. A combination of short-acting insulin before meals and intermediate long-acting insulin in the evening was initiated at the cessation of the infusion. The treatment goal was to obtain a fasting blood glucose of 5–7 mmol/l and a non-fasting of < 10 mmol/l. Group 2 (n=473) received the same initial insulin-glucose infusion. This was followed by glucose lowering treatment according to local practice without any protocol stated target glucose levels. Group 3 (n=306) constituted a control group receiving glucose lowering treatment according to local practice during the complete trial.

Hypoglycemia was defined as a blood glucose < 3.0 mmol/l and was recorded as with or without symptoms.
Concomitant treatment

The protocol stated that the use of concomitant treatment should be as uniform as possible according to evidence-based guidelines and that all patients without contraindications should receive aspirin, thrombolytic agents, beta-blockers, lipid-lowering drugs, angiotensin-converting enzyme (ACE)-inhibitors, and revascularization procedures when appropriate (123,124).

Laboratory assessments

Random blood glucose was obtained as soon as possible after admission. During the first 24 hours blood glucose was followed according to the infusion protocol in Groups 1 and 2 (at least every second hour during the complete infusion period and within one hour following dose adjustments and always in the presence of symptoms indicating hypoglycemia) while sampling was left at the discretion of the attending physician in Group 3. Thereafter, fasting blood glucose was recorded daily until hospital discharge and at each follow-up visit in all patients.

Samples for laboratory assessments including electrolytes, serum creatinine, blood lipids and glucose were analyzed locally. HbA1c was analyzed at a central core laboratory by high-performance liquid chromatography with an upper normal limit of 5.3% (Boehringer Mannheim Scandinavian AB, Bromma, Sweden).

Five hundred and seventy-five patients participated in a prespecified, biochemical substudy of the DIGAMI 2 trial. In these patients blood was collected at admission (before initiation of the glucose-insulin infusion), in the fasting state at the time of hospital discharge and at several occasions during follow up. Samples for DNA extraction and genetic analyzes were obtained at the time of hospital discharge. All specimens were stored in -70 °C pending analysis.

Endpoint adjudication

Event adjudication during the original part of DIGAMI 2 was performed by a group of three experienced cardiologists blinded to group belonging. Myocardial infarction was diagnosed according to the joint recommendations of the ESC and ACC (125). A reinfarction was defined as a new event >72 hours from the index infarction. Stroke was defined as unequivocal signs of focal or global neurological deficit of sudden onset and a duration of >24 hours that were judged to be of vascular origin. Deaths were verified with death certificates, hospital records and explaining letters from the physicians in charge when asked for by the adjudication committee members and autopsy reports when available. Sudden cardiovascular deaths were those that occurred within 24 hours following onset of symptoms and without any other obvious reason for the fatal outcome. Deaths were labeled as cardiovascular or non-cardiovascular and those without any obvious non-cardiovascular cause were considered cardiovascular. Non-cardiovascular deaths, including malignancies, were adjudicated according the same principles as cardiovascular events. Events during the extended follow-up (Study III) were adjudicated by two of the investigators, who were blinded to group belonging, by means of hospital records and/or death certificates.
Studies I - V

Patient populations

As outlined in Figure 8 the patients in Studies I-V are based on the 1253 participants in DIGAMI 2 (Study I) or different subgroups (Studies II-V). Study II comprises the 1181 subjects who survived the index hospitalization while Study III is based on 1145 patients in whom information from the extended follow-up was available. In this study 1073 patients, representing hospital survivors with long-term follow-up data, were analyzed separately. Studies IV and V are based on patients enrolled in the biochemical program. Copeptin and IGFBP-1 were analyzed in 393 patients. S-MBL was determined at hospital admission in 387 and typing for the MBL2 gene was performed in 287 of these patients.

![Figure 8. Flow chart outlining the origin of the patients in Studies I-V.](image_url)

Laboratory assessments

Copeptin, IGFBP-1 and S-MBL were analyzed from admission samples in the biochemical cohort.

Copeptin was measured with a sandwich immunoassay (LUMI test C-terminal pro-AVP: BRAMHS AG, Henningsdorf/Berlin, Germany, lower detection limit 0.4 pmol/l, functional assay sensitivity [<20% interassay coefficient of variation] <1 pmol/l) (93,94).

The IGFBP-1 concentrations in serum were determined by RIA (sensitivity 3 μg/l and cardiovascular intra- and interassays 3 and 10%) according to Povoa et al. (126).

S-MBL levels were measured using an in-house time-resolved immunofluorometric assay (127). The lower detection level was 10 μg/l, and the intra-assay and inter-assay coefficient of variation were below 10%.

Six single nucleotide polymorphism (SNPs) located within the promoter or coding regions of the MBL2 gene were analyzed using real-time polymerase chain reaction (rt-PCR)
with TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) (128-130). The genotyping was determined by measuring the end-point fluorescence on a 7900 HT Sequence Detection System using the SDS version 2.3 software. The presence of one of the three structural mutations within exon 1 at codons 52, 54 or 57 in the MBL2 gene, the D, B, and C variants (designated as “O”), significantly reduces circulating MBL. Of the promoter polymorphisms, comprising two variants in the 5’ regulatory region at positions -550 (H/L) and -221 (X/Y), and one in the 5’ untranslated region at position -4 (P/Q), only the X/Y polymorphism influences S-MBL, causing reduced MBL levels (108).

Endpoints
In Studies I-III the primary endpoints were total mortality and cardiovascular events (death, reinfarction or stroke). Furthermore analyses on cardiovascular death and cardiovascular morbidity (reinfarction or stroke) were performed. In Studies IV – V the primary endpoint was cardiovascular events (cardiovascular death and non-fatal myocardial infarction and stroke). In addition separate analyses of the components of this endpoint were performed.

Statistical methods
Continuous variables are presented as the median and interquartile range (IQR) and categorical variables as percentages unless otherwise stated. Differences between groups were assessed with Wilcoxon-Mann-Whitney, Kruskal-Wallis or Jonckheere-Terpstra tests. For comparison between randomized groups Chi Square test was used for non-ordered categorical variables. The association between continuous variables was studied with the Spearman’s Rank Correlation.

The Cox proportional hazard regression was the basis for analyzing mortality and morbidity in Studies I - V and is presented as hazard ratios (HR) and 95% confidence intervals (CI). In Study III odds ratios (OR) for non-fatal events were calculated by logistic regression analysis since the dates of these events were unavailable during the period of extended follow up.

In Study I “updated hypoglycemia” was the main variable (131). The term “updated” relates to when the hypoglycemic event occurred, during 0–24 hours, 24–48 hours or 48 hours–9 days respectively. In the adjusted multivariable models in Study I the following covariates were included: age, gender, smoking habits, previous myocardial infarction, previous congestive heart failure, pharmacological treatment, serum creatinine, diabetes duration, blood glucose and coronary interventions (PCI or CABG) before admission and during hospitalization. Stepwise logistic regression was used to predict hypoglycemic episodes during the initial 48 hours of hospitalization.

In Study II covariates known to be predictive from the literature and the DIGAMI 2 study (72) were applied. The same set of variables was used for all endpoints, thus no model building procedures were performed. In the adjusted multivariable models predicting endpoints, the following covariates remained significant: age, smoking habits, previous myocardial infarction, previous congestive heart failure, creatinine at randomization, sex, coronary interventions before admission and during hospitalization, and blood glucose at randomization. No interaction terms were used due to limited power. The statistical models were tested for consistency by adding a propensity score comprising diabetes duration, heart failure, hypertension, blood glucose and lipid lowering drugs as covariates. The inclusion of the linear term of propensity score did not change the results of Study II.
Adjustments were done for the prognostic variables as well as insulin status at discharge. In addition models including updated blood glucose and updated insulin treatment, in order to account for variability in glucose control and the use of insulin, were performed. The use of insulin was updated at each visit until the last one preceding an event or end of the initial period of follow up (December 2003). Updated blood glucose was expressed as the average of all glucose values from the time for hospital discharge until the end of the original follow up.

The same sets of covariates as in Study II were used in Study III. Glucose lowering agent (i.e. insulin, metformin and sulphonylureas) and blood glucose were used as time dependent (updated) variables in the Forest plot. In Study IV and V known independent predictors of outcome in the DIGAMI 2 trial (72) (age, creatinine at admission, glucose at admission, previous heart failure) were adjusted for. In addition gender, BMI, previous myocardial infarction, hypertension and HbA1c at admission were included in the outcome models in Study V. Due to skewed distribution, Copeptin, IGFBP-1 and S-MBL values were log transformed prior to analysis.

For the outcome analysis in Study V, S-MBL was dichotomized below or above a level of 1000 μg/l (122,132). Patients were also classified according to their genotypes in relation to the median S-MBL concentration for respective genotype: high/above = high genotype with S-MBL above the median for this genotype; high/below = high genotype with S-MBL below the median for this genotype; low/above = low genotype with S-MBL above the median for this genotype; low/below = low genotype with S-MBL below the median for this genotype.

Kaplan-Meier curves were drawn in Study I-V to illustrate time trends in mortality and cardiovascular morbidity. Log-rank test for trend were applied to assess differences in event patterns between strata.

Two-tailed statistical tests were used at a 5% significance level. The SAS version 8:02 were used for all statistical analyses in Studies I-II and version 9.2 in Studies III- V.

Ethical considerations

The DIGAMI 2 study conformed to good clinical practice guidelines and followed the recommendations of the Helsinki Declaration. Local ethics review boards approved the protocol. Written informed consent was obtained from all patients prior to enrolment.
Baseline characteristics

The most important clinical characteristics of the different patient cohorts included in this thesis are presented in Table 3.

### Table 3. Baseline characteristics for the patients in Studies I - V.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I (n=1253)</th>
<th>Study II (n=1181)</th>
<th>Study III (n=1145)</th>
<th>Study IV (n=393)</th>
<th>Study V (n=387)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.4 (11.0)</td>
<td>67.9 (11.0)</td>
<td>68.8 (10.9)</td>
<td>68.8 (10.5)</td>
<td>68.9 (10.5)</td>
</tr>
<tr>
<td>Male gender</td>
<td>837 (67)</td>
<td>791 (67)</td>
<td>765 (67)</td>
<td>269 (68)</td>
<td>264 (68)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 (4.7)</td>
<td>29 (4.7)</td>
<td>28 (4.6)</td>
<td>28 (4.7)</td>
<td>28 (4.6)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7.9 (8.3)</td>
<td>8.0 (8.3)</td>
<td>8.1 (8.4)</td>
<td>8.7 (8.8)</td>
<td>8.7 (8.6)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>135 (25)</td>
<td>136 (25)</td>
<td>135 (25)</td>
<td>133 (23)</td>
<td>133 (24)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76 (15)</td>
<td>76 (15)</td>
<td>76 (16)</td>
<td>74 (16)</td>
<td>75 (15)</td>
</tr>
<tr>
<td>Previous medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>423 (34)</td>
<td>397 (34)</td>
<td>400 (35)</td>
<td>148 (38)</td>
<td>149 (39)</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>563 (45)</td>
<td>529 (45)</td>
<td>525 (46)</td>
<td>201 (51)</td>
<td>202 (52)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>220 (18)</td>
<td>208 (18)</td>
<td>214 (19)</td>
<td>78 (20)</td>
<td>77 (20)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>607 (49)</td>
<td>576 (49)</td>
<td>568 (50)</td>
<td>215 (55)</td>
<td>214 (55)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>396 (32)</td>
<td>383 (33)</td>
<td>366 (32)</td>
<td>135 (34)</td>
<td>134 (35)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>300 (24)</td>
<td>293 (25)</td>
<td>264 (23)</td>
<td>84 (22)</td>
<td>84 (22)</td>
</tr>
<tr>
<td>Medication prior to admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>390 (31)</td>
<td>375 (32)</td>
<td>376 (33)</td>
<td>135 (34)</td>
<td>141 (36)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>274 (23)</td>
<td>257 (22)</td>
<td>263 (23)</td>
<td>119 (30)</td>
<td>117 (30)</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>512 (41)</td>
<td>484 (41)</td>
<td>487 (43)</td>
<td>175 (45)</td>
<td>176 (46)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>624 (50)</td>
<td>589 (50)</td>
<td>574 (50)</td>
<td>206 (52)</td>
<td>204 (53)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>390 (31)</td>
<td>373 (32)</td>
<td>365 (32)</td>
<td>124 (32)</td>
<td>122 (32)</td>
</tr>
<tr>
<td>Lipid lowering</td>
<td>349 (28)</td>
<td>338 (29)</td>
<td>325 (29)</td>
<td>117 (30)</td>
<td>119 (31)</td>
</tr>
<tr>
<td>Biochemistry at admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>12.7 (4.5)</td>
<td>12.5 (4.3)</td>
<td>12.8 (4.5)</td>
<td>12.3 (4.5)</td>
<td>12.3 (4.6)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 (1.8)</td>
<td>7.7 (1.8)</td>
<td>7.7 (1.8)</td>
<td>7.3 (1.7)</td>
<td>7.4 (1.8)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>104 (46)</td>
<td>103 (45)</td>
<td>104 (44)</td>
<td>100 (48)</td>
<td>100 (49)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.2 (1.3)</td>
<td>5.2 (1.3)</td>
<td>5.2 (1.3)</td>
<td>5.1 (1.3)</td>
<td>5.1 (1.3)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>2.2 (2.0)</td>
<td>2.2 (2.0)</td>
<td>2.2 (2.0)</td>
<td>2.3 (2.1)</td>
<td>2.3 (2.1)</td>
</tr>
</tbody>
</table>

For categorical variables n (%) is presented.
For continuous variables mean (SD) is presented.
Study I

**Hypoglycemia during the hospital phase in DIGAMI 2**

Hypoglycemic episodes during hospitalization was recorded in 153 of the 1253 DIGAMI 2 patients (12%) and 45 (29%) of these episodes were reported as symptomatic. The majority of episodes occurred during the first 24 hours and mainly in patients randomized to glucose-insulin infusions. There were 111 episodes (23% symptomatic) in study Groups 1 and 2, compared to three episodes (33% symptomatic) in the routinely treated patients in Group 3 (Figure 9).

![Bar chart showing hypoglycemia by treatment group during hospitalization](image)

**Figure 9.** Hypoglycemia (blood glucose <3.0 mmol/l) by treatment group during the initial hospitalization. The shaded area in each bar represents the proportion with symptoms.

Patients experiencing hypoglycemia were older, had a longer duration of diabetes, a lower body weight and body mass index and more often presented with a history of heart failure. HbA1c, admission blood glucose and glucose lowering treatment at admission did not differ between patients with or without hypoglycemia. Body weight (+1 kg; OR 0.97; 95% CI 0.95 to 0.98; p< 0.0001) and diabetes duration (+1 year OR 1.03; 95% CI 1.01 to 1.05; p=0.0085) were independent predictors of hypoglycemia.

**Hypoglycemia and prognosis**

During 2.1 years of follow-up 277 (22.1%) patients died, the majority for cardiovascular reasons (n=233) while 217 (17.3%) patients suffered from a nonfatal re-infarction or stroke. As can be seen in Figure 10 the event rate did not differ between patients with or without hypoglycemia besides for patients with symptomatic hypoglycemia, who were at an increased risk of death (total and cardiovascular). This increased risk for mortality disappeared following adjustment for confounders as described in the statistics section.
Myocardial infarction and diabetes mellitus

Studies II - III

Mortality and morbidity in relation to glucose lowering therapy

The 1181 patients discharged alive after the index infarctions were treated according to the DIGAMI 2 protocol for a median of 2.3 years. At discharge 436 patients were prescribed oral glucose lowering agents, 690 insulin and 176 did not receive any pharmacological glucose lowering treatment (Figure 11).

---

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total mortality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted: HG</td>
<td>1.22 (0.87 to 1.71)</td>
<td>0.2579</td>
</tr>
<tr>
<td>Adjusted: HG</td>
<td>0.91 (0.63 to 1.32)</td>
<td>0.6183</td>
</tr>
<tr>
<td>Unadjusted: symptomatic HG</td>
<td>1.99 (1.20 to 3.29)</td>
<td>0.0076</td>
</tr>
<tr>
<td>Adjusted: symptomatic HG</td>
<td>1.09 (0.64 to 1.87)</td>
<td>0.7403</td>
</tr>
</tbody>
</table>

**Cardiovascular mortality**

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted: HG</td>
<td>1.32 (0.92 to 1.89)</td>
<td>0.1316</td>
</tr>
<tr>
<td>Adjusted: HG</td>
<td>0.99 (0.67 to 1.46)</td>
<td>0.9590</td>
</tr>
<tr>
<td>Unadjusted: symptomatic HG</td>
<td>2.06 (1.20 to 3.53)</td>
<td>0.0090</td>
</tr>
<tr>
<td>Adjusted: symptomatic HG</td>
<td>1.20 (0.69 to 2.09)</td>
<td>0.5181</td>
</tr>
</tbody>
</table>

**Death/stroke/reinfarction**

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted: HG</td>
<td>1.05 (0.79 to 1.40)</td>
<td>0.7249</td>
</tr>
<tr>
<td>Adjusted: HG</td>
<td>0.87 (0.64 to 1.18)</td>
<td>0.3572</td>
</tr>
<tr>
<td>Unadjusted: symptomatic HG</td>
<td>1.35 (0.84 to 2.16)</td>
<td>0.2129</td>
</tr>
<tr>
<td>Adjusted: symptomatic HG</td>
<td>0.87 (0.53 to 1.42)</td>
<td>0.5705</td>
</tr>
</tbody>
</table>

**Figure 10.** Effect of hypoglycemic events (HG), with and without symptoms, during the initial hospitalization on subsequent mortality and morbidity.

**Figure 11.** Distribution of patients on insulin, sulphonylureas and metformin including various combinations of these drugs at the time for hospital discharge.
During follow-up 206 patients died while 162 had a non-fatal myocardial infarction and 54 a stroke. After adjustments for confounders, including glucose control, the risk for non-fatal myocardial infarction and stroke was significantly higher in patients on insulin after discharge whereas this risk was lower among those on metformin and neutral with sulphonylurea (Figures 12 and 13). None of the glucose lowering treatments influenced mortality. A separate analysis on patients with newly instituted insulin (n=317), as outlined in Figure 14, and those randomly allocated to newly instituted insulin (original Group 1; n=245; HR 2.22; 95% CI 1.46–3.35; p=0.0002) confirmed the relation between a higher rate of non-fatal cardiovascular events and insulin treatment.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metformin (200/981)</strong></td>
<td></td>
</tr>
<tr>
<td>Death (33/173)**</td>
<td>0.91 (0.61-1.34)</td>
</tr>
<tr>
<td>CV death (24/139)**</td>
<td>0.93 (0.60-1.43)</td>
</tr>
<tr>
<td>Death/reinfarction/stroke (56/304)**</td>
<td>0.78 (0.58-1.04)</td>
</tr>
<tr>
<td>Reinfarction/stroke (28/176)**</td>
<td>0.63 (0.42-0.95)</td>
</tr>
<tr>
<td><strong>Sulphonylurea (268/913)</strong></td>
<td></td>
</tr>
<tr>
<td>Death (51/155)**</td>
<td>1.08 (0.78-1.50)</td>
</tr>
<tr>
<td>CV death (41/122)**</td>
<td>1.15 (0.80-1.64)</td>
</tr>
<tr>
<td>Death/reinfarction/stroke (80/280)**</td>
<td>0.93 (0.73-1.20)</td>
</tr>
<tr>
<td>Reinfarction/stroke (40/164)**</td>
<td>0.81 (0.57-1.14)</td>
</tr>
<tr>
<td><strong>Insulin (690/491)</strong></td>
<td></td>
</tr>
<tr>
<td>Death (134/72)**</td>
<td>1.12 (0.83-1.51)</td>
</tr>
<tr>
<td>CV death (105/58)**</td>
<td>1.05 (0.75-1.46)</td>
</tr>
<tr>
<td>Death/reinfarction/stroke (243/117)**</td>
<td>1.42 (1.13-1.78)</td>
</tr>
<tr>
<td>Reinfarction/stroke (145/59)**</td>
<td>1.73 (1.26-2.37)</td>
</tr>
<tr>
<td><strong>Any glucose lowering drug (1005/176)</strong></td>
<td></td>
</tr>
<tr>
<td>Death (176/30)**</td>
<td>0.89 (0.61-1.31)</td>
</tr>
<tr>
<td>CV death (139/24)**</td>
<td>0.84 (0.55-1.29)</td>
</tr>
<tr>
<td>Death/reinfarction/stroke (311/49)**</td>
<td>1.04 (0.77-1.41)</td>
</tr>
<tr>
<td>Reinfarction/stroke (179/25)**</td>
<td>1.19 (0.78-1.83)</td>
</tr>
</tbody>
</table>

* Number of patients using drug/number of patients not using drug at discharge
** Number of endpoints for patients using drug/number of endpoints for patients not using drug

**Figure 12.** Effect of different updated glucose lowering treatments on mortality and morbidity. Cardiovascular (CV).

*The impact of extended follow-up*

Morbidity and mortality data from the extended period of follow-up (median duration 4.1 years; IQR 2.6 - 5.4 and a maximum of 8.3 years) was available in 1145 patients whereof
1073 patients had been discharged alive from the index hospitalization. Total mortality was 34% (72% cardiovascular). The impact of glucose-lowering treatment on outcome remained. Thus insulin had a negative impact on non-fatal cardiovascular events (OR 1.90; 95% CI 1.38-2.63; p=<0.0001) and mortality was not influenced (Figure 15). In contrast patients on metformin had a lower total mortality compared with those without such treatment and also a lower risk of dying of malignancies.
The mortality and specific causes of death in relation to randomized treatment group during the whole study period including the extended follow-up are outlined in Table 4. There were no differences in total or cardiovascular mortality between the three randomized groups although non-cardiovascular deaths were more common in Group 1 than 2, HR 1.89 (95% CI 1.11-3.21; p=0.02). The total number of deaths due to malignant diseases was 37 (9.5% of all deaths). The risk of dying of malignancies was highest in patients randomized to long-term insulin; Group 1 compared to Group 2 HR 1.83 (95% CI 0.90-3.71; p=0.096) and Group 1 compared to Group 3 HR 3.57 (95% CI 1.22-10.39; p=0.02).

**Study IV**

*Copeptin and IGFBP-1*

In the 393 patients in Study IV copeptin varied between 0.97 and 1936 (median 21.8; mean 62.4) pmol/l and IGFBP-1 between 3.0 and 677.0 (median 23.0, mean 42.0) μg/l. There was a significant correlation between the two biomarkers as shown in Figure 16.

The impact of copeptin and IGFBP-1 on cardiovascular prognosis

During a median follow-up of 2.5 (1.04-3.00) years, there were 77 cardiovascular deaths (20%), 59 non-fatal myocardial infarctions (15%) and 25 non-fatal strokes (6%) in this subgroup of the DIGAMI 2 cohort.
Myocardial infarction and diabetes mellitus

Table 4. Mortality rates and specific causes of death.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n=431)</th>
<th>Group 2 (n=441)</th>
<th>Group 3 (n=273)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mortality(^a)</td>
<td>153 (35.5%)</td>
<td>147 (33.3%)</td>
<td>89 (32.6%)</td>
</tr>
<tr>
<td>Cardiovascular mortality(^b)</td>
<td>104 (68.0%)</td>
<td>115 (78.2%)</td>
<td>59 (66.3%)</td>
</tr>
<tr>
<td>MI(^c)</td>
<td>53 (51.0%)</td>
<td>61 (53.0%)</td>
<td>27 (45.8%)</td>
</tr>
<tr>
<td>Sudden Cardiac(^c)</td>
<td>29 (27.9%)</td>
<td>28 (24.3%)</td>
<td>20 (33.9%)</td>
</tr>
<tr>
<td>Stroke(^c)</td>
<td>9 (8.7%)</td>
<td>6 (5.2%)</td>
<td>4 (6.8%)</td>
</tr>
<tr>
<td>Long Term Congestive HF(^c)</td>
<td>9 (8.7%)</td>
<td>21 (18.3%)</td>
<td>9 (15.3%)</td>
</tr>
<tr>
<td>Other Cardiovascular(^c)</td>
<td>6 (5.8%)</td>
<td>6 (5.2%)</td>
<td>2 (3.4%)</td>
</tr>
<tr>
<td>Non-Cardiovascular(^b)</td>
<td>38 (24.8%)</td>
<td>21 (14.3%)</td>
<td>17 (19.1%)</td>
</tr>
<tr>
<td>Malignancies(^d)</td>
<td>21 (55.3%)</td>
<td>12 (57.1%)</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>Other non-CV(^d)</td>
<td>17 (44.7%)</td>
<td>9 (42.9%)</td>
<td>13 (76.5%)</td>
</tr>
<tr>
<td>Unknown cause of death(^b)</td>
<td>11 (7.2%)</td>
<td>11 (7.5%)</td>
<td>13 (14.6%)</td>
</tr>
</tbody>
</table>

For categorical variables n (%) is presented.
Percentage represents the number of event in comparison with
a total number of patient in respective group
b total mortality in respective group
c cardiovascular mortality in respective group
d non-cardiovascular mortality in respective group

Figure 16. The correlation between copeptin and IGFBP-1 (Spearman’s correlation coefficient 0.53; p<0.001).
When analyzed separately both copeptin and IGFBP-1 predicted fatal events. Moreover copeptin predicted non-fatal cardiovascular events. In a multiple model including both biomarkers copeptin was the only remaining predictor of cardiovascular events. In the final model, adjusting for age and creatinine clearance, copeptin remained as an independent predictor of all events.

Cardiovascular events increased by increasing copeptin tertiles (log-rank test \( p < 0.001 \); Figure 17). Furthermore cardiovascular deaths within 90 days were related to higher copeptin levels at baseline (Jonckheere-Terpstra test; \( p < 0.0001 \)) and patients with previous heart failure had higher copeptin levels compared to those without (34.9 (IQR 16.7-66.4) pmol/l vs. 19.4 (IQR 8.2-43.4) pmol/l <0.001).

### Table 5. Unadjusted and adjusted predictive ability of copeptin and IGFBP assessed by Cox's proportional hazard regression. (HR = Hazard Ratio; CI = Confidence Interval; MI = Non-fatal re-infarction).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiovascular event (CV death, MI or stroke)</th>
<th>Cardiovascular death</th>
<th>MI or stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Univariable unadjusted (n = 393)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Copeptin</td>
<td>138</td>
<td>1.59 (1.41-1.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log IGFBP-1</td>
<td>138</td>
<td>1.49 (1.26-1.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Multiple model including log Copeptin and log IGFBP-1 (n = 393)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Copeptin</td>
<td>138</td>
<td>1.53 (1.31-1.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log IGFBP-1</td>
<td>138</td>
<td>1.10 (0.90-1.34)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Multiple adjusted (n = 380)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Copeptin*</td>
<td>129</td>
<td>1.35 (1.16-1.57)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* adjusted for age, creatinine clearance
Myocardial infarction and diabetes mellitus

Study V

**MBL phenotype and genotype**

Serum (S)-MBL was determined at hospital admission in 387 patients and MBL2 genotyping was performed in 287 of these them. Median S-MBL was 1212 μg/l (IQR 346 – 2681 μg/l), without a significant gender difference. The S-MBL concentrations in patients grouped according to genotype (encoding high or low S-MBL) and S-MBL level (above or below median S-MBL concentration for respective genotype) are presented in Figure 18.

**Figure 17.** Kaplan-Meier curves for cardiovascular events by copeptin tertiles.

**Figure 18.** S-MBL concentrations in patients grouped according to genotype (encoding high or low S-MBL) and S-MBL level (above or below median S-MBL concentration for respective genotype). 1=high/above (n=78). 2=high/below (n=78), 3=low/above (n=65), 4=low/below (n=66); Jonckheere-Terpstra test; p<0.001.
The impact of MBL phenotype on prognosis

During the follow up period of 2.5 (1.04 – 3.00) years, 136 (35%) patients in the total cohort (n=387) had a cardiovascular event. The corresponding number in the subgroup with both S-MBL and genotype (n=287) was 86 (30%). S-MBL did not correlate with age, BMI, creatinine clearance, glucose or HbA1c. Moreover S-MBL did not predict events (HR 0.93; CI 95% 0.85-1.01; p=0.09). In a univariable Cox regression analysis patients with S-MBL above 1000 μg/l had lower event rates than those below (HR 0.68; 95% CI 0.48- 0.95; p=0.02; Figure 19) but in a multiple model adjusting for significant confounders, using a best subset selection criterion (age, BMI, admission glucose, creatinine clearance and myocardial infarction) the dichotomized S-MBL did not remain as an independent predictor of events (p=0.09).

Grouping the patients according to their genotype and S-MBL above or below the median for their genotype revealed a significant difference in survival free from cardiovascular events (Figure 20; log-rank test p= 0.01). Using the low/below group as a reference in Cox-regression analysis, patients with high/above had a significantly lower event rate (HR=0.49, 95% CI 0.26-0.92; p= 0.03). The prediction capacity of the geno- and phenotype model was only of borderline significance when applying a best subset selection criterion (age, BMI, creatinine clearance and previous myocardial infarction) (p=0.07).

Figure 19. Kaplan-Meier curves for cardiovascular events by S-MBL below (blue) or above (red) 1000μg/l (Log-rank test for trend p=0.02).
Figure 20. Kaplan-Meier curves for cardiovascular events in patients grouped according to genotype (encoding high or low S-MBL) and S-MBL level (above or below median S-MBL concentration for respective genotype) (log-rank test for trend p=0.01). 1=high/above, 2=high/below, 3=low/above, 4=low/below.
GENERAL DISCUSSION

Patients with diabetes are particularly prone to develop atherosclerosis and thereby various manifestations of cardiovascular disease not the least myocardial infarction. They are subsequently and despite some therapeutic progress still at a considerably higher risk for fatal and non-fatal complications than patients without diabetes (10,133). The present thesis focuses on factors of potential prognostic implication in this context: hypoglycemia, choice of glucose lowering agents and novel risk markers. These factors were studied in patients with type 2 diabetes and myocardial infarction, all participating in the second DIGAMI trial.

The DIGAMI 2 study population

All studies in this thesis are epidemiological reports based on the DIGAMI 2 cohort. The main hypothesis in DIGAMI 2, that insulin-based, strict metabolic control in patients with diabetes and myocardial infarction would reduce mortality and morbidity compared to conventional treatment, could not be confirmed. The most obvious reason, an important limitation in the randomized part of DIGAMI 2, was the lack of a difference in glucose control between the three treatment groups. This shortcoming did, however, open for the possibility to combine the DIGAMI 2 patients into a single, epidemiological cohort suited for post-hoc analyses as mirrored by the present thesis. The database, one of the largest of its kind, offered a possibility to study a carefully defined group of patients with diabetes and myocardial infarction. Considering the very few exclusion criteria the results should be representative for such patients in a general perspective. This is further underlined by similar baseline characteristics in the different subgroups in Studies I-V and the total patient cohort (Table 3). The characteristics of the DIGAMI 2 patients are very close to those reported from the Swedish Coronary Care Unit database from 1995-2002 (10). Another benefit is the substantial number of thoroughly adjudicated endpoints (mortality and non-fatal myocardial infarction and stroke) and that none of the patients was lost during the initial period of follow-up. Moreover glucose lowering treatment, glucose control and hypoglycemic episodes during the hospital phase, all important parts in Studies I-V, were prospectively collected and updated over time. These factors are all advantages compared with reports based on retrospectively analyzed registries. Still it should be noted that although the present data are prospectively collected, they are not derived from randomized treatment. Thus the findings should be interpreted with caution as hypothesis generating observations in need of confirmation in prospective trials.

Hypoglycemia during hospitalization

Hypoglycemia is a dreaded complication to intensive glucose control. The fear for hypoglycemia has been mentioned as an obstacle in attempts to achieve normoglycemia in a long and short term perspective (72,73,75). The risk to develop hypoglycemia is indeed increased in patients prescribed intensive glucose lowering treatment (134), in one study 5.2% compared with 0.8% in conventionally treated patients (135). The risk for hypoglycemia was reported as high as 15 % in patients with diabetes and myocardial infarction prescribed glucose-insulin infusions with the intention to reach normoglycemia (70). A meta-analysis
reported on a nearly three times higher risk compared with controls (136). The potential negative cardiovascular effects of hypoglycemia include abnormal myocardial repolarisation due to increasing plasma adrenalin and low potassium causing QT-prolongation, arrhythmias (137) and aggravation of myocardial ischemia (138,139).

A limitation when studying the impact of hypoglycemia is the lack of a generally accepted glucose level below which hypoglycemia is defined (140,141). Symptoms usually starts at 2.8 – 3.2 mmol/l but the threshold is dynamic with inter- and intraindividual variations depending on age, existing comorbidities, diabetes duration and the actual glucose control. Traditionally the diagnosis is based on Whipple’s triad comprising a combination of confirmed low blood glucose and symptoms typical for hypoglycemia that are relieved by normalizing the glucose levels (142). Symptoms do, however, not occur in all patients making the definition of hypoglycemia according to Whipple less useful in clinical trials. Another limitation is that the border for potentially harmful cardiovascular effects is unknown. This was the reason to define hypoglycemia as the recording of a pre-specified glucose level <3 mmol/l, with or without symptoms in Study I. Applying an even lower cut-off level of 2.7 mmol/l in patients receiving a glucose-insulin infusion did not influence the results.

The main finding in Study I is that symptomatic hypoglycemia is associated with a worse outcome, however, not per se but rather by expressing other factors of a more direct prognostic implication. These results gain support from other studies, among them a recent report from the Health Facts database on patients with acute myocardial infarctions revealing a J-shaped relationship between average glucose and mortality, which was increased both by hypo- and hyperglycemia recorded during hospitalization (143). The authors suggested that the higher risk of death related to low blood glucose may be explained by concomitant conditions that worsens the prognosis. A subsequent report from the same registry expanded these observations by only including patients suited for glucose lowering treatment because of hyperglycemia (≥ 7.8 mmol/l). Patients, who developed an episode of hypoglycemia defined as a random glucose of less than 3.3 mmol/l had a higher in-hospital mortality than patients without such episodes (144). However, hypoglycemia did not relate to increased mortality in patients given insulin since following multivariable adjustment it only remained as a predictor in those without such treatment. Pinto et al. (57,61), pooling data from several trials on STEMI patients, noted a U-shaped relation between 30-day mortality and blood glucose. The risk of death was two- to four-times higher with high or low blood glucose compared to normoglycemia. The impaired outcome among patients with low glucose seemed to identify people at high risk for other reasons among them heart failure and low body weight. Only a minor proportion of these patients had a diagnosis of diabetes reducing the probability of insulin-induced hypoglycemia. Svensson et al. studied the association between glucose values during hospitalization of 713 patients with diabetes and unstable angina or non-Q-wave myocardial infarction (58). Hypoglycemia during hospitalization was an independent predictor of mortality during two years of follow up in this retrospective analysis.

One may speculate that there are different pathophysiological mechanisms between iatrogenic and spontaneous hypoglycemia. A number of conditions such as shock, multi-organ failure, sepsis, renal and liver dysfunction and malnutrition are associated with low blood glucose (145,146). It is reasonable that such patients are vulnerable. Accordingly hypoglycemia may be a marker of severe underlying disease rather than to trigger mechanisms causing an increased risk for mortality. Indeed Fischer et al. already in 1986 showed that hospital
mortality was related to the degree of hypoglycemia during hospitalization, however it was not apparently caused by low glucose in itself but rather related to comorbidities (147).

It should, however, not be ruled out that hypoglycemia may be more serious for the critically ill patient, often with a compromised counter regulatory capacity (148,149). Moreover spontaneous hypoglycemia seems to relate to, and in some studies explain, the increased mortality in the intensive care unit (148,150). In DIGAMI 2 patients with hypoglycemia during hospitalization, in contrast to those without such episodes, were older with lower body weight, longer diabetes duration and a higher prevalence of heart failure suggesting an increased risk to induce hypoglycemia in patients with predisposing conditions. Furthermore patients with longer diabetes duration might have a more advance disease and defect counter regulatory defense against hypoglycemia.

An explanation to the discrepancies between Study I, in which hypoglycemia in itself did not relate to an unfavorable long-term prognosis, and the reports on a J- or U-shaped relation between blood glucose and prognosis in patients with acute myocardial infarction is that DIGAMI 2, due to the study objective, is much more likely to reflect insulin-induced rather than spontaneous hypoglycemia than the other studies. Moreover the DIGAMI 2 patients were, as instructed in the protocol, cautiously followed with the intention to rapidly detect and intervene against hypoglycemic episodes. In registry based, retrospectively followed patient populations low glucose might only have been detected if the patient became symptomatic with the implication that reported episodes may have been profound and/or long lasting and thereby more harmful (57,58,61,143).

The lack of prognostic impact of hypoglycemia in Study I, in which the episodes were recorded during hospitalization, cannot be transferred to an out-patient setting where hypoglycemia may not be as easily detected.

In conclusion Study I indicates that the fear of increasing cardiovascular morbidity by the induction of hypoglycemia should not be looked upon as a contraindication to improve metabolic control in patients under continuous supervision.Vulnerable patients should be treated with special attention or maybe not at all with an intensive glucose lowering. Moreover this study shows that it is possible to supervise a continuous insulin-infusion during hospitalization by means of intermittent tests of venous blood.

Glucose lowering treatment and prognosis

Cardiovascular mortality and morbidity

Until recently glucose lowering drugs were mainly tested in short term investigations focusing on their ability to control hyperglycemia. Few clinical trials studied their effects on mortality and cardiovascular events explaining the lack of evidence based glucose lowering strategies. The UKPDS Group was the first to report on mortality benefits of metformin in overweight patients with newly diagnosed type 2 diabetes (79). Patients randomized to metformin had lower all-cause mortality and fewer strokes than those receiving chlorpropamide, glibenclamide or insulin. Further support was provided by the 10 year extended follow-up of this study reporting a highly significant, 33% reduction of myocardial infarction (p=0.005) and 27% of total mortality (p=0.002) in patients originally treated with metformin compared to conventional treatment (76). In contrast data from a pooled analysis of controlled clinical trials of thiazolidinediones demonstrated controversial results concerning a potential risk of cardiovascular events, mortality and heart failure (82,83). The importance to further explore
potential cardiovascular benefits or drawbacks with glucose lowering agent caused the US Federal Drug Agency (FDA) to issue recommendations to the industry that all new glucose lowering drugs should be evaluated as regards their impact on mortality and cardiovascular events. Until such trials have been completed we have to rely on data from post hoc analysis and the few prospective trials available.

The DIGAMI 2 epidemiological database offered an opportunity to contribute knowledge on the impact of different glucose lowering strategies on cardiovascular outcomes in patients with type 2 diabetes and myocardial infarction. Despite inherent limitations with post-hoc analyses in a general perspective the present material offers important information. As previously mentioned the patients were prospectively followed as regards glucose lowering treatment and glucose control. Although the outcome should be interpreted with caution Studies II and III clearly indicate that the agent used to achieve glucose control has prognostic implications with insulin seemingly associated with an impaired cardiovascular prognosis while metformin appears protective. Thus Study II revealed that non-fatal cardiovascular events were significantly more common in patients on insulin even after adjustments for a number of confounders including updated glucose control and concomitant treatment. Further analyses were performed to rule out the effect of already ongoing insulin therapy, which may reflect a more long-standing or severe diabetes. This did not affect the outcome. On the contrary the risk appeared even stronger in patients on newly instituted insulin (HR 1.95; 95% CI 1.35–2.82; p=0.0003) and in those, who according to the protocol were randomized to such treatment (HR 2.22; 95% CI 1.46–3.35; p=0.0002). During the initial period of observation lasting up to 3 years (median 2.3) the increased risk for non-fatal events was not accompanied by an increased mortality. It was, however, speculated that this could happen during an extended follow-up. Study III, including information on 91% of the original DIGAMI 2 cohort during a follow up to 8.3 years (median 4.1), confirmed the increased risk of non-fatal events associated with insulin treatment. At this time there was a trend towards increased mortality among patients on insulin (OR 1.30; 95% CI 0.94-1.80; p=0.11) and it may be that even longer periods of follow-up are needed to disclose such effect.

The present findings, consolidates observations from registry based reports that exogenous insulin may increase the risk for myocardial infarction and impair the prognosis (86,87,151). As an example the Euro Heart Survey on diabetes and the heart enrolled 4676 patients with coronary artery disease of whom 1425 had known diabetes. The impact of different glucose lowering modalities on cardiovascular mortality was followed during one year. Insulin treated patients with known diabetes had an adjusted hazard ratio 2.23 (95% CI 1.24-4.03; p=0.006) compared to those on oral glucose lowering drugs (151). Insulin treatment has also been associated with an increased risk for heart failure and increased mortality in heart failure patients (84,85).

There are several potential mechanisms that may contribute to these harmful effects. As already discussed hypoglycemia may be one explanation. Another mechanism may relate to direct effects of insulin on the vessel wall and hemodynamics. Patients with type 2 diabetes requiring insulin treatment are in an insulin resistant state with high levels of endogenous insulin to which the exogenous insulin is added. As discussed in the introduction the hormone might act via the MAPK pathway leading to anabolic, vasoconstrictive and subsequent pro-atherosclerotic effects. Moreover exogenous insulin has been related to endothelial dysfunction (152), increased inflammatory activation (153) and platelet dysfunction (154). Finally insulin may act on the IGF-1 receptor with subsequent anabolic effects (155).
Since high levels of insulin seems to have negative effects concerns have also been raised as regards drugs increasing endogenous insulin, so called insulin providers, for example sulphonylureas (80,86,87,156). Sulphonylureas may in addition have direct negative effects on the myocardium by inhibiting the opening of ATP-sensitive potassium channels not only in the pancreatic β-cell but also in myocytes thereby interacting with ischemic preconditioning, coronary vasorelaxation and diminishing myocardial contractile strength (157). Reviews on the theme seem reassuring especially with the use of the second generation of sulphonylureas such as glimepiride, claimed to be more specific to the pancreatic ATP-dependent potassium channels and less active in myocardial and vascular tissue (158,159). Data have, however, so far been inconclusive for patients with myocardial infarction. This makes the findings in Studies II and III of particular interest, supporting the notion that sulphonylureas do not affect the prognosis, at least not when compared with other compounds such as insulin and metformin.

Of the different glucose lowering alternatives analyzed within the context of DIGAMI 2 metformin was the most beneficial. Although this observation originates from a non randomized post-hoc analysis the present results strongly supports previous reports on beneficial effects of metformin in patients with newly diagnosed diabetes (79) extending them to patients with already established cardiovascular complications. In addition to the decreased risk of non-fatal cardiovascular events patients on metformin had a lower mortality during the prolonged follow-up in Study III. It is most likely that the beneficial effect is related to a combination of mechanisms. As reviewed by Bailey metformin may have anti-atherosclerotic effects independent of glucose control (160). Metformin stimulates the AMP-activated kinase (AMPK), a key regulator of cellular energy balance and substrate metabolism, inhibiting the hepatic gluconeogenesis, contributing to improved endothelial function and increased insulin sensitivity in adipose tissue and peripheral muscles (161-164). It has been suggested that the AMPK effect in endothelial cells is mediated by an activation of the PI3K pathway (162).

**Malignancies**

A concerning finding in the original report from DIGAMI 2 (72) was the higher death rate due to malignancies, although small in number, among Group 1 patients, randomized to insulin based treatment, than in those in Groups 2 and 3. Thus another reason to perform an extended follow up was to observe if this increased risk remained over time. This study (III) revealed that although total mortality was similar in the three randomized treatment groups patients randomized to insulin (Group 1) were at a higher risk of dying of malignant diseases than those randomized to non-insulin based glucose lowering therapy (Group 3). Despite the low number of such deaths the difference reached statistical significance. A separate analysis on the impact of long-term insulin treatment revealed a trend towards a higher rate of deaths in malignant conditions. In contrast patients on metformin had a significantly lower likelihood to die of malignancies.

The present findings must be taken with great caution. The patients were not randomized to metformin and interaction analysis could not be performed due to the small number of events. The finding that insulin may be associated with mortality caused by malignancies does, however, gain support in a recent registry study in which patients on insulin or insulin providers were more likely to develop solid cancers than those on metformin. Adding metformin to insulin or sulphonylurea reduced the risk of cancer (165). Diabetes per se is,
related to malignant diseases (30-33) and several reports have advocated that insulin might further increase this risk (165-170). It has been discussed whether insulin induces new malignancies or acts as an accelerator of already transformed cells. As already described high levels of circulating insulin caused by insulin resistance may change the cellular response to insulin altering growth signals perhaps via the MAPK-kinase or activation of IGF-1 receptors. It may also cause resistance to apoptosis, which predisposes to the survival and proliferation of malignant cells (171-173).

An alternate interpretation of the findings in Studies II and III is that the seemingly negative impact of insulin on malignant diseases may be explained by a beneficial effect of the drugs to which insulin is compared. Both alternatives have support from mechanistic investigations. Metformin has been reported to protect against cancer (165,167,174). The beneficial effects of metformin are thought to be mediated by the AMPK pathway, perhaps via growth inhibition (164,175,176).

In conclusion the present observations make it of great importance to further study the impact of glucose lowering agents not only on their capacity to lower glucose but also their influence on cardiovascular morbidity and mortality and malignant conditions.

**Mortality after a myocardial infarction**

The high mortality in the DIGAMI 2 cohort further emphasizes the importance of searching for management strategies with the capacity to improve the poor prognosis in patients with diabetes and myocardial infarction (10,133). The already impressive mortality of 18.4% in the original follow up (72) increased to 34% in the extended follow up (median 4.1 year). The majority of deaths were caused by cardiovascular reasons, 72%, but other factors such as malignancies were also important.

The results of Studies II and III may also illustrate how difficult it is to improve the prognosis in these patients. The DIGAMI 2 patients were on extensive, evidence based treatment by the end of the original follow-up (72) (beta-blockers >80%, aspirin 80%, ACE-inhibitors/angiotensin receptor blockers 65%, and lipid-lowering drugs 75%). Moreover almost all patients eligible for acute revascularization received such treatment, mostly as thrombolysis. It is important not to see the high mortality as a result of ineffectiveness of evidence based treatment. These drugs are well documented as prognostically beneficial and patients with diabetes benefit as much as those without (177). Early start of multifactorial intervention in patients with established type 2 diabetes complicated by microalbumuria is remarkably rewarding as shown by the STENO 2 study (178,179). Moreover patients with diabetes and stable angina eligible for revascularization had a similar prognosis if they were treated with optimal medical treatment (including lipid lowering, antihypertensive treatment, lifestyle interventions and insulin sensitizers or insulin providers) compared with revascularization (CABG or PCI)(180,181).

**Novel risk markers**

Epidemiologically risk expresses that exposure to a certain factor (e.g. hyperglycemia) increases the probability to fall ill in a defined disease (e.g. retinopathy). Two terms not infrequently used without defining the difference them in between are “risk factor” and “risk marker”. Risk factor usually expresses a lifestyle aspect, environmental exposure or characteristic which on the basis of epidemiological evidence is associated with health-
related conditions considered important to prevent (182). In contrast risk marker is often seen as an entity associated with an increased probability for unfavourable outcome or to acquire a disease although the exact pathogenetic mechanism may be unknown. In the present context the term risk marker was preferred when, as in Studies IV and V, novel variables of prognostic implications were searched for, not the least since potential causal relationships, as will be further discussed, are largely unknown. The benefit of novel risk markers in comparison to traditional risk scores has been questioned. In a study evaluating a large number of biomarkers in the populations based Framingham Heart Study the “multimarker score” resulted in a very modest increase in the ability to classify risk compared to the standard risk model (183). However, risk evaluation in patients with established diabetes and cardiovascular disease may be more rewarding in particular if one, as in Studies IV-V, searches for markers of potential pathogenetic and thereby future therapeutic implications. These subjects are as already discussed at a particularly high risk for fatal and non-fatal cardiovascular events that only to a limited extent has improved with modern management.

**Copeptin and IGFBP-1**

High levels of IGFBP-1 are related to impaired prognosis in patients with myocardial infarction (100) or critically illness (102). The mechanism that increases IGFBP-1 in these states is still unclear. IGFBP-1 is mainly produced by the liver (99) and largely regulated by the inhibitory effect of insulin (184). The IGFBP-1 and insulin ratio is increased in the above-mentioned states (185) perhaps as a consequence of hepatic insulin resistance induced by hypoxia and pro-inflammatory cytokines (185,186). The present observation of a correlation between the levels of copeptin and IGFBP-1, in combination with the previously described stimulatory effect of desmopressin on the serum levels of IGFBP-1 (103), suggests that there is a pathogenetic relationship between vasopressin and IGFBP-1. This assumption gains further support by the observation in Study IV that IGFBP-1 loses its predictive value for cardiovascular events when copeptin is introduced into the multivariable model.

Copeptin, a surrogate marker of vasopressin secretion, is a good prognostic marker of cardiovascular outcome after myocardial infarction (95). It had indeed a greater power to predict mortality than BNP and NT-proBNP in patients who developed heart failure (96). There are several mechanisms by which activation of the AVP system may be detrimental after a myocardial infarction among them increasing left ventricular after- and preload due to vasoconstriction and reabsorption of water in the renal tubules respectively (91) or by cardiac remodelling (187).

**Study IV** adds IGFBP-1 as a new effector of the vasopressin-mediated stress response in myocardial infarction, at least in patients with diabetes. The way in which vasopressin induces IGFBP-1 in patients such as those in the present study remains to be established. Vasopressin acts via three types of receptors: $V_{1a}$ with mainly vasopressor effects, $V_2$ that exerts an antidiuretic effect and the $V_{1b}$ receptor that modulates ACTH release (91). IGFBP-1 mediation warrants further investigation not the least since it may have therapeutic implications. Clinical trials with vasopressin receptor antagonists (vaptans) have produced mixed results, so far without beneficial cardiovascular effects. The present vaptans act primarily on the $V_2$ receptor (Lixivaptan, Tolvaptan and Satavaptan) and Conivaptan is the only dual receptor antagonist (91). It may well be that an unspecific dual effect of a vaptan is needed in the present clinical scenario by interfering with the new pathogenic mechanism that this study suggests.
An increased level of serum copeptin has recently been proposed as an early marker in patients with acute myocardial infarction (188). It is therefore not surprising that the copeptin levels in the present patient population are much higher than those in healthy individuals (94). Interestingly, the levels were not higher than in other studies of patients with myocardial infarction (95,96,188), even though the levels of copeptin are particularly high in patients with diabetes (95). This may reflect the very high proportion, about two thirds, of patients with glucometabolic perturbations in patients with coronary artery disease (5,6). That copeptin was higher in patients with previously known heart failure is in accordance with previous reports (96-98,189). The pathogenic relationship is further confirmed since heart failure, an independent event predictor in the original DIGAMI 2 analyses (72), disappeared in the present model adjusted for copeptin.

A potential limitation with Study IV is that although this study was a prospectively planned biochemical part of DIGAMI 2 it is still of observational character, thereby limited to the available subpopulation. The lack of a measure of hemodynamic confounders such as serum osmolality may be seen as draw back. However, copeptin and IGFBP-1 were intentionally sampled soon after hospital admission i.e. before the initiation of study related or other treatments that could have influenced the biomarkers.

In conclusion Study IV supports previous findings on a correlation between the vasopressin and the IGF-1 system (103) revealing that copeptin, a surrogate marker of vasopressin, at least partially may explain the prognostic impact of IGFBP-1 in patients with diabetes and myocardial infarction. The findings expand previous knowledge that copeptin is a prognostic predictor in patients with myocardial infarction by verifying this in a population of patients with type 2 diabetes, even adjusting for glucose control at admission, and it may open for novel therapeutic attempts.

MBL

The primary aim with Study V was to characterize geno-and phenotypes in patients with type 2 diabetes and myocardial infarction. Circulating MBL is strongly genetically determined with, by unknown reasons, different genotype distribution in different populations. In European populations a majority of persons (≥60%) have the AA genotype, followed by AO (one allele mutation; ≥36% and OO (two allele mutations; ≥4%). In contrast Quechua Amerindians in Peru have the opposite pattern with 7%, AA, 28% AO and 65% OO (107,108).

Although individual MBL levels are quite stable it acts as a modest acute phase reactant influenced by stress hormones, glucocorticoids and growth hormones (112). This property may be an explanation for the rather high MBL levels (median of 1212 µg/l) seen in Study V, in which it was measured at admission for an acute myocardial infarction. In an out-patient setting the median S-MBL was 666 µg/l in patients with type 2 diabetes and 728 µg/l in healthy controls (132). In a study of patients with acute coronary syndrome and matched controls, the patients with myocardial infarction had higher MBL (median 855 µg/l) than controls (median 441 µg/l; p<0.0001) supporting this explanation (116).

The role of MBL in cardiovascular diseases appears two sided. Studies on microvascular disease in diabetes and reperfusion/ischemia injury in animal models and humans have related low MBL to a better prognosis, suggesting less inflammatory and complement activation. Study V does not provide an answer to the question whether a low or a high S-MBL and which genotype that is harmful in a cardiovascular perspective among patients with type 2 diabetes and myocardial infarction but adds some information. In the unadjusted analysis, S-MBL below 1000 µg/l and
the combination of a low genotype with S-MBL below the median for this genotype related to a worse outcome. However following adjustments for covariates neither S-MBL alone nor the dichotomized S-MBL (below or above 1000 µg/l) or the combination of geno- and phenotype had a definite prognostic implication. The reason to use 1000 µg/l as a cut-off was based on experiences from previous studies in which it provided a high sensitivity and specificity for risk prediction (122,132). The attempt to use the combination of genotype and MBL levels below or above the median for the respective genotype was the interest in combining information on genetic susceptibility and ongoing inflammation.

A limitation with **Study V** is that patients with available geno- and phenotypes had a lower event rate than the total cohort. A possible explanation is that genotypes were measured at the time of hospital discharge, eliminating patients who died during the hospital phase from the genotyped subgroup. The results of the correlation and regression analyses for continuous S-MBL were, however, similar in the two groups, suggesting that findings in the genotyped group are representative of all patients. Still it cannot be excluded that the inability of the geno- and phenotype combination to predict risk in the present cohort may be due to lack of power. Several studies have, however, reported on similar findings as those in **Study V**, linking a low S-MBL or genotypes associated with low S-MBL to cardiovascular disease (119-122,190). **Study V** also indicates that easily available factors such as age and renal function are more useful as clinical risk markers than S-MBL.

In conclusion **Study V** indicates that patients with type 2 diabetes and myocardial infarction have a similar MBL2 genotype distribution as the general population and that S-MBL does not significantly add information on cardiovascular prognosis beyond that contributed by traditional risk markers.

**Future implications**

The present thesis shows that the prognosis for patients with diabetes and myocardial infarction remains poor underlining the need to improved management strategies. These strategies should not be restricted to glucose control but focus on a comprehensive and target driven multifactorial intervention. Although hypoglycemia during hospitalization does not affect the prognosis per se it is preferable to avoid such complication. Improved tools to guide the glucose lowering treatment would be beneficial especially in vulnerable patients. They would hopefully decrease the risk of insufficient glucose control caused by fear of the health care professionals to induce hypoglycemia. Continuous glucose monitoring by microdialysis is a promising technology (191).

That glucose lowering agents affect the prognosis highlights the importance to evaluate the cardiovascular effects of such drugs as soon as possible during drug development and marketing. The potentially negative effects of insulin deserve further evaluation. In the mean time it is important to lower glucose sufficiently when insulin is used not to end up with the possible negative effects in addition to the known negative effects of hyperglycemia. Since metformin seems to have positive effects on the cardiovascular system and also on tumour cells drugs involving similar mechanisms such as insulin sensitization, probably via the AMPK pathway, are of interest. Furthermore new treatments should be investigated. The finding of a prognostic impact of vasopressin system and the relation to IGF-1 system offers a potential treatment target. Activation of the innate immune system measured by the key player MBL did not affect the prognosis in the present cohort but several other pathways, including activators and inhibitors, are involved in this part of the inflammatory system and remains to be further explored.
CONCLUSIONS

1. Hypoglycemia during hospitalization for a myocardial infarction is not an independent risk factor for future mortality and cardiovascular morbidity but is more prevalent in patient at high risk for other reasons.

2. Glucose lowering agents seems to impact cardiovascular morbidity, mortality and deaths from malignancies in patients with type 2 diabetes surviving an acute myocardial infarction. Insulin appears to be harmful while metformin appears beneficial.

3. Copeptin, a surrogate marker for vasopressin, appears to explain at least some of the prognostic impact of IGFBP-1 in patients with diabetes and myocardial infarction. This observation may open for new therapeutic attempts.

4. MBL, an activator of the complement system, did not have a significant impact on prognosis in patients with type 2 diabetes and acute myocardial infarction.
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