Acute myocardial infarction and glucose abnormalities

Novel risk markers and characteristics

Märit Wallander
The figure on the cover, which is a reprint of a wood engraving from Lübeck 1519, represents medical diagnosis gained by investigation of the urine. The diagnosis of diabetes mellitus was based on the sweet taste. Reprinted with permission from the Hagströmer medico-historical library at Karolinska Institutet.
Acute myocardial infarction and glucose abnormalities - novel risk markers and characteristics

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Stockholm 2009
ABSTRACT

**Background:** There is a strong relationship between abnormal glucose tolerance (AGT) and the occurrence of an acute myocardial infarction (AMI). The identification of novel risk markers and pathophysiological disease characteristics may add important information to our understanding of the reasons for the disease pattern and thereby open the door to new therapeutic opportunities in this high-risk group of patients.

**Aims:**
1. To characterise patients with AMI and newly discovered AGT as regards their beta-cell function (Study I)
2. To investigate the long-term reliability of the early classification of glucose perturbations by means of an oral glucose tolerance test (OGTT) in patients with AMI without previously known glucose abnormalities (Study II)
3. To investigate the potential relationships between novel risk markers from the IGF-I system and the adipokines and future cardiovascular events and glucose tolerance in patients with AMI with and without glucose abnormalities (Studies III-V)

**Studies I-III and V:** A total of 181 AMI patients (the GAMI study: 125 men, 56 women; mean age 63.5 ± 9.4 years) were enrolled and 168 of them were classified by means of an OGTT before hospital discharge as having normal glucose tolerance (NGT, n=55), impaired glucose tolerance (IGT, n=58) or type 2 diabetes (n=55). Classifications were repeated three and 12 months thereafter. Age- and gender-matched subjects from the background population served as controls (n=185, 127 men, 58 women; mean age 64.4 ± 9.2 years). Beta-cell function was quantified as the insulinogenic index (IGI) 30 minutes after the glucose load. The associations between levels of IGF-I, IGF binding proteins 1 and 3 (IGFBP-1, IGFBP-3), leptin, adiponectin, glucose metabolism and future cardiovascular events were studied. The studies revealed that patients with AMI and AGT have reduced beta-cell function compared with patients with AMI and NGT (Study I). These patients can be detected and reliably classified from a long-term perspective using an OGTT when they are discharged from the coronary care unit (Study II). Furthermore, patients with AMI and glucose abnormalities have lower levels of IGF-I compared with patients with NGT and controls (Study III). Moreover, high levels of leptin on the first morning after the AMI are associated with the presence of AGT at discharge and with a more serious long-term prognosis (Study V).

**Study IV:** In the DIGAMI 2 trial, 1,253 patients with AMI and type 2 diabetes were randomised to one of three study arms receiving: a) a 24-hour insulin-glucose infusion followed by subcutaneous insulin-based, long-term glucose control, b) the same initial treatment followed by glucose-lowering treatment according to local practice or c) glucose-lowering treatment according to local practice. The main objective, to compare total mortality and morbidity between these management strategies, revealed no significant differences between the treatment groups regarding the primary (total mortality) or the secondary (mortality, non-fatal MI or stroke) endpoints. A total of 575 of the DIGAMI 2 patients participated in a biochemistry programme with repeated blood sampling during 12 months of follow-up. In these 575 patients, the associations between IGF-I, IGFBP-1 and future cardiovascular events were studied. A high level of IGFBP-1 at admission was a strong predictor of cardiovascular mortality and morbidity.

**Conclusion:** Glucose abnormalities in patients with AMI without previously known type 2 diabetes are related to impaired beta-cell function. These patients can be detected with an OGTT as early as day 4-5 after the AMI and the classification of the glucometabolic state is reliable in a long-term perspective. Furthermore, low levels of IGF-I are related to glucose abnormalities, while high levels of leptin are related to both glucose abnormalities and the subsequent prognosis in AMI patients without previously known type 2 diabetes. Moreover, high levels of IGFBP-1 are related to morbidity and mortality in patients with AMI and established type 2 diabetes. These findings add important information and will hopefully lead to studies aimed at improving management strategies and risk assessments in these patient groups.
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Novel risk markers and characteristics

Märit Wallander
To my parents

"It is that which we do know which is a great hindrance to our learning that which we do not know."

Claude Bernard (1813-1878)
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PAPER I-V
ABSTRACT

Background
There is a strong relationship between abnormal glucose tolerance (AGT) and the occurrence of an acute myocardial infarction (AMI). The identification of novel risk markers and pathophysiological disease characteristics may add important information to our understanding of the reasons for the disease pattern and thereby open the door to new therapeutic opportunities in this high-risk group of patients.

Aims
1. To characterise patients with AMI and newly discovered AGT as regards their beta-cell function (Study I)
2. To investigate the long-term reliability of the early classification of glucose perturbations by means of an oral glucose tolerance test (OGTT) in patients with AMI without previously known glucose abnormalities (Study II)
3. To investigate the potential relationships between novel risk markers from the IGF-I system and the adipokines and future cardiovascular events and glucose tolerance in patients with AMI with and without glucose abnormalities (Studies III-V)

Studies I-III and V
A total of 181 AMI patients (the GAMI study: 125 men, 56 women; mean age 63.5 ± 9.4 years) were enrolled and 168 of them were classified by means of an OGTT before hospital discharge as having normal glucose tolerance (NGT, n=55), impaired glucose tolerance (IGT, n=58) or type 2 diabetes (n=55). Classifications were repeated three and 12 months thereafter. Age- and gender-matched subjects from the background population served as controls (n=185, 127 men, 58 women; mean age 64.4 ± 9.2 years). Beta-cell function was quantified as the insulinogenic index (IGI) 30 minutes after the glucose load. The associations between levels of IGF-I, IGF binding proteins 1 and 3 (IGFBP-1, IGFBP-3), leptin, adiponectin, glucose metabolism and future cardiovascular events were studied. The studies revealed that patients with AMI and AGT have reduced beta-cell function compared with patients with AMI and NGT (Study I). These patients can be detected and reliably classified from a long-term perspective using an OGTT when they are discharged from the coronary care unit (Study II). Furthermore, patients with AMI and glucose abnormalities have lower levels of IGF-I compared with patients with NGT and controls (Study III). Moreover, high levels of leptin on the first morning after the AMI are associated with the presence of AGT at discharge and with a more serious long-term prognosis (Study V).

Study IV
In the DIGAMI 2 trial, 1,253 patients with AMI and type 2 diabetes were randomised to one of three study arms receiving: a) a 24-hour insulin-glucose infusion followed by subcutaneous insulin-based, long-term glucose control, b) the same initial treatment followed by glucose-lowering treatment according to local practice or c) glucose-lowering treatment according to local practice. The main objective, to compare total mortality and morbidity between these management strategies, revealed no significant differences between the treatment groups regarding the primary (total mortality) or the secondary (mortality, non-fatal MI or stroke) endpoints. A total of 575 of the DIGAMI 2 patients participated in a biochemistry programme with repeated blood sampling during 12 months of follow-up. In these 575 patients, the associations between IGF-I, IGFBP-1 and future cardiovascular events were studied. A high level of IGFBP-1 at admission was a strong predictor of cardiovascular mortality and morbidity.

Conclusion
Glucose abnormalities in patients with AMI without previously known type 2 diabetes are related to impaired beta-cell function. These patients can be detected with an OGTT as early as day 4-5 after the AMI and the classification of the glucometabolic state is reliable in a long-term perspective. Furthermore, low levels of IGF-I are related to glucose abnormalities, while high levels of leptin are related to both glucose abnormalities and the subsequent prognosis in AMI patients without previously known type 2 diabetes. Moreover, high levels of IGFBP-1 are related to morbidity and mortality in patients with AMI and established type 2 diabetes. These findings add important information and will hopefully lead to studies aimed at improving management strategies and risk assessments in these patient groups.
LIST OF ORIGINAL PAPERS

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

I

II

III

IV

V
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>AGT</td>
<td>Abnormal Glucose Tolerance</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute Myocardial Infarction</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DIGAMI 2</td>
<td>Diabetes mellitus and Insulin Glucose infusion in Acute Myocardial Infarction</td>
</tr>
<tr>
<td>EASD</td>
<td>European Association for the Study of Diabetes</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>FFAs</td>
<td>Free Fatty Acids</td>
</tr>
<tr>
<td>FSIGT</td>
<td>Frequently Sampled Intravenous Glucose tolerance Test</td>
</tr>
<tr>
<td>GAMI</td>
<td>Glucose tolerance in patients with Acute Myocardial Infarction</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon Like Peptide 1</td>
</tr>
<tr>
<td>HDL</td>
<td>High-Density Lipoprotein</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>HHomeostasis Model Assessment of Insulin Resistance</td>
</tr>
<tr>
<td>HS-CRP</td>
<td>High-sensitivity C-Reactive Protein</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like Growth Factor I</td>
</tr>
<tr>
<td>IGFBP-1,3</td>
<td>Insulin-like Growth Factor Binding Protein 1 and 3</td>
</tr>
<tr>
<td>IGI</td>
<td>Insulinogenic Index</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
</tr>
<tr>
<td>NGT</td>
<td>Normal Glucose Tolerance</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen Activator Inhibitor 1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor alfa</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
INTRODUCTION

Diabetes mellitus

Historical background

The condition of diabetes mellitus has been known for more than three thousand years and the term “diabetes”, which means “to pass or run through”, has been credited to Demetrius of Apameia (1st or 2nd century BC), who referred to the large volumes of urine in patients with the disease [1].

The disease was commonly diagnosed by “water tasters”, since, at that time, the diagnosis was based on sweet-tasting urine. As a result, the Latin word for honeyed, “mellitus”, was added to the term diabetes by Thomas Willis (1621-1674). The disease was discovered in a similar way in various cultures, such as ancient India, Korea, China and Japan, where the words for diabetes are based on the same ideograms which mean “sugar urine disease”.

In the early 18th century, the first chemical tests were developed to identify and quantify the presence of glucose in the urine and, in 1776, Matthew Dobson (1732-1784) showed that the sweetness in urine was accompanied by a sweetness of the blood and diabetes started to be regarded as a disorder of nutrition [1]. A German student named Paul Langerhans (1849-1888) reported in his dissertation in 1869 that the pancreas contains two cellular systems and, several years later, one of these groups of cells was identified as the “islets of Langerhans”. In 1889, Oscar Minkowski (1858-1931) and Joseph Mering (1849-1908) showed in their pioneering work that pancreatectomised dogs developed diabetes. At the beginning of the 20th century, this finding resulted in large-scale attempts to isolate a glucose-lowering substrate from the pancreas. In 1922, Frederick Banting (1891-1941) and Charles Best (1892-1978) succeeded [2] and described their finding as “isletin”. The head of their laboratory, John Macleod (1876-1935), insisted on using the term “insulin” and, in 1923, Banting and Macleod shared the Nobel Prize in Medicine or Physiology for “the discovery of insulin”.

Back in 1875, it was suggested that there might be two subtypes of the disease, one affecting the young and one the elderly and overweight. However, it was not until fifty years later that Himsworth (1905-1993) showed that the levels of insulin varied in different patient categories, causing diabetes to be officially divided into two groups [3].


Terminology and classification of diabetes

The four principal forms of diabetes mellitus are “types 1 and 2”, “gestational diabetes” and “other specific types”. The term “type 1 diabetes” has replaced several former terms, including childhood-onset diabetes, juvenile diabetes and insulin-dependent diabetes (IDDM). In the same way, the term “type 2 diabetes” has replaced adult-onset diabetes, obesity-related diabetes and non-insulin-dependent diabetes (NIDDM).

Type 1 diabetes: accounts for approximately 5-10% of people with diabetes and is a disease caused by pancreatic beta-cell destruction, leading to an absolute insulin deficiency. The
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patho-physiological background is an autoimmune process triggering an inflammatory response within the islets of Langerhans and the production of antibodies to beta-cell antigens. The autoimmune destruction of the beta-cells has multiple genetic predispositions and is related to environmental factors that are so far poorly understood [5].

**Type 2 diabetes:** is the predominant form, accounting for approximately 90% of all people with diabetes. It is characterised by slowly progressing insulin resistance and relative insulin deficiency. The condition has a complex aetiology, but lifestyle-related habits resulting in obesity and a lack of physical activity are key elements [5].

**Gestational diabetes:** resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and activity. Gestational diabetes is a temporary condition requiring medical attention throughout the pregnancy and it is a risk marker for future type 2 diabetes [5].

**Other types of diabetes:** several rare causes of diabetes mellitus are not possible to categorise as type 1, type 2 or gestational. They include genetic disorders in the insulin system and drug- or chemical-induced diseases of the exocrine pancreas [5].

**Impaired glucose tolerance and impaired fasting glucose**

There are two intermediate conditions characterised by glucose levels that are too high to be considered normal, even though they do not meet the criteria for diabetes. They are labelled as **impaired glucose tolerance (IGT)**, marked by elevated postprandial glucose disclosed by an oral glucose tolerance test (OGTT), and **impaired fasting glucose (IFG)**, with an isolated elevation of fasting glucose. People belonging to these groups run a high risk of developing diabetes [5] and the conditions are sometimes referred to as “pre-diabetes”. The early detection of people at risk offers an opportunity to prevent or at least retard the progression to diabetes. The currently used criteria for glucometabolic classification according to the WHO [4] and ADA [5] are presented in Table 1.

<table>
<thead>
<tr>
<th>Glucometabolic state</th>
<th>Source</th>
<th>Classification criteria mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>WHO</td>
<td>FPG &lt; 6.1 + 2hPG &lt; 7.8</td>
</tr>
<tr>
<td></td>
<td>ADA</td>
<td>FPG &lt; 5.6</td>
</tr>
<tr>
<td>Impaired Fasting Glucose</td>
<td>WHO</td>
<td>FPG ≥ 6.1 and &lt; 7.0 + 2hPG &lt; 7.8</td>
</tr>
<tr>
<td></td>
<td>ADA</td>
<td>FPG ≥ 5.6 and &lt; 7.0</td>
</tr>
<tr>
<td>Impaired Glucose Tolerance</td>
<td>WHO</td>
<td>FPG &lt; 7.0 + 2hPG ≥ 7.8 and &lt; 11.1</td>
</tr>
<tr>
<td></td>
<td>ADA</td>
<td>FPG ≥ 7.0 or 2hPG ≥ 11.1</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>WHO</td>
<td>FPG ≥ 7.0</td>
</tr>
<tr>
<td></td>
<td>ADA</td>
<td>FPG ≥ 7.0</td>
</tr>
</tbody>
</table>

WHO, World Health Organization; ADA, American Diabetes Association; FPG, fasting plasma glucose; 2hPG, 2-hour post-challenge plasma glucose.
The global burden of diabetes
The WHO and the International Diabetes Federation (IDF) have estimated that 194 million people worldwide, or 5.1% of the adult population, currently have diabetes and that this number will increase to 333 million, or 6.3%, by 2025. The situation is exacerbated by the estimated number of people with IGT, currently at 314 million, or 8.2% of the adult population, which is expected to increase to 472 million, or 9.0%, by 2025. More than 13% of the adult population therefore suffer from glucose abnormalities. At present, the prevalence is highest in the European and Western Pacific Regions, but by 2025 the top-ranking region is expected to be South-East Asia. The estimates for both 2003 and 2025 show a female predominance, with a percentage of females that is about 10% (diabetes) and 20% (IGT) higher than that among males. As a result, glucose abnormalities represent a global health problem which has developed in parallel with a rapidly emerging socio-economic transition, including urbanisation, dietary changes, reduced demand for physical activity and other unhealthy lifestyle patterns [6].

Beta-cell dysfunction and insulin resistance in type 2 diabetes – pathological considerations
The development of type 2 diabetes is characterised by the progressive deterioration of glucose tolerance, with increasing insulin resistance and decreasing insulin secretion. Insulin normally suppresses glucose production, promotes glucose storage, increases triglyceride synthesis and the formation of very low density lipoprotein in the liver, increases glucose uptake and protein synthesis in skeletal muscle and suppresses free fatty acid release (FFAs) from the adipocytes. The normal beta-cell adjusts to increased or decreased insulin resistance by up- or down-regulating insulin secretion. If, for any reason, this mechanism is disturbed, blood concentrations of glucose and FFAs will increase (Figure 1).

![Figure 1](image.png)

Figure 1. Increased pancreatic insulin secretion reduces hepatic glucose output, enhances glucose uptake in skeletal muscles and suppresses the release of fatty acids from fat tissue. Reduced insulin secretion will reduce insulin signalling in the target tissues. Insulin resistance pathways affect the action of insulin in each of the target tissues, leading to increased circulating fatty acids and hyperglycaemia. In turn, the raised blood concentrations of glucose and fatty acids will have a negative impact on insulin secretion and resistance. Reprinted with permission from reference [7].
The relationship between insulin sensitivity and insulin secretion has been described as “hyperbolic”, with the implication that the product of insulin sensitivity and secretion is constant at any given level of glucose tolerance. Accordingly, any deviation from this state will lead to changes in glucose tolerance (Figure 2) [8]. The relative importance of and causal relationship between insulin resistance and beta-cell dysfunction in the pathogenesis of type 2 diabetes are still the subject of debate. However, both insulin secretion and insulin sensitivity are genetically and environmentally controlled and the impairment of each of them separately or together is associated with an increasing risk of type 2 diabetes [9-11]. At each stage of the development of type 2 diabetes, reduced insulin sensitivity and impaired insulin secretion are independent predictors of worsening glucose tolerance [12].

Many studies claim that insulin resistance is the primary dysfunction ultimately causing beta-cell failure, while other investigations reveal that decreased beta-cell function may already exist at fasting plasma glucose levels in the normal range. Independent of insulin resistance, beta-cell dysfunction may exist in the early stages of glucose abnormalities and, in obese patients, it may already be apparent in the presence of normal glucose tolerance (NGT) [13-15]. A study of Japanese patients with type 2 diabetes revealed that decreased insulin secretion was more important for glucose tolerance than insulin sensitivity [16]. At the time of diagnosing type 2 diabetes, beta-cell function was already compromised to approximately 50% of the original capacity (Figure 3) [17].
Figure 3. By the time diabetes is diagnosed, beta-cell function has already deteriorated over a period of many years. Adapted with permission from reference [17].

Regardless of which abnormality precedes the other, reductions in both insulin sensitivity and beta-cell function are present at an early stage during the development of type 2 diabetes [18]. Both conditions may therefore serve as targets for preventing or at least retarding the onset of the disease.

**Indices of insulin sensitivity and insulin secretion**

The golden standard for measuring insulin sensitivity and insulin secretion is a euglycaemic or hyperglycaemic glucose clamp. Both procedures have high sensitivity and reproducibility and are almost completely unaffected by confounding factors [19]. Due to the investigational complexity and the discomfort experienced by the examined subject, several simplified methods have been developed. The simplest are those derived from fasting measurements. Fasting insulin has, for example, been used as a surrogate for insulin sensitivity. During the last two decades, the homeostasis model assessment (HOMA) [20] has become a frequently used index. It takes both fasting glucose and fasting insulin into account, thereby providing estimates of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B). The HOMA-IR correlates well with the more complex techniques and has been used in many population studies, [21, 22].

The OGTT, which stimulates both insulin secretion and glucose disposal, can be used for estimating insulin secretion and sensitivity. To enable the application of advanced indices, blood sampling should be extended to incorporate samples before the glucose load and 30, 60, 90 and 120 minutes thereafter. The “advanced” OGTT methods for the estimation of insulin sensitivity include ISIcomp [23], MCRest [24]) and OGIS [25]. These indices deliver information that closely resembles that obtained by the clamp technique using a considerably simpler procedure [26]. The most common index for expressing beta-cell function from the OGTT is the insulinogetic index (IGI). This index is based on the increase in insulin at a certain time (usually 30 minutes) after the glucose load divided by the corresponding increase in blood glucose. The rationale behind IGI is that the early-phase beta-cell secretion of insulin is lower in patients with glucose abnormalities [27]. The IGI is a commonly utilised index of beta-cell function [27-31] which is closely correlated...
with the actual insulin secretion [32]. One drawback is the fact that IGI does not reflect any specific mechanisms and the results must always be interpreted with reference to all assumptions and simplifications included in the model.

A test, similar to the OGTT but somewhat more advanced, is the intravenous glucose tolerance test. Several protocols are available and they share the common denominator that blood glucose is frequently sampled during a defined time period following an intravenous bolus of glucose and the method is often referred to as a “frequently sampled intravenous glucose tolerance test” (FSIGT) [33]. Insulin sensitivity is expressed from the FSIGT by calculating the sensitivity index \( S_i \), which is a mathematical function. The commonly applied expression of beta-cell function from the FSIGT is the \( \Delta A I R_C \) (Acute Insulin Response), which is the mean concentration of insulin during the first peak between two and 10 minutes [34]. The OGTT and FSIGT do not express the same reactions to a glucose load. The oral test incorporates the impact of the release of incretins such as glucagon-like peptide 1 (GLP-1) from the gastro-intestinal tract, while the FSIGT provides an expression of the incretin-independent beta-cell function.

### Indices for insulin resistance:

#### Fasting:

\[
\text{HOMA-IR} = \frac{I_0 \times G_0}{(22.5 \times 6)}
\]

#### From OGTT:

\[
\text{ISI}_{\text{comp}} = \frac{10000}{G_2 \times I_0 \times G_m \times I_m}
\]

\[
\text{MCR}_{\text{est}} = 18.8 - 0.271 \times \text{BMI} - 0.0052 \times I_{120} - 0.27 \times G_{90}
\]

\[
\text{OGIS} = f(G_0, G_{90}, G_{120}, I_0, I_{90}, D_0)
\]

#### From FSIGT:

\( S_i \) and \( S_{\text{G}} \) (Minimal model) Computer program

#### From euglycaemic clamp:

Mean glucose infusion rate (M) = mg/min or \( \mu \text{mol/min IS-index} \) (mean glucose infusion rate at steady state)

### Indices for beta-cell function:

#### Fasting:

\[
\text{HOMA-B} = \frac{20 \times I_0}{(G_0 - 3.5) \times 6}
\]

#### From OGTT:

Insulinogenic index = \( \frac{\Delta I_{30}}{\Delta G_{30}} \) (or other points in time such as 5 or 120 minutes)

#### From FSIGT:

\[ \Delta A I R_{I_0} \] = mean insulin above basal during the first 2-10 minutes during the FSIGT.

#### From hyperglycaemic clamp:

Early insulin secretion = mean insulin above basal during the first 8-10 minutes during the clamp.

\( I = \text{Insulin (pmol/l)}, G = \text{Plasma Glucose (mmol/l)}, \) subscripted number = minutes as regards to the OGTT where 0 is fasting. \( m = \text{mean during OGTT. The OGIS is a more complex function which can be downloaded at http://www.isib.cnr.it/bioing/ogis/home.html where a web-based calculator is also available. OGTT = Oral glucose tolerance test, FSIGT = Frequently Sampled Intravenous Glucose tolerance test} \)
Since insulin resistance and beta-cell dysfunction interact in the pathogenesis of glucose abnormalities, the most accurate estimate of beta-cell function is that derived following adjustment for insulin resistance. Importantly, this adjustment should be made from measurements that are as independent from each other as possible (e.g. by means of FSIGT or clamps). The index for beta-cell function obtained after these adjustments is referred to as a disposition index [34].

Glucose abnormalities and cardiovascular disease

Epidemiology

Patients with type 2 diabetes run an increased risk of cardiovascular disease (CVD) [35] and type 2 diabetes has in fact been referred to as a “cardiovascular risk equivalent”, with the implication that a patient with type 2 diabetes runs a similar cardiovascular risk as a patient with a previous acute myocardial infarction (AMI) [36]. Atherosclerosis contributes to approximately 75% of deaths among individuals with both type 1 and type 2 diabetes [37].

During the last decade, it has become obvious that glucose is a continuous risk factor for cardiovascular mortality and that the risk is already increased at levels below those presently used for the diagnosis of diabetes [38]. More attention has therefore been focused on detecting “pre-diabetic” conditions such as IGT and IFG in order to initiate preventive measures directed against the atherosclerotic process at the earliest possible stage. The relationship between glucose perturbations and CVD should also be seen from another perspective. The very high prevalence of undiagnosed glucose abnormalities in patients with AMI, as reported in the GAMI (Glucose Tolerance in Acute Myocardial Infarction) study [39] and confirmed by the Euro and China Heart Surveys [40, 41] (Figure 4), makes it important to discover these aberrations and handle the patients accordingly.

Figure 4. Glucose abnormalities were more common than normoglycaemia in three studies of patients admitted to hospital with cardiovascular disease. The figures reflect patient cohorts which were not diagnosed with diabetes at the start of the study, but who underwent an oral glucose tolerance test (OGTT). GAMI, Glucose Tolerance in Patients with Acute Myocardial Infarction study [39]; EHS, Euro Heart Survey [42]; CHS, China Heart Survey [41]
The importance of these findings was further underlined when it became evident that post-infarction patients with newly detected disturbed glucose metabolism run an increased risk of future cardiovascular morbidity and mortality [42, 43].

**Diabetes and atherosclerosis**

Atherosclerosis, diabetes and inflammation are heterogeneous processes with many shared links. All manifestations of CVD, such as coronary heart disease, stroke and peripheral vascular disease, are more common in patients with type 2 diabetes than in those without it [44]. The onset of type 2 diabetes is usually preceded by several years of asymptomatic, postprandial hyperglycaemia, which may exist despite normal fasting glucose. Prolonged exposure to hyperglycaemia, even below the present threshold for diabetes, is in fact recognised as an important factor in the pathogenesis of diabetic complications [35]. It is widely accepted that atherosclerosis is an inflammatory disease in many respects [37]. The available evidence suggests that hyperglycaemia can cause a deterioration in endothelial function and aggravate inflammatory activity within atherosclerotic plaques. Inflammation is therefore thought to constitute a strong link between diabetes and atherosclerosis and many risk factors for CVD in type 2 diabetes appear to work via inflammatory activation [45]. In addition to inflammatory activation, hyperglycaemia promotes atherosclerosis by augmenting thrombosis and vasoconstriction [44]. Figure 5 demonstrates how an accumulation of products related to hyperglycaemia, such as blood glucose, very low density lipoproteins, advanced glycation end-products, angiotensin II and oxidised LDL, can cause oxidative stress and inflammatory activation, promoting the development of the atherosclerotic plaque.

![Figure 5](image)

**Figure 5.** The accumulation of (1) glucose, (2) advanced glycation end-products, (3) oxidised LDL, (4) free fatty acids and (5) angiotensin II, which are all common in type 2 diabetes, can induce the production of inflammatory cytokines and other molecules. INF = Interferon, NO = Nitric Oxide, AGES = Advanced Glycation End-products, RAGE = Receptor of Advanced Glycation End-products, IL = Interleukin, TNF = Tumour Necrosis Factor, PDGF = Platelet Derived Growth Factor, IGF = Insulin-like Growth Factor, NF-kB = Nuclear Factor kB
An alternate view is that diabetes and CVD share common pathways. It has in fact been speculated that chronic sub-clinical inflammation, and the resulting endothelial dysfunction, may be involved in the development of insulin resistance, thereby preceding the onset of type 2 diabetes [46]. It is possible to question whether the antecedents of endothelial dysfunction could depend on genetic or environmental factors that contribute to chronic inflammation and/or make the subject more susceptible to inflammatory activation in a general perspective. In fact, 10 years ago, Pickup and Crook already postulated that type 2 diabetes mellitus may be a disease of the innate immune system [47].

Risk factors and risk markers of CVD

Definition
In the epidemiological setting, risk expresses the fact that exposure to a certain factor, such as smoking, increases the probability of attracting a defined disease like myocardial infarction. Two terms that express this relationship are “risk factor” and “risk marker” and they are sometimes used without distinguishing the difference between them. Risk factor has been defined as “an aspect of personal behaviour or lifestyle, an environmental exposure, or an inborn or inherited characteristic which, on the basis of epidemiological evidence, is associated with health-related conditions considered important to prevent” [48]. A risk factor is usually defined as a condition with a causal relationship to the disease, although the exact details of this relationship may be only partially understood. To be accepted as a risk factor, there has to be a longitudinal observation that the exposure causes illness. Risk factors may be modifiable, with the implication that a reduction in or the elimination of the exposure will reduce the risk of becoming ill (such as smoking and myocardial infarction). A risk marker is a condition that is associated with an increased likelihood of becoming ill but without any (at least so far) established causal relationship. It may also be that a risk marker is detected in a cross-sectional investigation and that the longitudinal exposure that may transform a risk marker into a risk factor is lacking. To prevent misunderstandings, it may therefore be better to use the term “risk marker” when studying novel variables for which a possible causal relationship remains to be established.

Traditional risk factors and risk assessment
The major risk factors for CVD are hypertension, dyslipidemia, diabetes mellitus, cigarette smoking, obesity and physical inactivity [49]. They interact synergistically, as outlined in different risk charts of which the Framingham risk model [50] was the first, followed subsequently by several others like the European Heart SCORE [51]. The Oxford risk engine, which is based on the UK Prospective Diabetes Study, is specific to patients with diabetes [52]. Target-driven control of the major risk factors, primarily based on lifestyle modification but usually supplemented by pharmacological agents, is a key preventive element.
Novel risk markers in patients with type 2 diabetes

During the past decade, in parallel with increasing knowledge of the pathogenesis of CVD, there has been a large-scale search for novel “non-traditional” biomarkers of CVD. The benefit of novel markers in comparison with traditional risk scores is the subject of debate. One study evaluated 10 biomarkers (CRP, brain natriuretic peptide, N-terminal pro–atrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen-activator inhibitor type 1 (PAI-1), homocysteine and the urinary albumin-to-creatinine ratio) in 3,209 people from the Framingham Heart Study and the “multimarker score” only resulted in small increases in the ability to classify risk compared with the classic Framingham risk model [53].

Cardiovascular risk evaluations in patients with glucose abnormalities may, however, differ from those of the general population. Fifteen years ago, Stamler and co-workers already demonstrated that the increased cardiovascular risk that characterises patients with diabetes cannot be fully explained by an accumulation of the traditional risk factors [54]. Comparing men with and without diabetes, it was noted that the risk of cardiovascular mortality was increased by a factor of three to four times at any level of blood pressure or total cholesterol (Figure 6). Moreover, patients with diabetes have not benefited as much from recent progress in the management of traditional risk factors as their non-diabetic counterparts [55].

Accordingly, there is a need for new and improved risk markers for CVD, not least in patients with diabetes. The search should ideally originate from an assumed common denominator or at least a link between the abnormal glucose metabolism and vascular disease (Figure 7).
Although many studies indicate the possibility of a relationship between novel markers, CVD and diabetes, these observations have so far mainly been based on epidemiological investigations [56]. Of these markers, inflammatory factors such as C-reactive protein (CRP) and interleukin 6 (IL-6) have been extensively studied and have been found to be associated with an increased risk of CVD in patients both with and without diabetes [57-60]. In the Atherosclerosis Risk in Communities (ARIC) study, levels of albumin, fibrinogen, von Willebrand factor, factor VII and leukocyte count were predictors of coronary heart disease among patients with diabetes [61]. Recently, the Collaborative Atorvastatin Diabetes Study (CARDS) showed that ApoB and the ApoB:ApoA-1 ratio were associated with various manifestations of atherosclerotic disease in patients with type 2 diabetes [62].

This thesis addresses some novel risk markers, including early glucose abnormalities, members of the insulin-like growth factor system and adipokines.

**Insulin-like growth factors**

The insulin-like growth factor (IGF) system includes three ligands (insulin, IGF-I and IGF-II), three receptors: the insulin, the IGF-I and the mannosé-6-phosphate receptors respectively, together with six IGF binding proteins (IGFBPs 1-6). This family has been extensively studied due to its important role in both normal physiology and various diseases such as cancer and diabetes.

IGF-I is primarily synthesised in the liver in response to growth hormone and, in addition, locally in almost every tissue in the body. Most of the cellular effects of IGF-I are mediated by the IGF-I receptor, which has many similarities to the insulin receptor. In high concentrations, IGF-I stimulates the insulin receptor, as well as a hybrid insulin/IGF-I receptor with affinity for both insulin and IGF-I. IGF-I enhances the uptake of glucose in
skeletal muscle, improves insulin sensitivity and reduces the production of hepatic glucose [63-65]. The administration of IGF-I in humans reduces both the glucose and insulin concentrations [66].

Low levels of IGF-I have been related to the development of type 2 diabetes [67] and to AMI and angiographically assessed coronary heart disease [68-71]. In fact, numerous cardioprotective mechanisms have been proposed for IGF-I, in addition to the glucometabolic properties [72]. These findings are, however, not consistent, as there are indications that some patients with coronary heart disease have increased levels of IGF-I [73]. The complex relationship between IGF-I and health was emphasised in a recent review of 16 case-control studies, which revealed that patients in the upper quartile of IGF-I ran an increased risk of developing certain forms of cancer, while patients in the lower quartile ran an increased risk of ischemic heart disease and type 2 diabetes [74]. It has been suggested that there may be an optimal level of IGF-I for longevity (Figure 8) [75, 76].

![Figure 8. Apparent relationship between IGF-I activity and longevity, as proposed by Shimokawa et al. [76]. Figure adapted with from reference [75]](image)

The binding protein IGFBP-I has been described as the only acute inhibitor of the bioactivity of IGF-I [77-79]. Hepatic production is inhibited by insulin and, as a result, there is a correlation between low levels of IGFBP-1 and hyperinsulinemia, the latter relating to increased cardiovascular risk [80, 81]. However, the IGFBP-1 concentrations rise during the development of type 2 diabetes despite persisting hyperinsulinemia, indicating increased hepatic insulin resistance during disease progression [82, 83]. These observations were supported by a report showing that patients admitted to the intensive care unit with elevated IGFBP-1 had a poor prognosis, including increased mortality, a finding that was related to acute hepatic insulin resistance [84].

**Leptin and adiponectin**

Obesity has been known as a risk factor for CVD for more than twenty years [85]. At the same time, it was noted that systemic inflammatory activity was enhanced in obese patients [86]. In spite of this, it is only recently that the concept of adipose tissue as a passive organ
storing triglycerides and FFAs was abandoned. Adipocytes secrete hundreds of proteins, all involved in energy homeostasis [87]. Moreover, adipose tissue is a rich source of proinflammatory mediators that may contribute to vascular injury, insulin resistance and atherogenesis. The proinflammatory adipocytokines, or adipokines, include increased levels of CRP, IL-6, tumour necrosis factor-α (TNF-α), leptin, PAI-1 angiotensinogen and resistin, together with reduced levels of adiponectin. These mediators all influence the vasculature, promoting all stages of atherosclerosis such as endothelial dysfunction, plaque initiation, plaque progression and plaque rupture [88].

Leptin was identified in 1994 by cloning the ob gene, which determines the development of obesity in ob/ob mice [89]. From an evolutionary perspective, leptin has probably been important, as it protects the organism from starvation. Secreted from white adipose tissue, leptin acts on hypothalamic centres to regulate food intake and energy expenditure. Furthermore, leptin has multiple effects on metabolism, hormones and inflammatory and immune reactions, all processes involved in the development of both type 2 diabetes and CVD [90]. In 1999, Söderberg demonstrated that men with a first AMI had significantly increased levels of leptin prior to the event compared with matched controls [91]. Similar results were subsequently reported from the West of Scotland Coronary Prevention Study [92]. In a Swedish study of hypertensive men and women, comparing serum leptin in patients with a previous AMI and matched controls, leptin was significantly higher among the patients. This was particularly apparent in women, among whom leptin was the most important predictor of AMI [93].

Adiponectin is a complement factor that is expressed in adipocytes. Adiponectin gene expression is negatively regulated by glucocorticoids and TNF-α and positively by insulin and IGF-I [88]. There are several reports of associations between low plasma adiponectin and obesity, coronary artery disease and type 2 diabetes [94, 95]. It has been suggested that adiponectin is anti-atherogenic [96]. Low levels of adiponectin predicted AMI in the Health Professionals Follow-up study [97] but not coronary heart disease among American Indians [98] and it appears to be more consistently associated with the development of type 2 diabetes [99]. Recently, the leptin/adiponectin ratio was related to the carotid intima-media thickness in patients with type 2 diabetes and this ratio may function as an atherosclerotic index in this patient group [100].

Unresolved issues

The majority of patients with AMI suffer from newly detected glucose abnormalities [39]. In spite of this, detailed studies of novel prognostic risk markers beyond those already known are sparse in this particular group of patients. The IGF system and the adipokines may be of particular interest to explore, considering their close relationship with both type 2 diabetes and CVD. It is also important to investigate the importance of beta-cell dysfunction in patients with newly detected glucose perturbations and AMI. Historically, insulin resistance has been regarded as the main link between these conditions, but the possibility of a common denominator between the vasculature and the beta-cells deserves to be explored. It is also important to establish the time point at which a reliable glucometabolic classification of AMI patients may be performed in order to facilitate risk-reducing strategies at the earliest possible stage.
AIMS

1. To characterise patients with AMI and newly discovered abnormal glucose metabolism as regards their beta-cell function

2. To investigate the long-term reliability of the early classification of glucose perturbations by means of an oral glucose tolerance test in patients with AMI without previously known glucose abnormalities

3. To investigate the potential relationships between novel risk markers from the IGF-I system and the adipokines and future cardiovascular events and glucose tolerance in patients with AMI with and without glucose abnormalities
MATERIAL AND METHODS

Definitions

Acute myocardial infarction (AMI) was defined according to the joint recommendations of the European Society of Cardiology and the American College of Cardiology [101]. Patients were therefore diagnosed as having an AMI if they had two values of serum troponin T > 0.05 g/l or creatine kinase-MB > 10 μg/l, together with either typical symptoms (chest pain > 15 min; pulmonary oedema in the absence of valvular heart disease; cardiogenic shock; arrhythmia such as ventricular fibrillation or ventricular tachycardia) or new Q-waves in at least two of the twelve standard ECG leads, or ECG changes indicating acute myocardial ischaemia (ST elevation, ST depression or T-wave inversion). A myocardial infarction was regarded as severe in the presence of any or a combination of the following complications: congestive heart failure, cardiogenic shock, ventricular fibrillation or complete atrio-ventricular block, during the index hospitalisation.

Type 2 diabetes and impaired glucose tolerance (IGT) were defined according to the 1998 WHO classification (Table 1) [102].

Abnormal glucose tolerance (AGT) was defined as the presence of either IGT or type 2 diabetes.

Subjects, study protocols and laboratory methods

Studies I-III and V

Patients

Studies I-III and V are based on the GAMI study [39] which recruited patients (n=181; 125 men, 56 women) with AMI without known type 2 diabetes and blood glucose < 11.0 mmol/L when admitted to the two participating Swedish coronary care units. Before hospital discharge (on day 4-5 after admission), glucose metabolism was characterised by means of OGTT as NGT, IGT or type 2 diabetes in 168 of the patients. The OGTT was repeated after three (n=145) and 12 months (n=129). Biochemical and clinical variables were obtained during hospitalisation and at follow-up after three and 12 months following discharge.
Märit Wallander

Controls
Age- and gender-matched subjects (n=185), selected from the population registry in the recruitment area, served as controls. All of them had to be free from CVD apart from treated hypertension. Information regarding medical history, concomitant diseases and current medication was obtained by personal interviews using predefined questions and checked, if appropriate, by a review of available medical records.

Biochemical investigations
The patients had their blood glucose and creatinine measured as soon as possible after arrival at the coronary care unit and HbA1c was measured on the first morning after admission. Fasting blood glucose was measured on the following morning and repeated every morning until discharge.

The OGTTs were performed in the morning at the day of hospital discharge (day 4-5). The glucose load (75 g glucose in 200 ml water) was ingested as quickly as possible. Capillary blood glucose was measured before and 15, 30, 60 and 120 minutes after the glucose intake.

Plasma concentrations of insulin and proinsulin were analysed in fasting samples taken on the first morning after admission and during the OGTT at 0, 30 and 120 minutes. In addition, IGFBP-1 was analysed before and 120 minutes after the glucose load. Fasting values of IGF-1, IGFBP-1, IGFBP-3, total-, HDL- and LDL-cholesterol, triglycerides, highly sensitive C-reactive protein (hs-CRP) and cortisol were obtained on day two after admission and on the day of hospital discharge. Free fatty acids (FFAs), PAI-1 and fibrinogen were measured at discharge. Body mass index (BMI) was recorded at admission.

Subjects in the control group were investigated once in the fasting state at the research outpatient clinic. They were interviewed and underwent a physical examination. The OGTT and blood sampling was similar to that of the patients. Figure 9 presents the glucometabolic classification in patients at hospital discharge and after three months and in controls [39, 103].

![Figure 9](image.png)

**Figure 9.** Classifications after an OGTT in patients at hospital discharge and after three months and in controls [39, 103]. NGT = Normal Glucose Tolerance, IGT = Impaired Glucose Tolerance, DM = Type 2 Diabetes Mellitus
Laboratory methods

Blood glucose was analysed immediately in capillary whole blood using a HemoCue photometer (HemoCue, Ängelholm, Sweden). HbA1c was determined by high-performance liquid chromatography of capillary blood applied to filter paper with an upper reference limit of 5.3% (Boehringer Mannheim Scandinavian AB, Bromma, Sweden).

Insulin and proinsulin were quantified using enzyme immunoassays from Dako Diagnostics (Cambridgeshire, UK). Intra- and inter-assay coefficients of variation (CVs) for these analyses were 6 and 7% for insulin and 5 and 6% for proinsulin respectively.

Concentrations of IGF-I were determined in serum by RIA after separating IGFs from IGFBPs by acid ethanol extraction and cryoprecipitation. To minimise interference by the remaining IGFBPs, des (1-3) IGF-I was used as a radioligand [104]. The intra- and inter-assay CVs were 4% and 11% respectively. IGFBP-1 concentrations in serum were determined by RIA according to the method of Póvoa et al. [105]. The sensitivity of the RIA was 3 μg/l and the intra- and inter-assay CVs were 3% and 10% respectively. IGFBP-3 was quantified in heparinised plasma using IMMULITE 2000 IGFBP-3 (DPC, Germany), which is a solid-phase, enzyme-labelled chemiluminescent immunometric assay. The analytic sensitivity was 0.1 mg/l and the intra- and inter-assay CVs were 4% and 7% respectively.

Plasma levels of leptin and adiponectin were analysed with a double-antibody radioimmunoassay (Linco Res., St Louis, MO, USA). The total coefficient of variation (CV) for leptin was 4.7% at both low (2–4 ng/mL) and high (10–15 ng/mL) levels, while the corresponding values for adiponectin was 15.2% at low levels (2–4 μg/mL) and 8.8% at high (26–54 μg/mL) levels.

Events

Cardiovascular death was defined as death from myocardial infarction, stroke and sudden death without any obvious reason. A non-fatal re-infarction was defined as a non-fatal myocardial infarction occurring later than 72 h after the index infarction. Stroke was defined according to the WHO as a neurological deficit observed by a physician and persisting > 24 hours without any other disease explaining the symptoms. Severe heart failure was recognised when it caused hospital admission and intensified and/or additional treatment. A composite outcome was defined as a major cardiovascular event representing the first occurrence of stroke, re-infarction, severe heart failure or cardiovascular death. In all, 37 patients suffered from at least one major cardiovascular event during the 34 months of follow-up (re-infarctions n=16; stroke n=7; severe heart failure n=10; cardiovascular death n=12) [42].

Study IV

Patients

The patients in Study IV were all participants in DIGAMI 2, a multicentre, prospective, randomised, open trial with blinded evaluation comparing three different glucose-lowering strategies. Patients with established type 2 diabetes or an admission blood glucose of > 11.0
mmol/l admitted to the enrolling 44 coronary care units were recruited if they fulfilled the following criteria: suspected AMI due to symptoms (chest pain > 15 min during the preceding 24 hours) and/or recent ECG signs (new Q-wave and/or ST-segment deviations in two or more leads). A total of 1,253 patients were randomised to one of three study arms receiving: a) a 24-hour insulin-glucose infusion followed by subcutaneous insulin-based, long-term glucose control (Group 1, n= 474); b) the same initial treatment followed by glucose-lowering treatment according to local practice (Group 2, n=473); or c) glucose-lowering treatment according to local practice (Group 3, n=306). The median study duration was 2.1 (Q1, Q3: 1.0, 3.0) years and no patient was lost to follow-up. The primary and secondary objectives were to compare total mortality and cardiovascular morbidity (non-fatal myocardial infarction and stroke) between these management strategies [106]. As there were no differences in the endpoints between the three study arms, the three groups were combined into one epidemiological database for further studies.

A total of 575 of the DIGAMI 2 patients participated in a biochemistry programme, with repeated blood sampling at admission before initiation of the glucose-insulin infusion and in the fasting state at the time of hospital discharge and after three and 12 months (Figure 10).

**Figure 10. The DIGAMI-2 study design and the biochemistry population.**

Biochemical investigations

Blood samples for future analyses were obtained as soon as possible after hospital admission, at hospital discharge and six weeks, three, six and 12 months thereafter. Concentrations of IGF-I and IGFBP-1 were analysed from samples at admission, on hospital discharge and three and 12 months thereafter.

Laboratory methods

Blood glucose (whole blood glucose in mmol/l), S-creatinine, S-cholesterol and S-triglycerides were analysed locally at admission. HbA1c was analysed at a core laboratory (Department of Laboratory Medicine, Malmö Hospital, Sweden) by high-performance liquid chromatography on capillary blood applied to a filter paper with an upper normal limit of 5.3% (Boehringer Mannheim Scandinavian AB, Bromma, Sweden). Concentrations of IGF-I and IGFBP-1 were determined as already described in Study III.
Events
Myocardial infarction was diagnosed according to the joint recommendations of the ESC and ACC [101]. A re-infarction was defined as a new event > 72 h from the index infarction. Stroke was defined as unequivocal signs of focal or global neurological deficit of sudden onset and a duration of > 24 h that were judged to be of vascular origin. Deaths were verified with death certificates, hospital records and explanatory letters from the physicians in charge when requested by the adjudication committee members and autopsy reports when available. Sudden cardiovascular deaths were those that occurred within 24 h following the onset of symptoms and without any other obvious reason. Deaths were labelled as cardiovascular or non-cardiovascular, while those without any obvious non-cardiovascular cause were considered cardiovascular. Non-cardiovascular deaths, including malignancies, were adjudicated according to the same principles as cardiovascular events. An independent committee comprising three experienced cardiologists adjudicated all events blindly and could, as indicated, ask for any type of information they felt was needed to ensure the correct classification of the events and the reasons for mortality.

During the 36-month follow-up, 131 (23%) patients from the biochemistry group died, 102 (78%) from CVD, while 175 patients (30%) had at least one cardiovascular event. Within this biochemistry group, there were no significant differences in cardiovascular death or the occurrence of cardiovascular events between the three randomised treatment groups. Furthermore, there were no differences in prognosis (cardiovascular death or major cardiovascular event = cardiovascular death/re-infarction/stroke) between the biochemistry population and those for whom biochemistry was not available (Figure 11).

![Figure 11. Kaplan-Meier curves of major cardiovascular events in the biochemistry population (n=575) compared with those for whom biochemistry was not available (n=678).](#)
Calculations

In Studies I-III and IV, insulin resistance, expressed as the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in the fasting condition, was calculated according to Matthews et al. [20]. The constant 1.13 converts blood glucose to plasma glucose, while 6 converts pmol/L to mU/l.

\[
\text{HOMA-IR} = \frac{\text{plasma insulin} \times \text{blood glucose} \times 1.13}{22.5 \times 6}
\]

In Study I, the insulinogenic index (IGI) was calculated as the difference between plasma insulin during the OGTT at 0 and 30 minutes (ΔI30) divided by the difference between the corresponding glucose values (ΔG30).

\[
\text{IGI} = \frac{\Delta I30}{\Delta G30}
\]

As IGF-I decreases with age, a standardised IGF-I score was calculated as follows (Studies III and IV):

\[
\text{IGF-I SD} = \frac{\log \text{(IGF-I)} + 0.00625 \times \text{age} - 2.555}{0.104}
\]

The equation of the IGF-I SD originates from the regression line of IGF-I values in 247 healthy adult subjects [107]. In Study III The IGF-I SD was converted back to the IGF-I scale by applying a common age (mean 64 years) to the entire study population. Differences in the profiles of IGF-I in patients and controls with respect to glucose tolerance categories (Study III) were tested using a non-parametric test for interactions based on aligned ranks (program written in FORTRAN) [108]. BMI was calculated as weight/height\(^2\) (kg/m\(^2\)) (Studies I-V). The area under the curve for glucose (AUCg) was calculated by numerical integration using Hermite polynomials (Study I) [109].
Statistical methods

In Studies I-IV, continuous variables are presented as the median (lower and upper quartile) and categorical variables are presented as percentages and in these studies differences between groups of patients were compared using the chi-square test, Wilcoxon’s rank-sum test or Jonckheere-Terpstra’s test. In Study V, continuous variables are presented as means with 95% confidence intervals, categorical variables as percentages and differences between groups were compared using the chi-square test or ANOVA. In Studies I and V, Pearson’s correlation coefficients were calculated for pairs of continuous variables, whereas in Study II Spearman’s rank correlation coefficients were calculated. In Study II, concordance rates between OGTTs were calculated as weighted Cohen’s kappa.

Multivariate statistics were used in Studies I, III, IV and V according to the Cox proportional hazard regression (Studies III, IV and V), multiple linear regression (Study I) and logistic regression (Studies III and V). In all multivariate analyses, possible combinations of predictors with a \( p \)-value of < 0.2 were fitted into a best subset or stepwise analysis and the models were compared using the Akaike information criterion (AIC). The rationale behind the AIC is that, if the only difference between two models is that a chance predictor has been included, the values of the AIC for the two models will not differ much and will instead tend to increase. Moreover, the AIC is an approximate measure of the prediction accuracy. To limit the influence of extreme values, skewed continuous variables were generally log-transformed prior to analysis.

A two-sided \( p \)-value of < 0.05 was regarded as statistically significant. All analyses were conducted using SAS version 9.1.3 (SAS Institute) and Statistica 7.0 (StatSoft Inc.).

Ethical considerations

Both the GAMI study (study I-III and V) and the DIGAMI 2 trial (study IV) conformed to good clinical practice guidelines and followed the recommendations of the Helsinki Declaration. They were approved by the regional ethics committee at Karolinska Institutet. All patients provided written and oral informed consent before enrolment.
RESULTS

Baseline characteristics of GAMI patients and controls (Studies I, II, III and V)

Pertinent clinical and biochemical characteristics of patients versus controls and patients divided into glucose tolerance groups are presented in Tables 2 and 3 [39, 103].

Table 2. Pertinent clinical and biochemical characteristics of the patients at the time of hospital discharge and of the controls. Values are presented as the median (Q1, Q3). *OGTT was performed in 168 patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (n=181)</th>
<th>Controls (n=185)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.0 (57.0, 71.0)</td>
<td>64.0 (58.0, 72.0)</td>
<td>0.395</td>
</tr>
<tr>
<td>Sex (female; %)</td>
<td>31</td>
<td>31</td>
<td>0.932</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>34</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.2 (23.6, 29.3)</td>
<td>26.0 (23.6, 29.0)</td>
<td>0.989</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.2 (4.7, 5.5)</td>
<td>5.0 (4.6, 5.4)</td>
<td>0.061</td>
</tr>
<tr>
<td>Blood glucose 120 min (mmol/l)*</td>
<td>8.8 (6.9, 11.0)</td>
<td>7.0 (5.9, 8.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c at admission (%)</td>
<td>4.9 (4.6, 5.3)</td>
<td>4.6 (4.3, 5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>17.8 (8.1, 50.2)</td>
<td>1.7 (1.0, 3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (pmol/l)</td>
<td>1.0 (0.9, 1.2)</td>
<td>1.2 (1.0, 1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (pmol/l)</td>
<td>3.1 (2.5, 3.8)</td>
<td>3.9 (3.3, 4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.9 (1.6, 2.6)</td>
<td>1.2 (0.9, 1.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free fatty acids (mEq/l)</td>
<td>0.49 (0.35, 0.71)</td>
<td>0.54 (0.38, 0.71)</td>
<td>0.324</td>
</tr>
<tr>
<td>PAI-1 activity (IU/ml)</td>
<td>8.9 (3.5, 18.7)</td>
<td>7.3 (2.6, 16.6)</td>
<td>0.123</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>53 (35, 84)</td>
<td>47 (32, 74)</td>
<td>0.126</td>
</tr>
<tr>
<td>Proinsulin (pmol/l)</td>
<td>5.9 (4.3, 8.9)</td>
<td>2.5 (1.4, 4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR (mU · mmol/l)</td>
<td>2.3 (1.5, 3.6)</td>
<td>2.0 (1.4, 3.0)</td>
<td>0.071</td>
</tr>
</tbody>
</table>
Myocardial infarction and glucose abnormalities – novel risk markers

Table 3. Clinical and biochemical characteristics of the glucose tolerance groups at hospital discharge. Values are presented as the median (Q1, Q3). n.a = not applicable

<table>
<thead>
<tr>
<th></th>
<th>NGT (n = 55)</th>
<th>IGT (n = 58)</th>
<th>T2DM (n=55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.0 (54.0,67.0)</td>
<td>64.0 (57.0,72.0)</td>
<td>66.0 (57.0,71.0)</td>
<td>0.007</td>
</tr>
<tr>
<td>Sex (female; %)</td>
<td>20</td>
<td>29</td>
<td>36</td>
<td>0.058</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>40</td>
<td>38</td>
<td>27</td>
<td>0.163</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (23.1,28.3)</td>
<td>26.6 (23.1,29.7)</td>
<td>27.0 (24.2,29.7)</td>
<td>0.143</td>
</tr>
<tr>
<td><strong>Family history (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>17</td>
<td>18</td>
<td>33</td>
<td>0.038</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>56</td>
<td>46</td>
<td>62</td>
<td>0.505</td>
</tr>
<tr>
<td><strong>Previous disorders (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>13</td>
<td>24</td>
<td>20</td>
<td>0.333</td>
</tr>
<tr>
<td>Angina Pectoris</td>
<td>31</td>
<td>33</td>
<td>31</td>
<td>1.000</td>
</tr>
<tr>
<td>Hypertension (treated)</td>
<td>31</td>
<td>31</td>
<td>36</td>
<td>0.543</td>
</tr>
<tr>
<td>Hyperlipidemia (treated)</td>
<td>15</td>
<td>19</td>
<td>15</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Treatment during hospital stay and discharge (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>45</td>
<td>28</td>
<td>42</td>
<td>0.696</td>
</tr>
<tr>
<td>Aspirin</td>
<td>95</td>
<td>96</td>
<td>89</td>
<td>0.231</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>93</td>
<td>93</td>
<td>91</td>
<td>0.681</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>16</td>
<td>12</td>
<td>17</td>
<td>0.939</td>
</tr>
<tr>
<td>Statins</td>
<td>87</td>
<td>58</td>
<td>67</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>Biochemistry (at discharge if not stated otherwise)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adm. capillary b-glucose (mmol/l)</td>
<td>5.9 (5.1,7.1)</td>
<td>6.1 (5.6,7.4)</td>
<td>6.9 (6.0,7.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.8 (4.5,5.3)</td>
<td>5.1 (4.7,5.5)</td>
<td>5.6 (5.2,6.2)</td>
<td>n.a</td>
</tr>
<tr>
<td>Blood glucose 120 min (mmol/l)</td>
<td>6.5 (5.9,7.1)</td>
<td>9.0 (8.2,9.6)</td>
<td>12.0 (11.2,13.3)</td>
<td>n.a</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8 (4.5,5.2)</td>
<td>4.9 (4.5,5.2)</td>
<td>5.1 (4.6,5.5)</td>
<td>0.023</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>12.7 (5.3,28.0)</td>
<td>18.8 (8.7,57.6)</td>
<td>29.2 (14.1,80.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>5.5 (4.6,6.0)</td>
<td>5.0 (4.5,5.7)</td>
<td>5.1 (4.3,5.8)</td>
<td>0.161</td>
</tr>
<tr>
<td>High-density lipoprotein (mmol/l)</td>
<td>1.0 (0.9,1.3)</td>
<td>1.1 (1.0,1.3)</td>
<td>1.0 (0.9,1.2)</td>
<td>0.288</td>
</tr>
<tr>
<td>Low-density lipoprotein (mmol/l)</td>
<td>3.3 (2.6,3.9)</td>
<td>3.0 (2.5,3.6)</td>
<td>3.1 (2.4,3.8)</td>
<td>0.296</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.1 (1.5,2.6)</td>
<td>1.8 (1.5,2.6)</td>
<td>2.0 (1.6,2.7)</td>
<td>0.987</td>
</tr>
<tr>
<td>Free fatty acids (mEq/l)</td>
<td>0.39 (0.30,0.61)</td>
<td>0.54 (0.35,0.69)</td>
<td>0.59 (0.39,0.78)</td>
<td>0.005</td>
</tr>
<tr>
<td>PAI-1 activity (IU/ml)</td>
<td>6.4 (3.1,14.4)</td>
<td>9.1 (3.1,18.7)</td>
<td>11.7 (4.6,22.8)</td>
<td>0.054</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>486 (408,609)</td>
<td>473 (399,540)</td>
<td>491 (390,621)</td>
<td>0.729</td>
</tr>
<tr>
<td>S-Creatinine (µmol/l)</td>
<td>92 (82,100)</td>
<td>92 (81,104)</td>
<td>89 (77,100)</td>
<td>0.296</td>
</tr>
</tbody>
</table>

Study I

Insulin resistance and beta-cell function

All the parameters related to insulin resistance and beta-cell function at discharge and during follow-up are presented in Table 4. AGT was associated with increased HOMA-IR (p=0.003), fasting insulin (p=0.046) and 120-min insulin levels (p<0.001) while the 30-min insulin levels tended to decrease (p=0.079). Baseline levels of proinsulin were significantly increased in patients with AGT (p<0.001), but the proinsulin/insulin ratio was not (p=0.140).
Table 4. Insulin resistance and beta-cell function at the time of hospital discharge, three months and 12 months. Classifications of the three groups are all based on OGTT data obtained at the time of discharge. Values are presented as the median (Q1, Q3). IGI = (Δ30I/Δ30G; pmol/mmol).

<table>
<thead>
<tr>
<th>Variable</th>
<th>NGT (n = 55)</th>
<th>IGT (n = 58)</th>
<th>T2DM (n = 55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discharge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose 30 min (mmol/l)</td>
<td>8.7 (7.7,9.6)</td>
<td>9.1 (8.4,10.2)</td>
<td>9.8 (9.0,10.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin baseline (pmol/l)</td>
<td>52 (33,70)</td>
<td>53 (34,78)</td>
<td>60 (43,101)</td>
<td>0.046</td>
</tr>
<tr>
<td>Insulin 30 min (pmol/l)</td>
<td>328 (217,404)</td>
<td>313 (195,430)</td>
<td>267 (185,370)</td>
<td>0.079</td>
</tr>
<tr>
<td>Insulin 120 min (pmol/l)</td>
<td>236 (132,371)</td>
<td>489 (321,704)</td>
<td>574 (370,1080)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proinsulin baseline (pmol/l)</td>
<td>4.9 (3.9,6.8)</td>
<td>6.2 (4.6,9.1)</td>
<td>7.4 (5.0,10.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR (mU · mmol/l)</td>
<td>2.07 (1.34,2.71)</td>
<td>2.13 (1.49,3.67)</td>
<td>2.91 (1.69,4.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>IGI</td>
<td>70.1 (42.7,101.4)</td>
<td>48.7 (34.7,86.8)</td>
<td>38.1 (25.7,61.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proinsulin/Insulin ratio</td>
<td>0.10 (0.09,0.14)</td>
<td>0.12 (0.08,0.17)</td>
<td>0.12 (0.08,0.17)</td>
<td>0.140</td>
</tr>
<tr>
<td><strong>Three months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR (mU · mmol/l)</td>
<td>2.14 (1.63,3.01)</td>
<td>2.25 (1.57,3.18)</td>
<td>3.54 (1.88,5.18)</td>
<td>0.007</td>
</tr>
<tr>
<td>IGI</td>
<td>44.5 (32.9,70.6)</td>
<td>47.7 (35.6,64.2)</td>
<td>37.2 (20.6,57.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>Proinsulin/Insulin ratio</td>
<td>0.09 (0.07,0.14)</td>
<td>0.12 (0.09,0.16)</td>
<td>0.13 (0.09,0.18)</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Twelve months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR (mU · mmol/l)</td>
<td>2.61 (1.54,3.42)</td>
<td>2.87 (1.65,5.06)</td>
<td>4.15 (2.09,6.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IGI</td>
<td>50.0 (33.9,99.0)</td>
<td>52.7 (32.8,77.9)</td>
<td>38.3 (24.1,58.9)</td>
<td>0.048</td>
</tr>
<tr>
<td>Proinsulin/Insulin ratio</td>
<td>0.06 (0.04,0.12)</td>
<td>0.07 (0.05,0.10)</td>
<td>0.08 (0.06,0.13)</td>
<td>0.121</td>
</tr>
</tbody>
</table>

The beta-cell function expressed as IGI was significantly lower in patients with type 2 diabetes compared with patients with IGT or NGT (Table 4, p<0.001). When comparing the glucometabolic groups as classified at discharge, the pattern of increased insulin resistance and decreased beta-cell function among patients with AGT was still seen at three and twelve months.

As presented in Figure 12, IGI at discharge was related to admission capillary blood glucose ($r$=-0.218, $p$=0.010) and to the area under the curve for glucose (AUC$_{gl}$) in all patients at hospital discharge ($r$=-0.475, $p<0.001$).
When performing a multiple regression analysis using AUC$_g$ at discharge as the dependent variable and HOMA-IR, IGI, age and gender as independent variables, IGI explained more of the variability in AUC$_g$ than HOMA-IR (Table 5).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Partial R-square</th>
<th>Parameter Estimate</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGI</td>
<td>0.286</td>
<td>-177.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR (mU · mmol/l)</td>
<td>0.155</td>
<td>138.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (Female = 1)</td>
<td>0.048</td>
<td>96.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.022</td>
<td>3.5</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Adjusted insulinogenic index (previously unpublished data)

When IGI was adjusted for HOMA-IR, creating an adjusted insulinogenic index (a disposition index), the differences between the glucose tolerance groups increased (Figure 13).

Figure 13. Insulinogenic index, adjusted insulinogenic index and HOMA-IR in glucose tolerance categories among the GAMI patients. For the adjusted insulinogenic index, the differences between NGT and IGT and between IGT and type 2 diabetes were statistically significant (p < 0.001).
Insulinogenic index in the controls (previously unpublished data)

As in the patients, the controls with glucose abnormalities presented a reduced insulinogenic index. There were no significant differences between patients and controls within the same glucose categories (Table 6).

| Table 6. Beta-cell function measured as the insulinogenic index in patients (three months) versus controls (enrolment). Values are medians and Q1, Q3. IGI = (∆30I/∆30G, pmol/mmol) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | NGT Patients    | NGT Controls    | IGT Patients    | IGT Controls    |
| n               | 50              | 120             | 59              | 45              |
| IGI             | 52.1 (33.2, 93.3) | 43.4 (30.1, 63.7) | 45.3 (34.8, 65.1) | 40.1 (22.3, 52.5) |
| Diabetes        |                 |                 |                 |                 |
| Patients        | 36              | 20              | 24.8 (20.2, 41.4) | 27.5 (16.0, 40.8) |
| Controls        |                 |                 |                 |                 |

Study II

Glucose tolerance during follow-up

Study II is based on the 122 patients, who at all occasions during follow-up (discharge and three and 12 months) could be reclassified into the three groups. A comparison of the OGTT-based glucometabolic classifications is presented in Figure 14. The agreement between the OGTTs at discharge and after three and 12 months according to weighted kappa statistics were: discharge-three months: \( \kappa = 0.35, p < 0.001 \); discharge-12 months: \( \kappa = 0.43, p < 0.001 \) and three months-12 months: \( \kappa = 0.48, p < 0.001 \).

Biochemical characteristics at discharge

There were no significant differences in age, gender or BMI between the three glucose tolerance groups at baseline (data on file). Patients changing from NGT at discharge to IGT or type 2 diabetes at 12 months had significantly higher levels of HbA1c at discharge \( (p<0.001) \), while those with IGT at discharge who changed to type 2 diabetes during follow-up had significantly higher levels of fasting blood glucose \( (p=0.004) \), triglycerides \( (p=0.046) \) and lower adjusted IGI \( (p=0.037) \). Patients originally classified as type 2 diabetes who remained in this group had higher discharge levels of HbA1c \( (p<0.001) \), triglycerides \( (p<0.001) \), HOMA-IR \( (p=0.031) \) and lower adjusted IGI \( (p<0.001) \). There were no significant differences in BMI between the different glucose tolerance groups on any occasion (data on file).
**Tertiles of HbA₁c (previously unpublished data)**

Classifications after the OGTT at discharge and after 12 months by HbA₁c tertiles at discharge are presented in Table 7. In the lowest HbA₁c tertile (3.8–4.6%), none of the patients diagnosed with NGT at discharge changed to type 2 diabetes after 12 months. In the same way, no patient in the highest tertile (5.2–7.1%) diagnosed with type 2 diabetes at discharge was classified as NGT after 12 months.

![Diagram](image)

**Figure 14.** Patients with NGT, IGT, and type 2 diabetes at hospital discharge.

**Table 7.** Patients classified after an OGTT at hospital discharge (dis) and after 12 months (12M) by HbA₁c tertiles.

<table>
<thead>
<tr>
<th>HbA₁c</th>
<th>NGT dis</th>
<th>IGT dis</th>
<th>T2DM dis</th>
<th>NGT 12M</th>
<th>IGT 12M</th>
<th>T2DM 12M</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8–4.6%</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HbA₁c</th>
<th>NGT dis</th>
<th>IGT dis</th>
<th>T2DM dis</th>
<th>NGT 12M</th>
<th>IGT 12M</th>
<th>T2DM 12M</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7–5.1%</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>17</td>
<td>0</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

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Study III

Insulin-like growth factor I

Table 8 contains all the variables related to the IGF-I system. Patients had lower levels of IGF-I compared with controls (median [Q1, Q3] in μg/l: 117.0 [75.0, 145.0] vs. 122.0 [99.0, 143.0], \( p = 0.009 \)) and patients with AGT had lower levels of IGF-I compared with those with NGT (\( p = 0.002 \)) and with controls, irrespective of glucose tolerance (NGT: \( p < 0.001 \), AGT: \( p = 0.008 \)). However, patients and controls with NGT did not differ in terms of IGF-I levels. When dividing patients with AGT into IGT and type 2 diabetes and comparing these groups with patients with NGT, there was a significant difference between the three groups with the lowest IGF-I values among those with type 2 diabetes (median [Q1, Q3] in μg/l: NGT, 128.0 [93.0, 154.0]; IGT, 99.0 [70.0, 144.5], type 2 diabetes, 90.5 [67.0, 125.0]; \( p < 0.001 \)). As can be seen in Figure 15, the age-adjusted IGF-I levels in patients and controls differed significantly (\( p < 0.001 \) for the interaction) when plotted by glucose tolerance category.

In the multivariate analysis, IGF-I at hospital discharge remained a significant predictor of AGT both at the time of hospital discharge and 12 months later (OR=0.29, \( p = 0.022 \) and OR=0.29, \( p = 0.034 \) respectively).

### Table 8.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients</th>
<th>Controls</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGT (n=55)</td>
<td>AGT (n=113)</td>
<td></td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>128.0 (93.0, 154.0)</td>
<td>93.5 (68.5, 138.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>IGF-I SD</td>
<td>-0.66 (-2.00, 0.35)</td>
<td>-1.74 (-3.00, -0.02)</td>
<td>0.014</td>
</tr>
<tr>
<td>IGFBP-1 baseline (µg/l)</td>
<td>18.0 (10.0, 28.0)</td>
<td>20.0 (12.0, 31.0)</td>
<td>0.400</td>
</tr>
<tr>
<td>2-h IGFBP-1 (µg/l)</td>
<td>10.0 (5.0, 17.0)</td>
<td>11.5 (7.0, 19.0)</td>
<td>0.143</td>
</tr>
<tr>
<td>2-h IGFBP-1/IGFBP-1 baseline</td>
<td>0.52 (0.44, 0.75)</td>
<td>0.63 (0.46, 0.74)</td>
<td>0.218</td>
</tr>
<tr>
<td>IGFBP-3 mg/l</td>
<td>3.4 (2.7, 3.9)</td>
<td>2.9 (2.2, 3.6)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NGT (n=120)</td>
<td>AGT (n=65)</td>
<td></td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>122.0 (100.0, 143.0)</td>
<td>124.0 (90.0, 149.0)</td>
<td>0.971</td>
</tr>
<tr>
<td>IGF-I SD</td>
<td>-0.81 (-1.43, -0.21)</td>
<td>-0.47 (-1.53, 0.22)</td>
<td>0.250</td>
</tr>
<tr>
<td>IGFBP-1 baseline (µg/l)</td>
<td>16.0 (10.0, 26.0)</td>
<td>19.0 (12.0, 26.0)</td>
<td>0.345</td>
</tr>
<tr>
<td>2-h IGFBP-1 (µg/l)</td>
<td>8.0 (5.0, 12.0)</td>
<td>9.0 (6.0, 14.0)</td>
<td>0.155</td>
</tr>
<tr>
<td>2-h IGFBP-1/IGFBP-1 baseline</td>
<td>0.50 (0.43, 0.58)</td>
<td>0.50 (0.44, 0.62)</td>
<td>0.342</td>
</tr>
<tr>
<td>IGFBP-3 mg/l</td>
<td>3.6 (3.2, 4.2)</td>
<td>3.8 (3.1, 4.3)</td>
<td>0.546</td>
</tr>
</tbody>
</table>

\( ^a \) \( p \)-value for the difference between glucose tolerance groups in patients and controls respectively.

\( ^b \) \( p \)-value < 0.05 for the difference between patients and controls in the same glucose tolerance category.
**Insulin-like growth factor binding proteins-1 and 3**

Fasting levels of IGFBP-1 did not differ significantly between patients with NGT and AGT and between patients and controls (Table 8). The absolute decrease in IGFBP-1 during the OGTT was significantly less in patients with AGT than in controls (NGT: \( p < 0.001 \) and AGT: \( p = 0.004 \); Table 8), resulting in significantly higher 120-min IGFBP-1 levels in patients with AGT than in controls (NGT: \( p < 0.001 \), AGT: \( p = 0.048 \)).

Fasting levels of IGFBP-3 were significantly lower in patients compared with controls (median [Q1, Q3]: 3.1 [2.4, 3.7] vs. 3.7 [3.7, 3.2] mg/l, \( p < 0.001 \)). Patients with AGT had lower levels of IGFBP-3 compared with those with NGT (\( p = 0.009 \)) and with controls (NGT: \( p < 0.001 \) and AGT: \( p < 0.001 \), Table 8).

**IGF-I system and cardiovascular events**

The levels of IGF-I, IGFBP-1 and IGFBP-3 obtained at hospital discharge did not significantly predict subsequent major cardiovascular events. The HR for IGF-I recorded the first morning after admission and major CV events was 0.56 (95% CI: [0.3 – 1.2], \( p = 0.133 \)).
Study IV

Baseline characteristics of the biochemistry group

Table 9 presents pertinent clinical and biochemical characteristics of patients who participated compared with those that did not participate in the biochemistry sub-study of DIGAMI 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Available</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>575</td>
<td>678</td>
</tr>
<tr>
<td>Age [years; median (Q1, Q3)]</td>
<td>69.6 (60.3-76.7)</td>
<td>69.7 (61.2, 77.0)</td>
</tr>
<tr>
<td>Male gender [n (%)]</td>
<td>385 (67)</td>
<td>451 (67)</td>
</tr>
<tr>
<td>BMI [kg/m2; median (Q1, Q3)]</td>
<td>27.6 (25.1, 30.7)</td>
<td>27.7 (25.3, 31.1)</td>
</tr>
<tr>
<td>Diabetes duration [yrs; med (Q1, Q3)]</td>
<td>5.3 (0.6, 12.3)</td>
<td>6.1 (1.1, 11.9)</td>
</tr>
<tr>
<td>Blood pressure [mm Hg; (Q1, Q3)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130 (116, 150)</td>
<td>130 (120, 150)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73 (62, 84)</td>
<td>80 (70, 88)</td>
</tr>
<tr>
<td>Blood-glucose (mmol/l)</td>
<td>11.9 (9.4, 15.1)</td>
<td>12.6 (10.0, 15.3)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0 (6.1, 8.4)</td>
<td>7.0 (6.1, 8.3)</td>
</tr>
<tr>
<td>S-Creatinine (mmol/l)</td>
<td>91 (79, 112)</td>
<td>98 (83, 118)</td>
</tr>
<tr>
<td>S-Cholesterol (mmol/l)</td>
<td>5.0 (4.3, 5.9)</td>
<td>5.2 (4.3, 5.9)</td>
</tr>
<tr>
<td>S-Triglycerides (mmol/l)</td>
<td>1.7 (1.2, 2.6)</td>
<td>1.7 (1.2, 2.6)</td>
</tr>
</tbody>
</table>

Baseline characteristics of IGFBP-1 tertiles

Pertinent clinical and biochemical characteristics of the patients divided into tertiles of IGFBP-1 at admission are presented in Table 10. Patients in the highest tertile were older, more frequently female, had a lower BMI and lower blood pressure. In addition, they presented higher admission blood glucose and creatinine but lower levels of triglycerides and IGF-I compared with those in the lowest tertiles. Furthermore, the patients in the highest IGFBP-1 tertile were less frequently treated with metformin prior to the study.
Table 10. Clinical and biochemical characteristics of patients divided into tertiles of IGFBP-1 at admission. N.S = not significant

<table>
<thead>
<tr>
<th>Variable</th>
<th>IGFBP-1 tertiles</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 – 24.0 μg/L</td>
<td>25.0 – 42.0 μg/L</td>
</tr>
<tr>
<td>Number of patients</td>
<td>165</td>
<td>169</td>
</tr>
<tr>
<td>Age [years]</td>
<td>65.3 (57.3, 73.3)</td>
<td>68.8 (60.5, 75.6)</td>
</tr>
<tr>
<td>Male gender</td>
<td>121 (73.3)</td>
<td>123 (72.8)</td>
</tr>
<tr>
<td>BMI [kg/m2]</td>
<td>29.0 (26.7, 32.2)</td>
<td>27.5 (25.4, 30.2)</td>
</tr>
<tr>
<td>DM duration [years]</td>
<td>5.2 (1.2, 12.4)</td>
<td>5.4 (0.7, 12.5)</td>
</tr>
<tr>
<td>Blood pressure [mm Hg]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>134 (120, 150)</td>
<td>130 (116, 145)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76 (67, 85)</td>
<td>72 (65, 82)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>36 (21.8)</td>
<td>48 (28.4)</td>
</tr>
<tr>
<td>Medication prior to adm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>53 (32.1)</td>
<td>54 (32.0)</td>
</tr>
<tr>
<td>Metformin</td>
<td>54 (32.7)</td>
<td>45 (26.6)</td>
</tr>
<tr>
<td>Gilbenclamide</td>
<td>46 (27.9)</td>
<td>41 (24.3)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>63 (38.4)</td>
<td>69 (40.8)</td>
</tr>
<tr>
<td>Aspirin 75 mg</td>
<td>50 (30.3)</td>
<td>52 (30.8)</td>
</tr>
<tr>
<td>ACE-inhibitor</td>
<td>51 (30.9)</td>
<td>53 (31.4)</td>
</tr>
<tr>
<td>Lipid Lowering</td>
<td>53 (32.1)</td>
<td>48 (28.4)</td>
</tr>
<tr>
<td>Biochemistry at admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood-glucose (mmol/l)</td>
<td>11.4 (9.0, 14.0)</td>
<td>11.8 (9.3, 14.6)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.8 (6.1, 8.1)</td>
<td>7.0 (6.1, 8.3)</td>
</tr>
<tr>
<td>S-Creatinine (mmol/l)</td>
<td>84 (75, 97)</td>
<td>94 (81, 109)</td>
</tr>
<tr>
<td>S-Cholesterol (mmol/l)</td>
<td>5.1 (4.2, 5.8)</td>
<td>5.0 (4.3, 6.0)</td>
</tr>
<tr>
<td>S-Triglycerides (mmol/l)</td>
<td>2.1 (1.4, 3.0)</td>
<td>1.7 (1.1, 2.5)</td>
</tr>
<tr>
<td>S-IGF-I (μg/l)</td>
<td>141 (112, 169)</td>
<td>124 (93, 154)</td>
</tr>
</tbody>
</table>

**Prediction models for cardiovascular mortality and morbidity**

The univariate survival analysis of the IGF-I system showed that ln IGF-I at the time of hospital admission or discharge (day 4-5) and three or 12 months later did not relate to cardiovascular death in contrast to ln IGFBP-1 at admission (adm), discharge (DIS) and three (3M) and 12 months (12M) (HRadm: 1.9, p<0.001; HRdis: 1.8, p=0.002; HR3M:1.5, p=0.059; HR12M: 2.8, p=0.004). The analysis of the secondary endpoint revealed that ln IGF-I at admission and after three months related to future cardiovascular events (HR3M: 0.5, p=0.012) in contrast to ln IGF-I at discharge and after 12 months. Ln IGFBP-1 related to cardiovascular events on all occasions (HRadm: 1.5, p<0.001; HRdis: 1.5, p=0.002; HR3M: 1.5, p=0.012; HR12M: 1.7, p=0.035).

In the best subset analysis of predictors, ln IGFBP-1 at admission remained significantly related to cardiovascular death (HR: 1.5, p<0.001) and to cardiovascular events (HR: 1.2, p=0.028), together with age and ln creatinine at admission. The predictive power increased when updated mean blood glucose was entered into the models as an explanatory variable (data on file). Candidate predictors with p-values of < 0.2 that did not remain in the final model were admission blood glucose, IGF-I, BMI, gender, smoking status and previous coronary disease history. Crude survival curves for IGFBP-1 tertiles and cardiovascular survival are presented in Figure 16.
**Study V**

*Leptin in patients versus controls in GAMI*

Women had higher leptin levels than men and patients of both genders had higher levels of leptin on day two compared with controls (Figure 17).

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**Figure 16.** Crude Kaplan-Meier curves of tertiles of IGFBP-1 during follow-up related to cardiovascular events (cardiovascular death/reinfarction or stroke).

**Figure 17.** Leptin (day 2) in patients (n=180) and controls (n=184) stratified for gender. Values are presented as the crude mean and SD. * = p<0.05 (adjusted for age and BMI)
**Leptin as a predictor of cardiovascular events**

In a multiple regression analysis adjusted for age, gender, BMI, previous AMI or stroke, (ln) leptin was the only variable that remained a significant predictor of cardiovascular events (HR 1.75, \( p = 0.045 \)). The relationship between (ln) leptin and cardiovascular events remained (HR 1.78) but was not statistically significant (\( p = 0.075 \)) when AGT at discharge and “severe infarction” were forced into the multivariate model (HR 3.6, \( p = 0.041 \) and HR 2.9, \( p = 0.007 \) respectively). Ln leptin at hospital discharge and after three months was not significantly related to future cardiovascular events (univariate HRs 1.25, \( p = 0.299 \) and 1.15, \( p = 0.562 \) respectively).

Figure 18 presents crude Kaplan-Meier curves of time until a major cardiovascular event in all patients, divided into those above and those below the median leptin level on day two (women: 22.6 mmol/l, men: 8.9 mmol/l). Survival was lower in men and women with high levels of leptin (HR 2.29, \( p = 0.018 \)).

**Adiponectin and leptin/adiponectin ratio**

Neither adiponectin levels nor the leptin/adiponectin ratio differed between patients and controls (mean (95% CI): 14.2 [12.8, 15.6] vs. 13.0 [11.9, 14.1] mg/l) and did not relate to AGT or cardiovascular events (data on file).
GENERAL DISCUSSION

Main findings

Glucose abnormalities in AMI patients without previously known glucose perturbations are related to a substantial degree to beta-cell dysfunction. These patients can be reliably detected with an OGTT already four to five days after the infarction. Low levels of IGF-I are related to AGT and high levels of leptin in the acute phase of an AMI are related to the subsequent prognosis. Finally, high levels of IGFBP-1 predict a dismal prognosis in AMI patients with established type 2 diabetes.

Beta-cell dysfunction

Beta-cell dysfunction in patients with AMI and glucose abnormalities

The relationships between insulin resistance and beta-cell function and glucose tolerance were investigated by comparing the respective contributions of HOMA-IR and IGI to the AUCg. The findings in Study I underline the fact that not only insulin resistance but also beta-cell dysfunction is an important contributor to AGT in patients with AMI with newly detected glucometabolic disturbances. They confirm that hyperglycaemia in connection with an AMI is not only, as commonly assumed, a stress epiphenomenon but an expression of abnormal glucose regulation. It is generally accepted that type 2 diabetes is characterised by both insulin resistance and insulin deficiency [7], but the question of which of these disturbances is the initial one and how to distinguish them from each other is the subject of debate [110]. As a result, the treatment of patients with mild glucose abnormalities has focused on remedies that increase insulin sensitivity and it is only recently that attention has focused on beta-cell dysfunction when addressing glucose abnormalities [111].

In the GAMI cohort, beta-cell dysfunction (IGI) and insulin resistance (HOMA-IR), noted four to five days after the onset of an AMI in patients with AGT in comparison to those with NGT, persisted when investigated three and 12 months after hospital discharge. During this period, none of the patients was treated with glucose-lowering drugs. Other agents, not the least beta-blockers and ACE inhibitors, may impact the insulin and glucose response [112, 113], but, apart from statins that were more frequent in patients with NGT at discharge, the pharmacological treatment did not differ between patients with different levels of glucose tolerance.

Common pathways connecting type 2 diabetes with CVD have been suggested during the past decade [114, 115]. One hypothesis is that damage caused by inflammation and oxidative stress in the vascular endothelium may affect other cells, including the pancreatic
beta-cells, in parallel. So hyperglycemia caused by insulin resistance and reduced beta-cell function may cause a further deterioration in beta-cell function, creating a vicious circle which has been referred to as the “common soil theory” of diabetes and CVD (Figure 19) [115]. It has indeed been suggested that prolonged exposure to high blood glucose may result in beta-cell damage [116].

Furthermore, elevated plasma FFA concentrations have been observed to hamper insulin secretion through toxic effects on the beta-cells [117] and the long-term reduction of FFAs improves the acute insulin response and insulin-mediated glucose uptake [118]. In the GAMI study, concentrations of FFAs were significantly increased in patients with abnormal glucose tolerance [103] and may therefore have contributed to the beta-cell dysfunction.

Increased proinsulin levels are associated with beta-cell dysfunction [119]. As reported previously, proinsulin was significantly higher in the GAMI patients compared with controls and highest in those with AGT [103]. These findings, together with the “common soil theory”, indicated that patients with AMI and AGT may have even lower beta-cell function compared with controls with AGT, reflecting a vulnerability to oxidative stress in the vessel wall and beta-cells. In Study I, the IGI correlated to fasting levels of proinsulin and the proinsulin/insulin ratio at discharge (data on file). However, when IGI was analysed in the GAMI controls, it did not differ between patients and controls within the same glucometabolic category. The control subjects displayed a similar pattern, with decreasing IGI among those with AGT compared with those with NGT (supplementary results, page 34). The beta-cell dysfunction in patients with AMI and AGT was not worse...
than that of controls with AGT. Nevertheless, although it does not represent a unique feature among AMI patients, beta-cell dysfunction is still an important finding, directing novel primary and secondary prevention strategies which previously focused first and foremost on improving insulin resistance.

Proinsulin was an independent predictor of coronary heart disease mortality in a Swedish study with 27 years of follow-up [120]. The lack of a significant correlation between IGI and future cardiovascular events in Study I may therefore seem surprising. There was, however, a trend in this direction (HR 0.63, 95% CI: 0.35-1.15, data on file). The lack of statistical significance may relate to a type II error caused by a low event rate and limited follow-up period.

**Insulinogenic index and adjusted insulinogenic index**

As multiple samples were collected during the OGTTs in the GAMI study, the insulinogenic index (IGI; [ΔI30/ΔG30]), a commonly utilised index of beta-cell response [27, 29-31], was the natural choice in Study I. IGI was closely correlated to insulin secretion in comparative studies of several indices of beta-cell function [32].

In order to obtain a disposition index (adjusted IGI) that has been advocated in some studies [121, 122], adjustment for the actual level of insulin resistance [IGI/HOMA-IR = (Δ30I/Δ30G)/HOMA-IR] has been presented in this thesis (supplementary results, page 33). In the Finnish Botnia Study, this disposition index was the strongest metabolic predictor of subsequent type 2 diabetes in patients with NGT or IFG/IGT during a 10-year follow-up [123]. The differences that have already been demonstrated in beta-cell dysfunction between GAMI patients with AGT and NGT became even more apparent when the disposition index was used.

**Classification with OGTT in patients with AMI**

Study II revealed that classification based on an OGTT at the time of hospital discharge may serve as a reliable tool for the early detection of glucometabolic perturbations in patients with AMI. The results were particularly robust for the patients with type 2 diabetes and NGT. Among patients diagnosed with diabetes at discharge, the vast majority (93%) still had AGT 12 months later (type 2 diabetes: 64%; IGT: 29%). Likewise, only a minority (12%) of those with NGT at discharge had developed type 2 diabetes over time. Not surprisingly, the most unpredictable patients were those with IGT at discharge. In this group, a similar percentage changed to NGT or type 2 diabetes during follow-up. Patients with consistent type 2 diabetes already had a more pronounced diabetic phenotype at discharge compared with those that normalised their glucometabolic situation. Their HbA1C, triglycerides and HOMA-IR were higher and their beta-cell dysfunction was more apparent. Interestingly, the fasting plasma glucose at discharge did not differ between patients who still had diabetes at 12 months compared with those who, at that time, had normalised their glucose tolerance. Moreover, patients in the lowest tertile of HbA1c at admission (supplementary results, page 35) were more likely to have an NGT after 12
months, while those in the highest tertile most frequently had persistent AGT. An evaluation of the complete glucometabolic profile, most easily HbA1c, can therefore provide additional information in patients with borderline OGTT results and justify a subsequent re-evaluation.

The OGTT has historically been referred to as time consuming, inconvenient and expensive [124] and has been criticised for poor reproducibility [125]. Nevertheless, some studies have found that the reproducibility of post-load glucose measurements is no worse than that of fasting glucose [126, 127]. Furthermore, a German study that evaluated the cost effectiveness of type 2 diabetes screening in a general population, aged 55-74 years, concluded that the OGTT was the most effective single tool compared with HbA1c, fasting blood glucose or a combination [128]. Another argument for not performing an OGTT in hospitalised AMI patients has been the interpretation of hyperglycaemia as a “stress epiphenomenon” rather than an expression of the true glucometabolic state [129]. A previous report from the GAMI study revealed that the glucometabolic condition in patients with AMI had already stabilised during the time of hospitalisation. Biochemical parameters such as blood glucose, insulin, HbA1c and HOMA-IR obtained on days 4-5 were similar to those analysed three months later [130]. Moreover and as already discussed, the present investigation revealed that newly detected glucose abnormalities in AMI patients represent a manifest condition rather than being caused by temporary stress during the acute phase of the AMI (Study I).

One explanation for the somewhat low reproducibility of the OGTT is probably that glucometabolic classification is arbitrary in itself. For example, a patient with fasting plasma glucose of 6.9 mmol/l and 2-h glucose of 7.7 mmol/l is labelled as normal, while a patient with fasting plasma glucose of 6.9 mmol/l and 2-h glucose of 7.8 mmol/l is categorised as having IGT and, if the fasting plasma glucose is 7.0 mmol/l and the 2-h glucose 7.7 mmol/L, he or she is classified as suffering from type 2 diabetes. Obviously, these patients are not clinically different and any change in the classification after repeating the OGTT may be accidental. In the context that hyperglycaemia is a continuous risk factor [38, 131], such dichotomised borders become artificial and the clinically important information is that an early OGTT will help to identify patients running a higher (those with AGT) or lower risk (those with NGT) of future cardiovascular mortality and morbidity [42, 43]. Several studies have stressed the importance of the early discovery of patients with IGT, due to their enhanced risk of progression to type 2 diabetes. This development may be prevented or at least retarded by weight reduction combined with increased physical activity and, if lifestyle modification is difficult to accomplish, by pharmacological agents [132-134]. In the ESC/EASD guidelines on diabetes, pre-diabetes and cardiovascular disease [135], patients without known diabetes but with established CVD are recommended to be investigated with an OGTT. In contrast, the American Diabetes Association (ADA) does not recommend this test as a clinical routine. However, in 2007, the ADA lowered the threshold for impaired fasting plasma glucose (IFG) from > 6.1 to > 5.6 mmol/l in order to increase the likelihood of detecting patients with IGT by using only fasting glucose [5]. A report from the Euro Heart Survey showed that, even if the agreement between the WHO and the ADA criteria increased with this lower cut-off point, 29% of patients with diabetes revealed by an OGTT and 57% with IGT would still have remained undiagnosed using only fasting plasma glucose [136].
The insulin-like growth factor system

Relation to abnormal glucose tolerance

The IGF system is important for glucose homeostasis [137] and low levels of IGF-I relate to an increased risk of developing type 2 diabetes [67]. Study III revealed that AMI patients with newly detected AGT have lower levels of IGF-I and IGFBP-3 compared with patients with NGT and with controls, irrespective of glucose tolerance, and a low IGF-I predicted the glucometabolic state. The association between IGF-I and AGT did only exist in patients with AMI and not in controls. Furthermore, IGF-I was inversely related to the 120-min post-load blood glucose and 30 minutes insulin response but not to fasting blood glucose. This indicates that IGF-I is related to processes regulating postprandial glucose, such as first-phase insulin secretion and glucose uptake. Low IGF-I levels at the time of hospital discharge remained as a predictor of AGT at discharge and after 12 months, even after adjustment for several known risk factors for diabetes (FFAs, triglycerides and proinsulin) in the multiple logistic regression models. Moreover, in the best subset analyses, investigating the predictive values of all candidate predictors, IGF-I at discharge was a better predictor of AGT both at discharge and after 12 months than traditional risk factors such as age, HDL- and LDL-cholesterol, hs-CRP, BMI, insulin, proinsulin and HOMA-IR.

The present finding that low levels of IGF-I relate to disturbed beta-cell function (Study III) supports a previous report that suggests that IGF-I is important for the beta-cells as a regulator of apoptosis [138]. It has been suggested that polymorphisms in the IGF-I gene may be important for insulin secretion [139] and the same polymorphism is associated with low birth weight [140], a condition that increases the risk of type 2 diabetes and CVD [141]. Thus the combination of IGF-I and AGT may be a risk factor for AMI, suggesting polymorphism in several genes. Furthermore, in Study III, IGF-I was inversely correlated to FFAs, a finding which at least in part may explain the relationship between low IGF-I and beta-cell dysfunction, as increased levels of FFAs exert a toxic effect on the beta-cells [117].

Relation to cardiovascular events

Circulating levels of IGF-I, IGFBP-1 and IGFBP-3 have been proposed as risk factors for CVD in large population studies [68, 71, 142, 143]. In Study III, neither IGF-I nor IGFBP-1 measured at hospital discharge predicted future cardiovascular events, while low IGF-I two days after the AMI related to an increased risk, even if it was of borderline significance. When this trend was further explored, a significantly increased event rate was found in the lowest compared with the highest IGF-I tertiles (data on file). The difference in the predictive power of IGF-I measured on day two and at the time of hospital discharge (day 4-5) may possibly be explained by the fact that events occurred between these two time periods. In the light of previous reports on the relationship between a low IGF-I during the acute phase of a myocardial infarction and a poor outcome [144], the present findings may be interpreted as indicating that IGF-I could be of importance in regulating the magnitude of myocardial injury during profound ischaemia.

It was of interest to measure IGFBP-3 in the GAMI population, since the Danish DAN-MONICA study showed that patients with low IGF-I and high IGFBP-3 ran an increased
risk of ischaemic heart disease [68]. In Study III, patients with AGT had lower levels of IGFBP-3 compared with patients with NGT and controls and these levels correlated strongly with IGF-I. Previous studies have reported lower levels of IGFBP-3 in patients with both type 2 diabetes [145] and coronary heart disease [146] compared with healthy subjects. On the other hand, the ratio between IGF-I/IGFBP-3 has been related to the metabolic syndrome [147], indicating that high levels of IGFBP-3 are related to cardiovascular risk. In Study III, neither IGFBP-3 nor the ratio between IGF-I/IGFBP-3 related to future cardiovascular events (data on file).

The reason for studying the relationship between the insulin-like growth factor system in the DIGAMI 2 cohort (Study IV) in greater detail was that the total number of events in the GAMI population (Study III) was relatively limited, creating the possibility of type II statistical errors. In Study IV, including AMI patients with established type 2 diabetes, IGF-I measured at the time of hospital admission and after three months correlated to cardiovascular events. Likewise, IGFBP-1 measured at admission, discharge and after three and 12 months was a strong predictor of cardiovascular events that remained significant after multivariate adjustments.

There are many mechanisms linking IGF-I to coronary heart disease not only through glucometabolic control but also in relation to local effects in the myocytes or vessel wall promoting myocyte survival, endothelial function, vascular compliance, vascular smooth muscle cell proliferation and migration and the inhibition of macrophages, apoptosis and necrosis [72]. The exposure of the patients in Study III (GAMI) to AGT and low levels of IGF-I during a reasonably long period of time may therefore have made them susceptible to developing an AMI. Vaessen et al. identified a polymorphism in the promoter region of the IGF-I gene that is associated with low IGF-I levels and an increased risk of myocardial infarction that seemed particularly important in patients with type 2 diabetes [148]. Some studies have indicated that it is the free fraction rather than the total concentration of IGF-I that is the most valuable variable [149]. Free IGF-I was not measured in the present studies due to the lack of a reliable and commercially available method. There is however, a strong inverse correlation between free IGF-I and IGFBP-1 [150, 151] and low levels of free IGF-I would therefore be expected in patients with high levels of IGFBP-1. Along these lines, the prognostic predictability of IGFBP-1 in Study IV may be that IGFBP-1 mirrors the concentration of free IGF-I and the high affinity binding of IGFBP-1 to IGF-I would then attenuate the known beneficial effects of IGF-I [65].

An observation in Study III that could explain the results in Study IV is that the inhibition of IGFBP-1 during the OGTT was significantly less in patients with AGT compared with controls. This may be interpreted as an expression of increased hepatic insulin resistance [137]. Hepatic IGFBP-1 production is inhibited by insulin [152] and when insulin rises during the OGTT in the presence of normal hepatic insulin sensitivity, the IGFBP-1 concentrations fall. High levels of IGFBP-1 may therefore reflect enhanced hepatic insulin resistance. However, due to the complex relationship to insulin, it becomes difficult to interpret IGFBP-1 results. In different populations, high and low levels may be markers of increased cardiovascular risk. In patients with normal glucose tolerance or mild abnormalities and normal hepatic insulin sensitivity, low levels of IGFBP-1 may relate to an increased risk by signalling hyperinsulinemia [153]. As the disease develops into overt type 2 diabetes, IGFBP-1 rises as a result of persistent hyperinsulinemia [154]. In these circumstances, high levels of IGFBP-1 may relate to increased cardiovascular risk. In line with this, patients with severe hepatic cirrhosis have demonstrated high fasting levels of IGFBP-1 in the presence of elevated insulin levels. Interestingly, these patients had a less
pronounced insulin-mediated suppression of IGFBP-1 during an OGTT [155]. Van den Berghe et al. reported on a correlation between high levels of IGFBP-1 and mortality in critically ill patients who were unresponsive to insulin [156]. In Study IV, patients with high levels of IGFBP-1 were less well glucometabolically controlled, as indicated by their higher blood glucose and lower levels of IGF-I at admission. Furthermore, they had lower BMI, triglycerides and blood pressure, which may indicate a catabolic condition similar to that of patients at the intensive care unit.

A link between IGFBP-1 and pro-inflammatory cytokines has been demonstrated [157] and, since both type 2 diabetes and CVD are conditions with increased inflammatory activity, this correlation must be taken into account. Analyses of inflammatory factors were not performed in Study IV. However, in Study III (GAMI) IGFBP-1 in the acute phase did not correlate to hs-CRP.

A correlation to physiological stress could partially explain the findings in Study IV. After a 30-min infusion of epinephrine in healthy men, levels of IGFBP-1 increased but returned to basal after approximately two hours [158]. The levels of IGFBP-1 during follow-up predicted cardiovascular events, which makes it unreasonable to believe that stress would be an important explanation of the correlation between high levels of IGFBP-1 and a dismal prognosis.

Adipokines

Leptin, abnormal glucose tolerance and prognosis

There are several studies linking high levels of leptin to CVD [91-93, 159-161]. In spite of this, the relationship is far from obvious, as other studies have been unable to verify these correlations [162, 163]. In Study V, based on a population of men and women with fairly normal BMI and without previously established diabetes, leptin recorded during the acute phase of an AMI related to the prognosis. Interestingly, the predictive power of leptin was independent of many traditional risk markers such as gender, age, BMI, smoking, previous medical history, severity of the infarction, glucose tolerance, insulin resistance, proinsulin, dyslipidaemia and hs-CRP. There was also a trend towards a relationship between leptin at hospital discharge and subsequent cardiovascular events. It must be acknowledged that about 20% of all events occurred during hospitalisation. Thus, the power to establish a definite prognostic value for leptin measured at discharge was therefore too low for any definite conclusions. It has been suggested that leptin affects blood pressure regulation by stimulating the sympathetic nervous system. Furthermore, leptin affects local lipid balance and contributes to endothelial dysfunction, impaired fibrinolysis and increased oxidative stress, all important parts of the atherosclerotic process. An increase in leptin during the AMI could be a response to increased inflammatory activity [90, 164]. A relationship between CRP and leptin has been reported [165], but leptin did not correlate to hs-CRP in Study V. The increase in leptin is also in line with earlier studies and may indicate that leptin is associated with the metabolic response to acute illness [166]. Furthermore, a study of 30 patients with AMI showed that the levels of leptin increased during the first 24 hours, returning to normal five days later. In this study, there was no correlation between cortisol and leptin or between BMI and leptin. The increase in leptin was interpreted as the result of an acute rise in inflammatory cytokines such as TNF-alpha and IL-6, known to
stimulate leptin production [167]. Likewise, there was no correlation between cortisol and leptin in Study V (data on file).

Another finding from Study V was that increased leptin levels during the acute phase of the AMI were related to AGT detected at the time of hospital discharge. This relationship did not, however, remain when proinsulin or fasting blood glucose were entered into the statistical model. Leptin predicted the development of diabetes in Mauritian [168] and Japanese American men [169]. This may possibly be mediated by an impact on beta-cell function.

Leptin resistance has recently been identified as a possible mechanism behind the relationship between leptin and CVD and hyperinsulinemia. As with insulin resistance, this appears to depend on the link between obesity, type 2 diabetes and CVD [170]. Future attention to this research field is to be expected, as it appears that neither insulin resistance nor the inflammatory state can fully explain why some of the patients in the present patient material had a more serious prognosis than others. It can be hypothesised that leptin or leptin resistance represent integrated markers of metabolically active risk factors and may be useful in future clinical risk stratification.

Adiponectin

Study V did not disclose any relationship between adiponectin or the leptin/adiponectin ratio and AGT or future cardiovascular events. This was somewhat unexpected in the light of several previous reports on associations between low plasma adiponectin concentrations, obesity, coronary artery disease and type 2 diabetes [94, 95]. In fact, it has been suggested that adiponectin has anti-atherogenic properties [96]. Low levels of adiponectin predicted MI in the Health Professionals Follow-up study [97] but not coronary heart disease among American Indians [98]. It has been suggested that the association with the development of type 2 diabetes is more consistent [99]. The reason for these differences could be ethnic, first-ever versus recurrent events, the setting of AMI and differences in BMI which was fairly low in the present cohort. Analytical issues could also be important, as sub-fractions of high-molecular weight adiponectin could convey the increased risk. Another explanation might be the small size of the present material with a lack of power for detecting a relationship with future cardiovascular events and the fact that the patients in GAMI have glucose abnormalities that are detected at an early stage.

Study populations

The GAMI population represents a unique material comprising well-characterised patients that are collected prospectively and followed up over a fairly long period. In spite of this, it is still a relatively small cohort with rather few events, which limits the opportunity for prognostic studies to some extent. A major strength is that none of the patients was treated for glucose abnormalities during the study period, partly because glucose abnormalities in AMI patients were regarded as a transient phenomenon. In addition, there were no established routines for addressing newly detected AGT in patients with AMI at that time. Another strength is the recruitment of controls, making
GAMI a combination of a prospective cohort and a case-control study well suited for studies of biomarkers.

The DIGAMI 2 trial was a prospective, randomised clinical trial comparing three different management strategies in patients with type 2 diabetes and AMI. Due to the fact that there were no significant differences in primary or secondary endpoints between the three study arms, all the patients could be combined into a cohort useful for epidemiological studies. The DIGAMI 2 protocol included a biochemistry protocol in which centres could participate at different blood sampling levels. For logistical reasons, not all the centres were able to participate in this programme, which recruited 575 patients from 14 centres. Pertinent clinical characteristics and prognosis were similar in patients who did or did not participate in the biochemistry programme. As a result, the biochemistry population from DIGAMI 2 is a fairly large, well-characterised population of patients with type 2 diabetes and AMI running a high risk of subsequent mortality and morbidity. Another strength is that all the events were adjudicated by an independent committee according to firmly established criteria.

Final remarks and future implications

This thesis supports the idea of early oral glucose tolerance testing in patients with AMI, which is a reliable tool with the potential to discover previously unknown glucose abnormalities related to not only insulin resistance but also beta-cell dysfunction. The thesis further underlines the importance of the IGF system and the adipokines in the pathogenesis of CVD and glucose abnormalities.

It is important to investigate whether an improvement in glucose levels has the potential to improve the outcome for AMI patients with newly detected glucose perturbations. Lifestyle modification, an obvious measure, is difficult to accomplish and thereby of limited feasibility. Initial observations with acarbose are promising [171] and another pharmacological possibility is the early institution of insulin, which is currently being tested in the ORIGIN (Outcome Reduction with Initial Glargine Intervention) trial. Among other oral agents, metformin or the glitazones may be discussed. These drugs prevent or retard the onset of diabetes among patients with IGT [133, 172], but they have not been tested in this setting.

From the results of this thesis, it would be interesting to study whether an improvement in beta-cell function in AMI patients would have a positive effect on the prognosis. It is known that beta-cell function may improve, following treatment with some drugs, including insulin [173], sulfonylurea [174], acarbose [175], troglitazone [176] and rosiglitazone [177]. However, interest has recently focused in particular on the potential benefits of the GLP-1 system [178-182]. GLP-1 is a naturally occurring incretin hormone, produced in intestinal L cells and secreted as a response to food intake and it increases insulin secretion in the beta-cells [183].

Interestingly, treatment with IGF-I results in improved glucose and lipid metabolism and improvements in muscle and hepatic insulin sensitivity [184, 185]. However, historically low-dose subcutaneous treatment with IGF-I has been associated with unacceptable adverse events such as oedema in the face and hands, arthralgias and myalgias, fatigue, tachycardia,
flushing and orthostatic hypotension [186]. Nowadays, the combination of IGF-I and IGFBP-3 is always used in clinical settings and continuous subcutaneous infusion of this combination up to one week in patients with type 2 diabetes reduced fasting glucose significantly and the adverse events were few [187]. Future studies are needed to evaluate the clinical potential of IGF-I/IGFBP-3 as a glucose-lowering treatment strategy during the initial course of an AMI, for example.

With respect to the results in Study IV, a recent observation of fourteen patients with diabetes insipidus has attracted considerable interest. Following an injection of desmopressin (a vasopressin analogue), the levels of IGFBP-1 increased [188], with the implication that an increase in IGFBP-1 in AMI patients may mirror high levels of vasopressin. If this finding is confirmed in future studies, vasopressin-receptor blockers, which have favourable effects on patients with heart failure [189], may prove to have interesting therapeutic potential.

To summarise; patients with AMI and glucose abnormalities require further attention, due to their dismal prognosis in combination with a shortage of evidence-based treatment strategies that can improve outcome. The studies in this thesis focus on three novel risk markers for AMI patients with glucose abnormalities: beta-cell dysfunction, the IGF-I system and leptin. It appears that all of them, at different levels, are related to CVD and glucose abnormalities. As an expression of the heterogeneity of these disorders, they are inter-related to some extent and they are probably also related to other risk markers, as exemplified in Figure 7. It is important to remember that these risk factors or risk markers do not act in isolation but may indeed cluster and interact with each other in many ways and the findings in this thesis may be interpreted as pieces in a complex jigsaw puzzle. These novel markers may prove useful in future approaches to cardiovascular risk stratification in clinical practice and may have important implications in the search for novel cardiovascular therapeutic strategies.
CONCLUSIONS

1. Impaired beta-cell function is an important regulator of glucose abnormalities in patients with AMI and newly detected IGT and type 2 diabetes. This implies that dysglycaemia immediately after an AMI is not a stress epiphenomenon but a pre-existing disturbance. Future research must test whether therapies that improve beta-cell function can normalise glucose abnormalities and improve outcome.

2. The outcome of an OGTT before hospital discharge in patients with an AMI is a reliable measure of the glucometabolic state, even in a long-term perspective.

3. Low levels of IGF-I are related to glucose abnormalities in AMI patients without previously known type 2 diabetes and high levels of the binding protein IGFBP-1 at admission are associated with an increased risk of cardiovascular mortality and morbidity in patients with AMI and established type 2 diabetes. These findings add important information to our understanding of the pathophysiological processes underlying both AMI and glucose abnormalities and warrant further studies.

4. Elevated levels of leptin in the acute setting of an AMI are associated with the presence of AGT at discharge and to a more serious long-term prognosis. Leptin levels may therefore be useful in future clinical risk stratification and in the search for novel cardiovascular treatment strategies.
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