Metabolic Syndrome and associated pathologies

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1 Introduction

Metabolic syndrome is a combination of risk factors — abdominal obesity, hypertension, high blood sugar levels, insulin resistance (IR), abnormal cholesterol levels, hyperlipidemia and inflammatory states — that increase the risk of heart disease, stroke, diabetes type 2, fatty liver disease and PCOS (in young women). Having any of these conditions increase the risk of serious disease. If more than one of these conditions occur in combination, the risk is even greater.

Metabolic syndrome is now present in up to 40% of the United States adult population (prevalence on average 25% of the population and 40+ % from age 60 and higher) [2] and is associated with a nearly a two fold increase in cardiovascular events, independent of the presence of diabetes mellitus. Obesity is the dominant key feature of metabolic syndrome [1, 2], although patients of normal weight may also suffer from IR and metabolic syndrome, and obesity and metabolic syndrome do not always occur in concordance as there is some evidence for conditions of benign obesity [3–7]. The epidemic of obesity in in adults and children in both industrialized and third world countries is regarded as one of the most serious public health problems of the 21st century.

Many (especially overweight) people with IR are able to compensate for the increasing insulin requirement, so that the blood sugar does not rise at first. Late stage β-cell dysfunction then develops into clinically manifest type 2 diabetes in approximately one third of these patients [31]. Development of macrovascular damages may, therefore, already have developed before type 2 diabetes is clinically manifest (normal or impaired glucose tolerance test result). These macrovascular damages are, in part, irreversible, need to be treated lifelong and still are the actual cause of death in 75% of diabetes type 2 patients.

Up to now, the underlying pathomechanisms of metabolic syndrome are not detected at all or only very late and inadequately. In this review we discuss the latest clinical insights into Metabolic syndrome and related disease like diabetes type 2, fatty liver disease, coronary disease and PCOS. Conventional diagnosis is discussed alongside the use of new biomarkers. Three new biomarkers appear to be particularly suitable for early diagnosis and therapy selection due to their stability and studies that are presently available: intact Proinsulin, Adiponectin and hsCRP [29]. In addition, the effect of the therapy used on pathophysiological basic components can be checked by means of these new markers.
2 Metabolic syndrome

a. Definition and pathophysiology of metabolic syndrome

Metabolic syndrome is a cluster of risk factors — abdominal obesity, hypertension, high blood sugar levels, IR, abnormal cholesterol levels, hyperlipidemia and inflammatory states — that increase the risk of heart disease, stroke, diabetes type 2, fatty liver disease and PCOS (in young women). It has also been variously termed X syndrome, insulin resistance syndrome, metabolic syndrome X, cardiometabolic syndrome, syndrome X, Reaven’s syndrome, and CHAOS (in Australia). The diagnostic criteria for metabolic syndrome have been set out by different organizations with slight variations in these criteria as shown in Table 1 [8].

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<thead>
<tr>
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<tbody>
<tr>
<td>Presence of one of following:</td>
<td>Insulin resistance AND two or more of following:</td>
<td>Presence of three of following (2004):</td>
<td>Central obesity (11) AND any two of following:</td>
</tr>
<tr>
<td>DM / IGT / IFG / Insulin resistance</td>
<td>Central obesity (6)</td>
<td>Elevated waist circumference (7)</td>
<td>Raised TG (12)</td>
</tr>
<tr>
<td>AND two of the following:</td>
<td>Dyslipidemia (6)</td>
<td>Elevated TG (7)</td>
<td>▼ HDL (13)</td>
</tr>
<tr>
<td>BP ≥ 140/90</td>
<td>BP ≥ 140/90</td>
<td>Reduced HDL (6)</td>
<td>▲ BP (16)</td>
</tr>
<tr>
<td>Dyslipidemia (1)</td>
<td>FBG ≥ 6.1 mmol/L (110 mg/dL)</td>
<td>Elevated BP (9)</td>
<td>▲ FBG (17)</td>
</tr>
<tr>
<td>Central obesity (2)</td>
<td></td>
<td>Elevated fasting glucose (19)</td>
<td></td>
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<tr>
<td>Microalbuminuria (3)</td>
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Table 1: Comparison of definitions of the metabolic syndrome.


(1) TG ≥ 1.695 mmol/L and HDL ≤ 0.9 mmol/L (male), ≤1.0 mmol/L (female).
(2) Waist/hip ratio > 0.90 (male) or >0.85 (female), or body mass index > 30 kg/m².
(3) Urinary albumin excretion ratio ≥ 20 μg/min or albumin/creatinine ratio ≥ 30 mg/g.
(4) Waist circumference ≥ 94 cm (male), ≥80 cm (female).
(5) TG ≥ 2.0 mmol/L and/or HDL < 1.0 mmol/L or treated for dyslipidemia.
(6) Men, greater than 40 inches (102 cm) and women, greater than 35 inches (88 cm).
(7) Equal to or greater than 150 mg/dL (1.7 mmol/L).
(8) Men, Less than 40 mg/dL (1.03 mmol/L) and women, Less than 50 mg/dL (1.29 mmol/L).
(9) Equal to or greater than 130/85 mm Hg or use of medication for hypertension.
(10) Equal to or greater than 100 mg/dL (5.6 mmol/L) or use of medication for hyperglycemia.
(11) Defined as waist circumference with ethnicity specific values (if BMI is >30 kg/m², central obesity can be assumed and waist circumference does not need to be measured).
(12) TG > 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality.
(13) HDL < 40 mg/dL (1.03 mmol/L) in males, <50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality.
(14) Systolic BP > 130 or diastolic BP > 85 mm Hg, or treatment of previously diagnosed hypertension.
(15) FPG > 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes.
Oxidative stress, which is defined as imbalance between the production and inactivation of reactive oxygen species, has a major pathophysiological role in all the components of metabolic syndrome [20–24]. Oxidative stress and consequent inflammation induce insulin resistance (IR) which likely initiates metabolic syndrome and massive damage of pancreatic β-cell dysfunction.

The association between the metabolic syndrome and inflammation is well documented [9]. Welsh et al. [10] demonstrated that adiposity leads to higher levels of the “acute phase” inflammatory protein CRP (C-reactive protein) and accumulating evidence demonstrates a close link among metabolic syndrome, chronic inflammation and oxidative stress [11]. In fact, the oxidative stress-inflammation pathway has important roles in all the individual components of Metabolic syndrome including vascular alterations [11–15]. Figure 1 shows the link between obesity, inflammation, insulin resistance, β-cell dysfunction, and cardiovascular risk. [28, 29]. Adipo(cyto)kines (e.g. Adiponectin) and other factors produced by fat tissue and antiinsulinemic hormones play a key role in the process.

b. The role of adipokines

Multiple adipokines can be held responsible for the negative consequences of abdominal adipogenesis on insulin resistance. The growth of lipid tissue is induced by the differentiation of mesenchymal stem cells to become preadipocytes and finally mature lipid cells. In this stage peripheral monocytes/macrophages migrate into the lipid tissue and are kept at a constantly increased level of activation by the secretion of a whole pattern of proinflammatory cytokines from the pre-adipocyte. In consequence many adipokines have been identified which have been previously described to be associated with inflammatory conditions in other parts of the body, and which have a known negative influence on insulin sensitivity, e. g. IL-6 and TNFα. A list of some recently described prominent adipokines is provided in Table 2 [31].

Adiponectin and leptin have been studied most extensively and play a major role in lipid metabolism and the development of obesity. Further adipokines are resistin and visfatin that also seem to be linked to insulin resistance and metabolic syndrome. They are currently under evaluation regarding their clinical value.

Table 2: Recently described adipokines

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>Leptin</td>
<td>Free Fatty acids</td>
</tr>
<tr>
<td>Interleukine-6</td>
<td>PAI-I and tPA</td>
</tr>
<tr>
<td>Retinol Binding protein 4</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>Adipsin (Complementation factor D)</td>
<td>TNF-alpha</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Visfatin</td>
</tr>
<tr>
<td>Resistin</td>
<td>Vaspin</td>
</tr>
</tbody>
</table>

I. Adiponectin

Adiponectin owns an exceptional place in this listing. It is secreted by the mature adipocytes (and the connective tissue) and not by the pre-adipocytes and has a synergistic action to insulin. High plasma adiponectin concentrations result in an improvement of insulin sensitivity. An increase in body weight with differentiation of stem cells to pre-adipocytes is associated with a suppression of adiponectin concentrations in the circulation [38]. Other disease conditions that have been described to be correlated with a suppression of adiponectin levels include, but are not limited to metabolic syndrome, atherosclerosis and any kind of obesity. Adiponectin may, therefore, be regarded as an indicator of the activity of pre-adipocytes. Female patients have higher reference values than male patients. Several plasma sub-fractions have been described that are differentiated by the agglomeration of different numbers of single adiponectin molecules. However, they have as of yet not shown any difference in their changing behaviour following therapeutic interventions. For practical use it does, therefore, not really matter, whether “High Molecular Weight” or “Low Molecular Weight” adiponectin is determined for diagnostic purposes, as long as the same sub-fraction is used to draw any diagnostic or clinical conclusions [40]. Adiponectin levels respond quickly to changes in insulin resistance and the metabolic situation in the lipid tissue. It is, therefore, suitable to track slight changes in insulin resistance, e.g. the metabolic deterioration induced by the hormonal changes in women with polycystic ovary syndrome (PCOS) [41, 42].
II. Leptin

Leptin is a 16kDa non-glycosylated protein that is predominantly secreted by mature lipid cells, but can also derive in minor amounts from the stomach, intestine tract, muscle and breast tissue. The plasma leptin levels reflect the actual amount of lipid tissue, the size of the adipocytes and their triglyceride content. In consequence, plasma leptin concentrations are elevated in case of obesity and decrease with a loss in body weight [43]. These changes are influenced by the actual insulin and glucose concentrations and by inflammatory cytokines. In addition, leptin plays a role in the control of energy consumption, in angiogenesis, fertility, bone formation and many other endocrine body functions [44]. Leptin levels are higher in female patients, most probably because of the larger amount of subcutaneous lipid tissue and a higher stimulation by estrogens in women. Leptin concentrations decrease in a cold environment and during adrenergic stimulation. The brain uses leptin as an important control variable for appetite regulation. It carries the information, whether “sufficient” amounts of lipid tissue are prevalent and, therefore, leptin, like insulin, belongs to the lipostatic molecules. The lipostatic factors orchestrate together with the short-acting incretines (e.g. GLP-1, ghrelin, GIP, cholecystokinin (CCK), obestatin, PYY etc.) the nutritional behaviour of the human organism (see Figure 2, [45]).

c. Development into diabetes

Oxidative stress and consequent inflammation induce IR, which likely initiates metabolic syndrome. Afterwards, there are 2 principal pathways of metabolic syndrome development [16]:

A. With preserved pancreatic beta cells function and insulin hypersecretion which can compensate for insulin resistance. This pathway leads mainly to the macrovascular complications of metabolic syndrome.

B. With massive damage of pancreatic beta cells leading to progressively decrease of insulin secretion and to hyperglycemia (e.g. overt type 2 diabetes). This pathway leads to both microvascular and macrovascular complications.

Figure 3: The relationship between metabolic syndrome, insulin resistance, hyper-insulinemia and hyperglycemia (overt type 2 diabetes), adapted from [16]

An insulin-resistant state – as the key phase of metabolic syndrome – constitutes the major risk factor for the development of diabetes mellitus. Hyperinsulinemia appears to be a compensatory mechanism that responds to increased levels of circulating glucose. Fasting glucose is presumed to remain normal as long as insulin hypersecretion can compensate for insulin resistance. The fall in insulin secretion leading to hyperglycemia occurs as a late phenomenon.

3 Insulin resistance and diabetes mellitus type 2

In type 2 diabetes, fat, liver, and muscle cells do not respond correctly to insulin anymore. Due to this insulin resistance (IR), blood sugar does not enter these cells and consequently high levels of sugar build up in the blood. This is called hyperglycemia. Type 2 diabetes usually occurs slowly over time. Most people with the disease are overweight when they are diagnosed. Type 2 diabetes can also develop in people who are thin. This is more common in the elderly. Family history and genes play a large role in type 2 diabetes. Low activity level, poor diet, and excess body weight around the waist increase the risk.
Diabetes type 2 is one possible outcome of Metabolic syndrome. Diabetes type 2 and obesity are two diseases with continuously growing prevalence over the past decades that have both reached pandemic dimensions in their distribution. Approximately 4–5% of the world population are currently affected and the annual incidence in Western Europe is approximately 10%. Type 2 diabetes is conventionally diagnosed via elevated blood glucose and glycosylated hemoglobin (HbA1c) levels.

**a. Pathophysiology**

The major underlying mechanisms of diabetes type 2 are the development of systemic insulin resistance and a collapsed insulin secretion by pancreatic β-cells. IR is characterized by a general decrease of the insulin sensitivity of the peripheral cells, which on a receptor level is associated with a genetically determined change in the insulin receptor molecule and a reduction of the overall number of insulin receptors on the cells (post-receptor defects have also been described in literature). Figure 4, shows the relation between IR, decreased insulin secretion and adiposity.

**b. Development stages of β-cell dysfunction**

If a patient shows hereditary or acquired insulin resistance, this will initially be compensated for by an appropriate additional secretion of insulin. Insulin, however, is the only (known) physiological hormone that stimulates adipogenesis. As a consequence, this leads to a strong tendency to develop adipose tissue, especially with increased intake of calories. In advanced stage, the processing of the insulin precursor molecule proinsulin becomes insufficient and increasing amounts of intact proinsulin are being secreted. Proinsulin has only a fraction of the blood sugar reducing effect of insulin, but has the same adipogenic potency [3–5].

In consequence, elevated plasma intact Proinsulin levels are a highly specific direct indicator for advanced β-cell dysfunction and a highly specific indirect indicator for clinically relevant insulin resistance [21]. Using the fasting intact proinsulin concentrations and under consideration of the level of insulin resistance (e.g., by means of the HOMA score [22]), it is possible to introduce a clinically useful staging of β-cell dysfunction that allows for a differential diagnosis and selection of a pathophysiologically oriented differential therapy of type 2 diabetes mellitus [23]. This staging is presented in Figure 5 and further detailed in Table 3.

![Stage I - Stage II - Stage IIIa - Stage IIIb](image)

**Figure 5: Staging of β-cell dysfunction by means of insulin resistance and composition of the β-cell secretion product [23]**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Insulin</th>
<th>Proinsulin</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Insulin sensitive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>Insulin resistance without qualitative secretion disorder</td>
<td>Elevated</td>
<td>Normal</td>
<td>Normal or elevated</td>
</tr>
<tr>
<td>III a</td>
<td>Insulin resistance with major β-cell secretion disorder</td>
<td>Normal/Elevated</td>
<td>Elevated</td>
<td>Normal or elevated</td>
</tr>
<tr>
<td>III b</td>
<td>Collapsed β-cell secretion</td>
<td>Low</td>
<td>Elevated to normal (in end stage)</td>
<td>Elevated</td>
</tr>
</tbody>
</table>

**Table 3: Staging of β-cell dysfunction by means of insulin resistance and composition of the β-cell secretion product [23]**
II. HOMA score to assess early stages of \(\beta\)-cell dysfunction

The HOMA (Homeostasis model assessment) score is an easy method to estimate the degree of insulin resistance non-diabetic or early stage diabetic patients [24,25]. It can be applied only if intact proinsulin levels are in the normal range, as the total secretion activity of the \(\beta\)-cell is under this condition represented by the fasting insulin levels. The HOMA score is based on the assumption that under normal conditions, a normal blood glucose value is associated with a matching normal insulin level, which may vary individually from patient to patient. Insulin resistance is indicated, if at this normal insulin level, an elevated blood glucose is observed, or if more insulin is required to maintain blood glucose at its normal level.

After determining the fasting serum insulin and fasting plasma glucose levels the HOMA-IR score is calculated as follows:

\[
HOMA = \frac{\text{Insulin} [\mu U/ml] \times \text{Glucose} [\text{mmol/l}]}{22.5}.
\]

Insulin resistance is assumed if the score value exceeds 2 [25]. Of particular interest are changes in the HOMA score during therapeutic interventions, as a score reduction represents an improvement in insulin sensitivity. Again, the HOMA score should only be used in patients with stage I and II of \(\beta\)-cell dysfunction (see Figure 3), because intact Proinsulin is an additional marker for \(\beta\)-cell activity that is not considered in the HOMA-IR score equation.

III. Intact proinsulin testing to assess late stage \(\beta\)-cell dysfunction

Intact proinsulin is produced in the pancreatic \(\beta\)-cells and is normally further processed to insulin and C-peptide. It is only seen in low concentrations in the plasma of healthy subjects, as it is rapidly degraded (T1/2 is 15 minutes). Proinsulin cleavage products (like des32,33) are stable for several hours. As these fragments are inactive and can make up to 50% of total Proinsulin, only assays specifically measuring intact proinsulin are suitable to indicate advanced \(\beta\)-cell dysfunction and IR.

An increase in the insulin demand, as provided by insulin resistance in later stages of type 2 diabetes mellitus, can result in increased expression of proinsulin into the blood (see Figure 3). When intact proinsulin is secreted together with or instead of insulin in the fasting state (stage III of \(\beta\)-cells dysfunction, Figure 3) the HOMA score cannot be used to assess the level of \(\beta\)-cells dysfunction [26, 27]. Intact proinsulin is also able to lower glucose values but is not considered in the HOMA equations. Measurement of fasting intact proinsulin has been shown to be a very specific indicator and clinically significant IR.

In clinical practice, fasting morning intact proinsulin can be used as highly specific indicator of of late stage \(\beta\)-cell dysfunction and clinically relevant IR. It can also to serve as the basis for the selection of an insulin resistance therapy, and to monitor the therapeutic effect on \(\beta\)-cell dysfunction.
IV. Intact proinsulin combined with oral glucose tolerance testing to identify prediabetes patients.

Because of the blood sugar reducing effect of Proinsulin, some patients may already be suffering from β-cell dysfunction years before clinical manifestation of elevated blood sugar levels. It has been shown that elevation of fasting intact proinsulin is an indicator of insulin resistance and severe β-cell dysfunction. Earlier detection of prediabetic patients is important to prevent irreversible cardiovascular damages.

In a recent pilot study [57] it was investigated whether elevation of intact proinsulin after 1 and 2 hours in the course of an oral glucose tolerance test (OGTT) may be an indicator of the development of type 2 diabetes. Patients were enrolled based on previous results of OGTT: 11 healthy subjects (7 female, 4 male, age: 59 ± 20 yrs.), 10 patients with Impaired Glucose Tolerance (IGT; 6 female, 6 male, age: 62 ± 10 yrs.), and 10 patients with overt type 2 diabetes (6 female, 4 male, age: 53 ± 11 yrs.). Another OGTT was performed with measurement of glucose and intact proinsulin levels after 0h, 1 h and 2 hours. Five years later. The diabetes status of the patients was confirmed and correlated with the earlier OGTT results.

Patients with diabetes (Fig. 6) had elevated fasting glucose levels after 1 and 2 h (diabetes: 0/1/2 h: 121 ± 20/230 ± 51/213 ± 24 mg/dL; prediabetes: 102 ± 9/168 ± 57/149 ± 34 mg/dL; normals: 94 ± 8/140 ± 29/90 ± 24 mg/dL). Proinsulin values after 2 hours were elevated in diabetes and prediabetes vs. control 27 ± 10 pmol/L and 28 ± 6 pmol/L, respectively, vs. 10 ± 5 pmol/L (p<0.05 vs. both other groups).

Five years later, all patients with IGT and three normal subjects had developed overt type 2 diabetes. All manifesting patients had elevated intact proinsulin levels (> 11 pmol/L) after 1 and 2 h. A value > 20 pmol/L after 2 h was always indicative for β-cell dysfunction and progressive disease development. However, because of the multiple existing phenotypes of type 2 diabetes, a value < 20 pmol/L, however, does not automatically exclude diabetes in an individual patient.

Two out of 10 patients with initial IGT were in fact normal according to the WHO diabetes criteria during the second OGTT. However, proinsulin values were 33 and 36 pmol/L after 2 hours, confirming β-cell dysfunction and progressive disease development.

In conclusion: Five year after an initial oral glucose tolerance test, all patients with elevated intact proinsulin levels after 1 h and 2 h had developed overt Type 2 diabetes, irrespective of the observed blood glucose values in the OGTT. Increasing intact proinsulin levels after 1 h and 2 h in the OGTT indicate stress-related β-cell dysfunction and may be an effective predictor for type 2 diabetes development in a mid-term future.

This first study is very promising, as early detection of prediabetes using intact proinsulin as biomarker, may allow life style and other interventions to prevent irreversible cardiovascular damages in diabetes patients that are often the actual cause of death.
4 Diabetes related cardiovascular disease

Today, diagnosis and therapy of the type 2 diabetes mellitus is still based on blood sugar values and the associated values for glycosylated hemoglobin (HbA1c). But even with good blood sugar adjustment, patients still have an increased cardiovascular risk. Still, 75% of patients with type 2 diabetes die of cardiovascular events, whereas this is only true for 35% of patients with type 1 diabetes, although these patients also have an increased blood sugar level. Today, differences in mortality can be explained by the underlying pathologic developments in type 2 diabetes on a metabolic and vascular level.

a. Pathophysiology

Pathophysiologically, type 2 diabetes is characterized by insulin resistance and defective secretion of the pancreas. In particular, IR is closely associated with macrovascular complications, since insulin receptors exist on the endothelial cells of large vessels, whose function is not to absorb glucose, but to activate NO synthesis in the cells. NO is the mediator of numerous vasoprotective mechanisms and finally protects the organism against the development and progression of atherosclerosis [29]. In case of IR not only metabolic but also vascular receptors will be affected and, consequently, not only the insulin requirement or the blood sugar will rise, but usually there is a parallel decrease of the protection of the vessel cells against the deposition of foam cells, a key step in the development of atherosclerosis. Many (especially overweight) people with insulin resistance are able to compensate for the increasing insulin requirement, so that the blood sugar does not rise at first. An additional malfunction of the insulin-producing \(\beta\)-cells of the pancreas then leads to clinically manifest type 2 diabetes in approximately one third of these patients [31]. Development of macrovascular damages may, therefore, already commence in a stage of the disease in which type 2 diabetes is not yet clinically manifest, but where, for example, “only” a disturbed glucose tolerance is present. For this reason, many type 2 diabetes patients already show cardiovascular damages during the first clinical diagnosis of their disease and these damages are, in part, not reversible any more. They need to be treated lifelong and cardiovascular damages are often the actual cause of death.

IR-induced hyperinsulinemia leads to a strong tendency to develop adipose tissue (insulin is the only physiological hormone that stimulates adipogenesis), especially with increased intake of calories. If there is a simultaneous \(\beta\)-cell dysfunction, proinsulin is increasingly present in the secretion product, which has only a fraction of the blood sugar reducing effect of insulin, but has the same adipogenic potency [32-34] (Fig. 7A).

Both hormones also increase adipogenesis and lead to intensified differentiation of mesenchymal stem cells into pre-adipocytes and finally into adipocytes [35]. At this stage, adipose tissue, a highly active endocrine organ, secretes hormones directed against insulin, e.g. estrogens, which in turn enhances insulin resistance [36]. At the same time, the amount of circulating adiponectin is suppressed, a hormone of the white adipose tissue and the connective tissue, which has strong vessel-protective and anti-atherosclerotic properties [37,38]. A vicious circle is created within which insulin resistance, \(\beta\)-cell dysfunction and adipogenesis mutually affect each other negatively. The differentiated pre-adipocytes in turn secrete further molecules, which in their totality can maintain or even enhance the metabolic syndrome, e.g. angiotensin, IL-6, TNF\(\alpha\), free fatty acids, RBP4 or PAI-1 (Fig. 7B). The consequence is the development or enhancement of hypertension, dyslipidemia and increased macrophage activation which ultimately contributes to atherosclerosis [39]. These pathophysiological associations result in a higher atherosclerosis risk, especially if the insulin requirement rises further due to hyperglycemia and toxic concentrations of glucose occurring in the plasma, and, at the same time, the present insulin resistance seriously interferes with the vessel-protective NO-production in the endothelium (Fig. 7C).
Figure 7: The relation between insulin resistance, obesity and the development of cardiovascular risk.
b. Biochemical markers for diagnosis and therapy selection

Routine diagnostics of high blood pressure involves measurement of lipids, HbA1c and glucose. However, the underlying pathomechanisms described above are not detected at all or only very late and inadequately. Therefore, numerous new laboratory markers for classification of metabolic and vascular risk have been investigated and described in recent years. Three of these markers appear to be particularly suitable for diagnosis and therapy selection due to their stability and studies that are presently available: intact Proinsulin, Adiponectin and hsCRP [29].

I. Proinsulin – a blood glucose independent marker to assess insulin resistance and β-cell dysfunction

The significance of intact proinsulin as β-cell function marker has already been described in chapter 3. Intact proinsulin occurs in plasma in increased fasting levels only if a clinically significant IR exists already [27]. Thus, intact Proinsulin is an indirect, but highly specific marker for late stage β-cell dysfunction.

Many (especially overweight) people with insulin resistance are able to compensate for the increasing insulin requirement, so that the blood sugar does not rise at first. Late stage β-cell dysfunction then develops into clinically manifest type 2 diabetes in approximately one third of these patients [31]. Development of macrovascular damages may, therefore, already have developed before type 2 diabetes is clinically manifest (normal or impaired glucose tolerance test result). These macrovascular damages are, in part, irreversible and need to be treated lifelong (and often are the actual cause of death). Measurement of Proinsulin allows for early detection and treatment of these prediabetic patients to prevent irreversible cardiovascular damages.

In view of this it appears to be reasonable to adapt the therapy to the β-cell dysfunction. Prospective studies have demonstrated already that intervention by mobility, Metformin, glitazones or insulin will protect the β-cell and lead to a decrease of the proinsulin level, an effect that could not be observed with sulfonylurea [46-48].

Only assays specifically measuring intact Proinsulin are suitable. Fasting values < 11 pmol/L are considered as normal. Fasting values > 11 pmol/L are indicative of β-cell dysfunction, insulin resistance and cardiovascular risk.

Increasing intact proinsulin levels after 1 and 2 hours in an oral glucose tolerance test are highly indicative for stressrelated β-cell dysfunction and may be a strong predictor for type 2 diabetes development and cardiovascular damages in a mid-term future (see chapter 3 [57]). A proinsulin value > 20 pmol/L at any time point (1 or 2 hours) is indicative for β-cell dysfunction and progressive disease development.

II. Adiponectin – a visceral adipose tissue activity marker to predict cardiovascular risk

The fat and connective tissue hormone adiponectin is a reverse indicator of visceral adipose tissue activity. As such it is regarded as a good marker of insulin resistance and metabolic syndrome. High plasma adiponectin concentrations result in an improvement of insulin sensitivity. Even though adiponectin appears to be less suitable for the initial diagnosis of insulin resistance than intact proinsulin [49], it is an excellent indicator of the metabolic overall situation, which responds very sensitively to successful interventional therapeutic approaches. An increase of adiponectin under therapy shows an improvement of the risk profile.

Clinical studies showed that values below 7 mg/l were associated with an increased risk of cardiovascular events [37, 38]. Values between 7 and 10 mg/L are regarded as grey zone, values > 10 mg/L are considered as normal.
III. hsCRP – a chronic inflammation marker to predict cardiovascular risk

The increasing amount of abdominal lipid tissue exposes the patient to an increased macrovascular risk. The proinflammatory adipokines deriving from the pre-adipocyte activate the immune system not only locally but also systematically, i.e. mononuclear cells in the circulation are also alerted. Especially in the postprandial state, these monocytes/macrophages may be loaded with LDL particles. At the same time, these cells play a key role in the pathophysiology of atherosclerosis, as they penetrate into the vessel wall by means of further inflammatory proteins and enzymes, which finally leads to cholesterol deposit and plaque formation. A known, “acute phase” inflammatory protein involved in this process is C-reactive protein (CRP), which is produced in the liver.

Whereas the application of intact proinsulin and adiponectin for therapy selection and therapy control is just beginning to assert itself now in type 2 diabetes, the use of highly sensitive C-reactive protein (hsCRP) as inflammatory marker of cardiovascular risk especially in cardiology has already reached a high level of general acceptance. While CRP has been considered to be an unspecific indicator of inflammation of any origin in the past, it could be shown that hsCRP-values, stratified into three risk groups, have their own predictive value for cardiovascular risk in the low measurement range (< 10 mg/l) [50]. Values in this range, when determined with a highly sensitive test method (therefore: “high sensitivity CRP” or “hsCRP”), describe a stepwise increased cardiovascular risk in patients with and without diabetes mellitus (Table 5 [50, 52]). This staging has been confirmed in numerous studies and meta analyses and has been included in the official diagnosis criteria of the American Heart Association [51].

A reduction of the hsCRP in the course of the observation shows an improvement of the cardiovascular risk profile [53].

<table>
<thead>
<tr>
<th>hsCRP fasting value</th>
<th>Cardiovascular risk</th>
</tr>
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<tbody>
<tr>
<td>&gt; 10 mg/L</td>
<td>No assessment possible</td>
</tr>
<tr>
<td>&gt; 3-10 mg/L</td>
<td>High</td>
</tr>
<tr>
<td>&gt; 1-3 mg/L</td>
<td>Average</td>
</tr>
<tr>
<td>0-1 mg/L</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table 5: hsCRP cardiovascular risk groups

5 Other metabolic syndrome-associated diseases

a. Fatty liver disease

With the increasing prevalence of obesity and Metabolic syndrome an increase of nonalcoholic fatty liver disease (NAFLD) is obvious. Patients with insulin resistance and other symptoms of metabolic syndrome should therefore be screened for NAFLD and its progressive and chronic form NASH (non-alcoholic steatohepatitis) [56]. While most patients with steatosis tend to have a benign clinical course, a significant proportion of those with NASH have a progressive disease with a risk of developing liver cirrhosis and hepatocellular carcinoma [12]. In the USA, 7 % of all liver transplants are based on diagnosis of NASH [23]. The diagnostic challenge is to predict NAFLD patients that are likely to progress into liver disease, initiate therapy and life style changes and to monitor the efficacy of the measures.

Conventional markers of liver damage, liver transaminases (AST/ALT), frequently provide incorrect information about liver damage. For example, it could be shown that up to 25–30 % of the patient with fibrosis liver damage have normal transaminase levels [59, 60]. Hepatocyte cell death, specifically hepatocyte apoptosis, is considered to play a crucial role in the formation of liver fibrosis or liver cirrhosis. Numerous studies have demonstrated that hepatocyte apoptosis can be specifically assessed by means of caspases cleaved fragments of Keratin 18 (ccK18), a major intermediate filament protein, expressed by hepatocytes. The biomarker CcK18 as determined by the M30 Apoptosense® ELISA allows prediction of the level of fibrosis (staging), steatosis and NASH and can improve decisions on therapeutic regiments for patients.

Canbay et al. concluded that serological investigation, including the biomarker ccK18 can predict progression of NAFLD into NASH in obese patients [55].
Liver damage can also be assessed using other biomarkers. A list of some recently described liver damage biomarkers is shown in Table 6.

<table>
<thead>
<tr>
<th>Liver damage biomarker</th>
<th>Cardiovascular risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspases cleaved Keratin 18 (ccK18: M30 Elisa)</td>
<td>Hepatocyte apoptosis</td>
</tr>
<tr>
<td>Keratin 18 (cleaved and uncleaved: M65 Elisa)</td>
<td>Hepatocyte apoptosis and necrosis</td>
</tr>
<tr>
<td>Alpha Glutathione STransferase (α GST, serum)</td>
<td>Hepatocyte damage</td>
</tr>
<tr>
<td>Pi Glutathione S-Transferase (π GST, serum)</td>
<td>Bile duct damage</td>
</tr>
<tr>
<td>Collagen IV (serum)</td>
<td>Increased collagen deposition</td>
</tr>
</tbody>
</table>

Table 6: Various liver damage biomarkers

In a recent study by Pfützner et al [58], liver transaminases (ALT/AST) and liver damage biomarkers ccK18, K18 and α GST were measured in 32 diabetic patients and 36 healthy subjects. ALT/AST levels were elevated in only 22/13 % of diabetic patients. In contrast, all biomarkers were highly elevated in up to 65 % of the cases (above reference range, ccK18: 50 %; K18: 65 %; α GST: 58 %) and showed similar behavior in most patients (Figure 8), indicating ongoing liver damage. This pilot study supports the use of liver damage biomarkers in diabetic patients to diagnose fatty liver disease and predict possible progression to NASH.

b. PCOS: polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is induced by a deterioration of the hormonal regulation in female patients and is characterized by chronic anovulation and hyperandrogenism. It is one of the most frequent endocrine disorders in young female patients (prevalence 6 %). PCOS is often associated with obesity and IR. During the PCOS development increased LH levels induce an increased synthesis of steroids in the ovaries, which in turn leads to an increased modification of androgens into estrogens in the lipid tissue. The acyclic production of these estrogens results in increased secretion of LH from the pituary gland. Another source of increased androgen concentrations in patients with PCOS is a suppression of the production of sex-hormone-binding globulin (SHBG) in the liver, which leads to increased formation of biologically active androgens. The increased formation of all these “anti-insulinemic” hormones may frequently result in development of a metabolic insulin resistance and an increased insulin secretion to compensate for the higher needs.

Insulin resistance does not represent the only cause for PCOS development, but the accompanying hyperinsulinemia supports the development by the acceleration of ovarian and adrenal androgen production. This understanding of the pathophysiological disease background has led to the use of insulin sensitizing drugs in the affected patients. Therapy with metformin resulted in a significant decrease in circulating androgen levels, an increase in SHBG concentrations, a normalization of the menstrual cycle and an improvement in the fertility [54]. Similar effects have been reported for intervention with glitazones (rosiglitazone, pioglitazone). The key parameters for diagnosing insulin resistance in PCOS patients appear to be the HOMA score and adiponectin or intact proinsulin.

Canbay et al. demonstrated that patients with PCOS also have increased risk for developing non-alcoholic steatohepatitis (NASH). Due to this association they advise to investigate female NASH patients for PCOS and PCOS patients for NASH [55].
6 Risk assessment and therapy of metabolic syndrome-associated pathologies

a. Diagnosis and risk assessment

Up to now, the underlying pathomechanisms described above are not detected at all or only very late and inadequately. As discussed in chapter 3, intact Proinsulin, Adiponectin and hsCRP appear to be particularly suitable for diagnosis and therapy selection due to their stability and studies that are presently available [29]. In addition, the effect of the therapy used on pathophysiological basic components can be checked by means of these new risk markers.

The values for intact proinsulin, adiponectin and hsCRP can be used to assess:

- β-cell function,
- insulin sensitivity
- patient’s individual cardiovascular risk

Increased proinsulin and hsCRP levels and low adiponectin values indicate insulin resistance with β-cell dysfunction and impending macrovascular complications. Adiponectin increase, on the other hand, is accompanied by significant improvement of metabolic status and cardiovascular prognosis. In addition biomarkers for liver damage and progression to NASH can be included like ccK18, K18, α GST and collagen IV (see chapter 4a).

b. Therapy and monitoring

Proinsulin, adiponectin and hsCRP are independent risk factors for type 2 diabetes as well as for macrovascular complications. According to present knowledge, all three risk factors will be improved in particular through changes in lifestyle (weight reduction and more physical activity) and through pathophysiologically oriented medicinal treatment. Improvements have been observed especially with pioglitazone, GLP-1 analogs, SGLT-2 inhibitors and insulin (Table 7, Pfützner et al [58]). Such positive evidence is not available for other oral antidiabetic drugs, for example, sulfonylurea [11, 13, 14, 19].

<table>
<thead>
<tr>
<th>Intervention</th>
<th>β-Cell Dysfunction</th>
<th>Visceral tissue activity</th>
<th>Chronic systemic inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet &amp; Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphonylurea/Glinides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPIV-Inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 Analogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGLT-2 Inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (early)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (late)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7: effect of various therapies on biomarkers
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Intact Proinsulin (TECO®)

Cat. No.: TE1012  
Tests: 96  
Method: ELISA  
Range: 3 – 100 pmol/l  
Sensitivity: 0.3 pmol/l  
Incubation time: 2.5 hours  
Sample volume: 50 μl  
Sample type: Serum, EDTA / Heparin plasma, cell culture  
Sample preparation: Fasting blood sample collection. Due to higher stability, EDTA or heparin plasma samples are preferred to serum samples.  
Plasma: the sample collection can take place in HbA1c-tubes. These samples are stable at room temperature and should be centrifuged within 48 hours. Plasma should be used in the assay or can be stored in aliquots, stable > 2 years at -20 °C.  
Serum: centrifuge whole blood within 4 hours. Proteases degrade intact proinsulin in serum, do not store longer than 1 day at 2–8 °C. Serum should be used in the assay or can be stored in aliquots at -20 °C. Avoid repeated freeze/thaw cycles.  
Reference values: After fasting: mean 3.99 pmol/l +/- 1.58 SD  
≤ 11 pmol/l (normal secretion)  
> 11 pmol/l (dysfunction of secretion)  
Species: Human  
Specificity: No cross-reactivity has been observed:  
| Human Insulin | < 10 000 pmol/l |  
| Human C-Peptide | 50 000 pmol/l |  
| Des (31,32) - Proinsulin | < 200 pmol/l |  
| Split (32,33) - Proinsulin | 5000 pmol/l |  
| Des (64,65) - Proinsulin* | 200 pmol/l |  
| 1000 pmol/l | Split (65,66) - Proinsulin |  

*not present in Serum and Plasma samples

Intended use: Proinsulin is produced in the pancreatic β-cells and is normally further processed to insulin and C-peptide. It is only seen in low concentrations in the plasma of healthy subjects. An increase in the insulin demand, as provided by insulin resistance in later stages of type 2 diabetes mellitus, can result in increased expression of proinsulin into the blood. Intact proinsulin is rapidly degraded, but is considered to be an independent cardiovascular risk factor. The intact molecule and its degradation products are known to block fibrinolysis because of plasminogen-activator inhibitor (PAI-1) stimulation. In clinical practice, fasting morning intact proinsulin can be used as a highly specific indicator of clinically relevant insulin resistance, to serve as the basis for the selection of an insulin resistance therapy, and to monitor the therapeutic effect on β-cell dysfunction.

Patients with type 2 diabetes mellitus and with elevated fasting intact proinsulin levels should be regarded and treated as insulin resistant, in order to reduce the risk for further cardiovascular damage. Elevated fasting intact proinsulin levels may also be seen in patients with insulinoma, a benign insulin producing tumor of the pancreas.

- Diabetes II  
- Staging of insulin resistance and β-cell dysfunction  
- Therapy selection  
- Therapy monitoring  
- Identification of high risk patients for CAD  
- Polycystic ovary Syndrome (PCOS)  
- Insulinoma
Adiponectin high sensitive (TECO®)
Total Human Adiponectin

Cat. No.: TE1013
Tests: 96
Method: ELISA
Range: 1 – 100 ng/ml native Adiponectin
Sensitivity: < 0.6 ng/ml
Incubation time: 2 hours
Sample volume: 5 μl (dilute >1:300 serum and plasma).
For other biological fluids see protocol for dilutions
Sample type: Serum, heparin plasma, breast milk, urine, saliva, CSF, cell culture
Sample preparation: Blood collection - fasting is recommended.
Samples are stable for maximum 2 days at room temperature.
Long-term storage stable for maximum 2 years at -20 °C.
Max. 5 freeze and thaw cycles.

Reference values:

<table>
<thead>
<tr>
<th></th>
<th>mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men Adult</td>
<td>8-10</td>
</tr>
<tr>
<td>Female Adult</td>
<td>10-12</td>
</tr>
<tr>
<td>Cut-off</td>
<td>10</td>
</tr>
</tbody>
</table>

Comprehensive clinical reference data related to age and gender are available for this test.

Species: Human

Intended use:
Adiponectin is a 30kDa protein and its percentage in serum proteins is 0.01 %. In vivo, it appears with different oligomers and it is mainly synthesized by adipocytes. Until now, IGF-1 is the only known natural inductor of synthesis.
Low Adiponectin levels are closely associated with insulin resistance and metabolic syndrome as well as an increased risk of type 2 diabetes mellitus and cardiovascular disease.
Today, Adiponectin is thought to act as an endogenic insulin sensitizer by decreasing excessive glucose levels without increasing insulin concentrations and by stimulating the burning of adipose tissue in muscle and liver.

Adiponectin is associated with glucose and lipid metabolism and is assumed to have direct antiatherogenic characteristics.
Furthermore, it is involved in inflammatory processes.

Clinical significance:
- Obesity
- Arteriosclerosis
- Energy metabolism
- Coronary diseases
- Metabolic syndrome
- Polycystic ovary syndrome (PCOS)
**Adiponectin, Mouse**  
**Total Adiponectin**

Cat. No.: E091M  
Tests: 96  
Method: **ELISA**  
Range: 0.025 - 1 ng/ml native Adiponectin  
Sensitivity: ~ 0.01 ng/ml  
Incubation time: 3 hours  
Sample volume: 100 μl (after dilution 1:10'000)  
Sample type: Serum and plasma  
Sample preparation: Generally, samples should be refrigerated as soon as possible following collection. Samples are stable maximum 2 days at room temperature. Long-term storage up to 2 years at -20 °C or below. Maximum 5 freeze and thaw cycles.  
Species: Mouse

**Adiponectin, Rat**  
**Total Adiponectin**

Cat. No.: E091R  
Tests: 96  
Method: **ELISA**  
Range: 0.25 – 10 ng/ml native Adiponectin  
Sensitivity: ~ 0.01 ng/ml  
Incubation time: 3 hours  
Sample volume: 100 μl (after dilution 1:1’500)  
Sample type: Serum and plasma  
Sample preparation: Samples are stable for maximal 2 days at room temperature. Long-term storage up to 2 years at -20 °C or below. Maximum 5 freeze/thaw cycles.  
Species: Rat
### hsCRP

<table>
<thead>
<tr>
<th><strong>Cat. No.:</strong></th>
<th>7033</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests:</strong></td>
<td>96</td>
</tr>
<tr>
<td><strong>Method:</strong></td>
<td><strong>Sandwich ELISA</strong></td>
</tr>
<tr>
<td><strong>Range:</strong></td>
<td>0.005 – 0.1 mg/L (0.5 – 10 mg/L), standardisation NIBSC 85/506</td>
</tr>
<tr>
<td><strong>Sensitivity:</strong></td>
<td>0.1 mg/L</td>
</tr>
<tr>
<td><strong>Incubation time:</strong></td>
<td>65 minutes</td>
</tr>
<tr>
<td><strong>Sample volume:</strong></td>
<td>5 µL (1:100 diluted)</td>
</tr>
<tr>
<td><strong>Sample type:</strong></td>
<td>Serum</td>
</tr>
<tr>
<td><strong>Sample preparation:</strong></td>
<td>Centrifuge collected blood within 60 minutes. Specimen which cannot be assayed within 24 hours, should be frozen at minus 20°C or lower, and will be stable for up to 6 months. Specimen should not be repeatedly frozen and thawed before testing. Avoid grossly hemolytic, lipemic or turbid samples.</td>
</tr>
<tr>
<td><strong>Species:</strong></td>
<td>Human, Monkey</td>
</tr>
</tbody>
</table>

**Intended use:**
CRP is synthesized in the liver and is a well established indicator for inflammatory processes. CRP assays provide useful information for the diagnosis, therapy and monitoring of inflammatory processes and associated diseases. Measurement of low level CRP by using hsCRP assays is useful in the risk assessment of coronary heart diseases, diabetes mellitus type 2 and metabolic syndrome (American Heart association).
### Leptin, human (TECO®)

<table>
<thead>
<tr>
<th>Cat. No.:</th>
<th>TE1015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests:</td>
<td>96</td>
</tr>
<tr>
<td>Method:</td>
<td>ELISA</td>
</tr>
<tr>
<td>Range:</td>
<td>1 – 100 ng/ml, recombinant Leptin WHO NIBSC 97/594</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td>0.2 ng/ml</td>
</tr>
<tr>
<td>Incubation time:</td>
<td>2 hours</td>
</tr>
<tr>
<td>Sample volume:</td>
<td>20 μl</td>
</tr>
<tr>
<td>Sample type:</td>
<td>Serum, heparin and EDTA plasma, urine, saliva, cell culture.</td>
</tr>
<tr>
<td>Sample preparation:</td>
<td>Normal food intake rhythm provided, samples should be collected till 2 p.m. Leptin shows a moderate circadian variation with a peak at 2 a.m., the leptin values at that time are about 30 to 100 % higher. This variation together with the influence of food intake needs to be taken into account when blood samples are collected. Whole blood should be refrigerated as soon as possible following collection. Samples are stable for maximal 2 days at room temperature. Long-term storage stable for maximal 2 years at -20 °C. Max. 5 freeze and thaw cycles.</td>
</tr>
</tbody>
</table>

### Reference values:
Leptin levels depend on age and gender and must be referred to the percentage body fat (such as BMI). Comprehensive clinical reference data are available for this test.

### Species:
Human

### Intended use:
Leptin, the product of the ob gene, is a recently discovered proteohormone. It is almost exclusively produced by differentiated adipocytes and is thought to play a key role in the regulation of body weight. Leptin has an influence on the central nervous system, mainly on the hypothalamus, by suppressing food ingestion and increasing energy consumption. Beside its influence on food intake, leptin has been shown to have a strong effect on reproduction and a number of metabolic and endocrine axes.

As leptin is of great importance for reproductive functions, infertility may be due to inadequate leptin production. The most important variable determining the circulating leptin concentration is the body fat mass as leptin level and fat mass increase exponentially. Due to its pleiotropic effects, leptin is a valuable parameter with regard to:

- Metabolic syndrome
- Obesity
- Cachexia and other metabolic disorders
- Eating disorders
Leptin, Mouse/Rat

Cat. No.: E06
Tests: 96
Method: ELISA
Range: 25 – 1600 pg/ml
Sensitivity: 10 pg/ml
Incubation time: 3.5 hours
Calibration: WHO NIBSC 97/626 (39, 40)
Sample volume: 100 μl (rat: after 1:5 – 1:10 dilution; mouse: after 1:20 dilution)
Sample type: Serum, plasma, cell culture
Sample preparation: Serum samples could be stored at -20 °C.
Avoid repeated freezing/thawing of specimens.
Species: Mouse, rat

Intended use:
This mouse/rat-Leptin EIA provides a tool for investigation of leptin effects on energy metabolism. Beside energy metabolism leptin influences several further endocrine axes.
In male mice leptin reduces the effect of starvation on testosterone, ACTH and corticosterone. In female mice leptin delays starvation induced ovulation.

Leptin, the product of the ob gene, is a recently discovered proteohormone. It is almost exclusively produced by differentiated adipocytes and is thought to play a key role in the regulation of body weight. Leptin has an influence on the central nervous system, mainly on the hypothalamus, by suppressing food ingestion and increasing energy consumption. Beside its influence on food intake, leptin has been shown to have a strong effect on reproduction and a number of metabolic and endocrine axes. As leptin is of great importance for reproductive functions, infertility may be due to inadequate leptin production. The most important variable determining the circulating leptin concentration is the body fat mass as leptin level and fat mass increase exponentially.
**GLP-1, Total**  
*Total Glucagon-like peptide 1*

<table>
<thead>
<tr>
<th>Cat. No.:</th>
<th>KT-876</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests:</td>
<td>96</td>
</tr>
<tr>
<td>Method:</td>
<td>ELISA</td>
</tr>
<tr>
<td>Range:</td>
<td>1.8 – 55 pmol/l</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td>0.91 pmol/l</td>
</tr>
<tr>
<td>Incubation time:</td>
<td>20 – 24 hours</td>
</tr>
<tr>
<td>Sample volume:</td>
<td>100 μl</td>
</tr>
<tr>
<td>Sample type:</td>
<td>EDTA plasma, serum, cell culture</td>
</tr>
<tr>
<td>Sample preparation:</td>
<td>Fasting sample collection by using a Vacutainer EDTA plasma tube. Separation of plasma within 1 hour after blood collection. The use of a protease inhibitor cocktail is required. DPP-4 inhibitor should be added right after blood collection. Recommended is the BDTM P700 Blood Collection and Preservation System containing DPP-4 protease inhibitor. Extraction of the samples is strongly recommended by using Oasis® HLB 3 cc Cartridge, Extraction Kit KT-910, or ethanol protein precipitation. Store maximum 3 hours at 2 – 8 °C. For longer storage at -70 °C. Maximum 3 freeze and thaw cycles.</td>
</tr>
<tr>
<td>Reference values:</td>
<td>Depending on blood collection fasting or none fasting the values are different.</td>
</tr>
<tr>
<td>Species:</td>
<td>Human, rat, mouse, goat</td>
</tr>
</tbody>
</table>
| Specificity: | GLP-1 (7-36) 100 %  
GLP-1 (9-36) 100 %  
GLP-1 (9-37) < 0.1 %  
GLP-1 (7-37) < 0.1 %  
GLP-1 (1-36) < 0.1 %  
GLP-2 < 0.1 %  
Glucagon < 0.1 % |
**GLP-1 (7-36), Active Human**

*Active Glucagon-like peptide 1 (7-36)*

<table>
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<tr>
<td>Method:</td>
<td>ELISA</td>
</tr>
<tr>
<td>Range:</td>
<td>2.11 – 158.3 pg/ml = 0.64 - 48 pmol/l</td>
</tr>
<tr>
<td>Sensitivity:</td>
<td>1 pg/ml = 3.298 pmol/l</td>
</tr>
<tr>
<td>Incubation time:</td>
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</tr>
<tr>
<td>Sample type:</td>
<td>Plasma</td>
</tr>
<tr>
<td>Sample preparation:</td>
<td>Fasting sample collection by using a Vacutainer EDTA plasma tube. Separation of plasma within 1 hour after blood collection. The use of a protease inhibitor cocktail is required. DPP-4 inhibitor should be added right after blood collection. Recommended is the BDTM P700 Blood Collection and Preservation System containing DPP-4 protease inhibitor. Extraction of the samples is strongly recommended by using Oasis® HLB 3 cc Cartridge, Extraction Kit KT-910, or ethanol protein precipitation. Store maximum at 2 – 8 °C for 3 hours. For longer storage at -70 °C. Avoid freeze and thaw cycles.</td>
</tr>
<tr>
<td>Reference values:</td>
<td>Depending on blood collection fasting or none fasting the values are different.</td>
</tr>
<tr>
<td>Species:</td>
<td>Human</td>
</tr>
<tr>
<td>Specificity:</td>
<td>GLP-1 (7-36) 100 %</td>
</tr>
<tr>
<td></td>
<td>GLP-1 (9-36) &lt; 0.1 %</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>GLP-1 (7-37) &lt; 0.1 %</td>
</tr>
<tr>
<td></td>
<td>GLP-1 (1-36) &lt; 0.1 %</td>
</tr>
<tr>
<td></td>
<td>GLP-2 &lt; 0.1 %</td>
</tr>
<tr>
<td></td>
<td>Glucagon &lt; 0.1 %</td>
</tr>
</tbody>
</table>
Resistin

Cat. No.: E50
Tests: 96
Method: ELISA
Range: 20 – 1000 pg/ml
Sensitivity: 12 pg/ml
Incubation time: 4 hours
Sample volume: 15 μl (dilute 1:21; recommended)
Sample type: Serum, plasma, cell culture medium, saliva, breast milk and urine.
Sample preparation: Haemolytic samples appear to show falsely high Resistin levels. Whole blood should be chilled as soon as possible following collection. Serum and plasma samples are stable for maximal 2 days at room temperature. Long-term storage at -20 °C, stable for maximal 2 years. Maximum 3 freeze/thaw cycles.

Reference values: Women: 7.0 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m²)  
Men: 6.0 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m²)

Species: Human

Intended use: 
Resistin (FIZZ3) is a hormone influencing fat metabolism and inflammation processes. In humans, it is expressed in bone marrow and transported by macrophages into adipose tissue. Resistin stimulates pre-adipocyte proliferation and lipolysis of mature adipocytes probably by influencing MAPK signaling. With regard to the importance of Resistin in disorders of energy metabolism, a significant reduction could be shown in patients with anorexia nervosa. It has been demonstrated that Resistin enhances the expression of specific cell markers such as VACM-1 and ICAM-1 and thus may influence endothelial inflammatory processes, and thereby arteriosclerosis. Moreover, due to its association with Endothelin-1, Resistin also plays a role in cardiovascular diseases.

Resistin is relevant to medical conditions such as:
- Obesity
- Insulin resistance, diabetes
- Arteriosclerosis
- Inflammation
- Lipolysis
Fetuin-A, Human

Cat. No.: KT-800
Tests: 96
Method: ELISA
Range: 12.5 – 370 ng/ml
Sensitivity: 5.0 ng/ml
Incubation time: 3 hours
Sample volume: 10 μl (dilute 1:10,000)
For other biological fluids see protocol for dilutions
Sample type: Serum
Sample preparation: Serum should be separated within 3 hours after blood collection, measure or store at -20 °C. Max. 3 freeze and thaw cycles.

Reference values:

<table>
<thead>
<tr>
<th>mg/l</th>
<th>Mean (g/l)</th>
<th>SD (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35 – 0.95</td>
<td>0.57</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Species: Human

Intended use:
Fetuin-A synthesized in the liver is secreted into the blood stream and it is deposited, accumulated as a non-collagenous protein in mineralized bones and teeth.
Fetuin-A acts as an important circulating inhibitor of ectopic calcification, a frequently seen complication in degenerative diseases. Low Fetuin-A level may be associated with higher cardiovascular mortality in chronic renal failure, liver cancer and liver cirrhosis patients on long-term dialysis.

Human Fetuin-A represents a natural inhibitor of tyrosine kinase activity of the insulin receptor. Fetuin-A may play a significant role in regulating post-prandial glucose disposition, insulin sensitivity, weight gain, and fat accumulation and may be a novel therapeutic target in the treatment of type 2 diabetes, obesity, and other insulin-resistant conditions.

- Fetuin-A level (< 0.35 g/l) indicates a higher risk of cardiovascular calcification and increase mortality in ESRD-patients.
- Fetuin-A level (> 1.00 g/l) in elderly population, an independent risk factor of type II diabetes.
- Fetuin-A is an important predictor of death in acute myocardial infarction.
- Involved with the regulation of calcium metabolism and osteogenesis.