

Clinical and Technical Review

Metabolic Syndrome

Diabetes and Obesity

Differential diagnosis for patients
with type 2 diabetes mellitus

Bone and Diabetes

always your partner

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1 Diabetes and Obesity

Diabetes mellitus type 2 and obesity are two diseases with continuously growing prevalence over the past decades that have both reached pandemic dimensions in their distribution. Type 2 diabetes is diagnosed via an elevated blood glucose level (WHO: fasting glucose > 127 mg/dl, 2 h after an oral 75 g glucose challenge > 200 mg/dl), and (morbid) obesity is defined by increased body fat mass, which exceeds by far the normal reference range of a given population. Both conditions can be considered chronic diseases, which lead to impaired quality of life and a high morbidity and mortality risk, and which require long-term care by the health-care system.

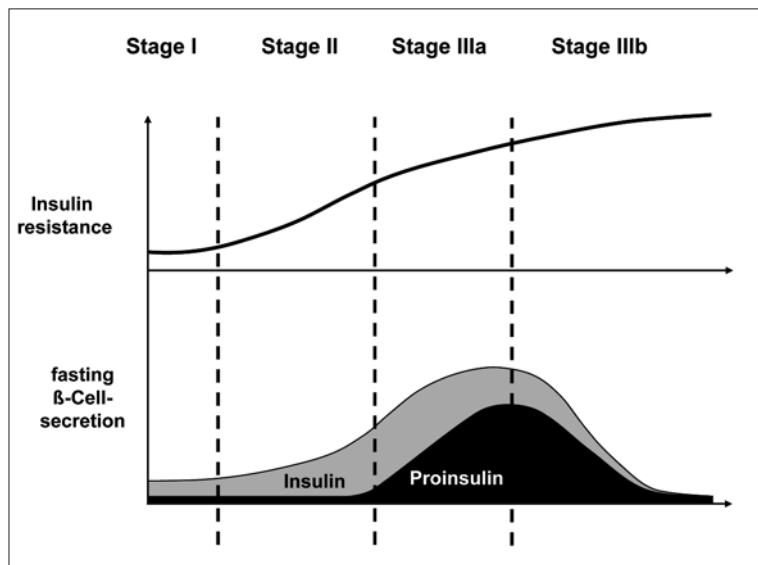
During the past years, scientific research has contributed to a better understanding of the interrelations between the two diseases, and to define new laboratory biomarkers for their classification. New biomarkers of this class are the proteins adiponectin, intact proinsulin, high sensitivity C-reactive protein (hsCRP) and leptin. They play important roles in the pathophysiological development of both diseases.

Type 2 diabetes mellitus is one of the most frequent diseases worldwide. Approximately 4 – 5 % of the world population are currently affected and the annual incidence in Western Europe is approximately 10 %. The major underlying mechanisms are the development of systemic insulin resistance and secretion disorder of the insulin-producing pancreatic β -cells. Insulin resistance 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. Several post-receptor defects have also been described in recent literature [1, 2].

1.1 Intact Proinsulin:

Insulin resistance and β -cell dysfunction result in a significant increase of insulin production. In advanced stage, the processing of the insulin precursor molecule proinsulin becomes insufficient and increasing amounts of intact proinsulin are being secreted in parallel to insulin into the circulation. 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 [3]. Using the fasting intact proinsulin concentrations and under consideration of the level of insulin resistance (e. g. by means of the HOMA score [4]), 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 [5]. This staging is presented in Figure 1.

Figure 1:
Staging of β -cell dysfunction by means of insulin resistance and composition of the β -cell secretion product [5]



1.2 HOMA score: Determination of insulin resistance by means of the fasting reference ranges of insulin and blood glucose

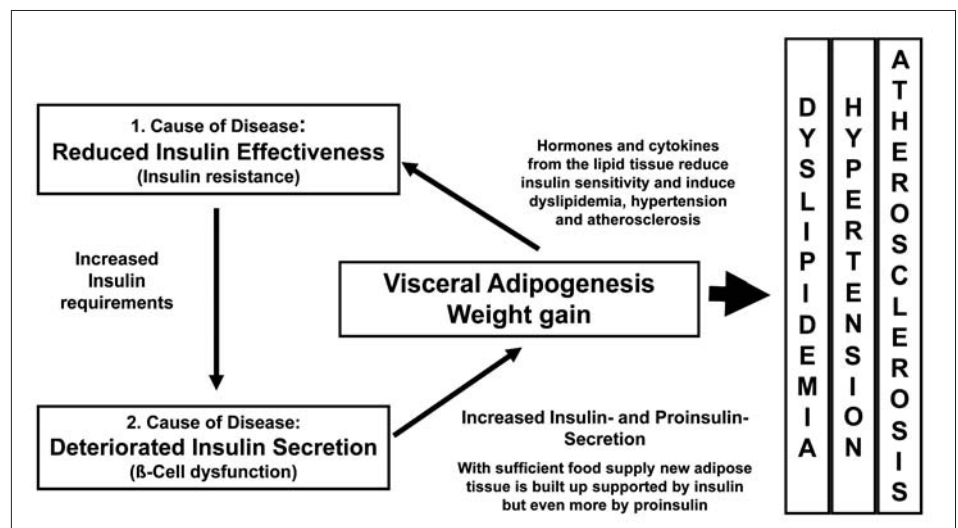
The HOMA-IR score is an easy to be performed clinical method to estimate the degree of insulin resistance in non-diabetic or early stage diabetic patients [18, 19]. It can be applied if intact proinsulin levels are in the normal range, as the total secretion activity of the β -cell is under this condition 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. The mathematical procedure to calculate the HOMA-IR score is defined as follows:

$$\text{HOMA-IR} = \text{Insulin } [\mu\text{U/ml}] \times \text{Glucose } [\text{mmol/l}] / 22.5$$

The factor of 22.5 is an empirical factor that has been introduced to achieve handier numeric values. Based on the publication by Hedblad et al., 2000 [19], insulin resistance is assumed if the score value exceeds 2. Of particular interest are changes in the HOMA score during therapeutic interventions, as a score reduction represents an improvement in insulin sensitivity. The HOMA score should preferably be used in patients with stage I and II of β -cell dysfunction (see Figure 1), because intact proinsulin is a further measure of β -cell activity that is not considered in the HOMA-IR score equation.

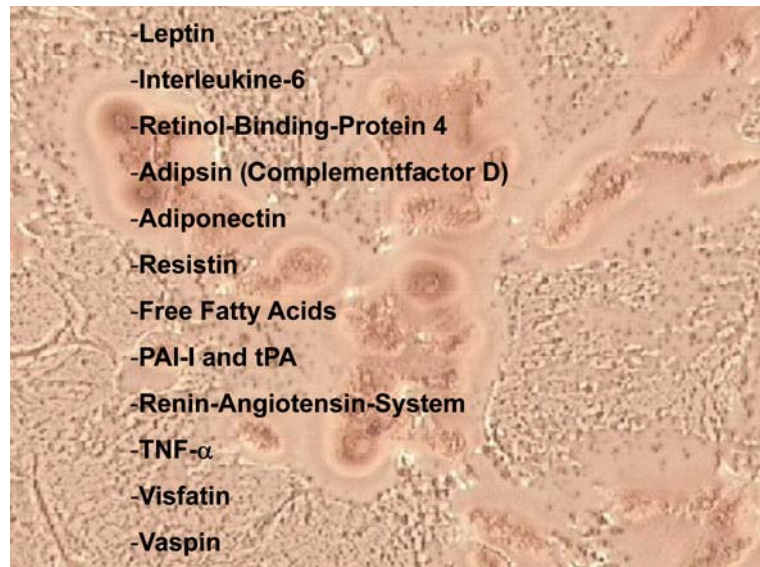
Both elevated insulin and proinsulin have a strong stimulating effect on lipid tissue growth. When patients eat more than their personally required caloric uptake demands to cover energy consumption, an increase in body fat mass is almost inevitable. The consequence is obesity with a significant contribution by the visceral fat mass. In turn, a life-style induced increase in body weight leads to insulin resistance and increased risk for diabetes. It has been described in recent years that the growing adipose tissue is a highly active endocrine organ that secretes a large amount of hormones and cytokines (referred to as “adipokines”), which contribute to the induction or aggravation of hypertension, atherosclerosis and dyslipidemia and which further increase insulin resistance. Thus, a circle is closed (as shown in Figure 2) that may help to explain why so many patients with type 2 diabetes and obesity die from macrovascular events, if no appropriate therapy is applied to stop further deterioration.

Figure 2:
Relation between insulin resistance, β -cell dysfunction, obesity and the resulting complications [6, 7]



Multiple adipokines can be held responsible for the negative consequences of visceral adipogenesis on insulin resistance. The growth of lipid tissue is induced by the differentiation of mesenchymal stem cells to become pre-adipocytes 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. This situation is not entirely understood but may e. g. serve to recognize and immediately destroy potentially developing cancer cells in the differentiation process. 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 TNF α . A list of some recently described prominent adipokines is provided in Figure 3 [8].

Figure 3:
Recently described adipokines [8]



1.3 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 [9]. 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 [10]. Adiponectin levels react very sensitive to changes in insulin resistance and in 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). It has been shown that next to the HOMA-IR score, adiponectin is a very good biomarker for this metabolic condition [11, 12].

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.

1.4 PCOS: polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is induced by a deterioration of the hormonal regulation in female patients, which is associated with insulin resistance. It is one of the most frequent endocrine disorders in young female patients. As set forth by the International Consensus Workshop the PCOS diagnosis is confirmed if two of the following criteria are met:

- prevalence of polycystic ovaries,
- oligo- or anovulation and
- clinical or laboratory signs of hyperandrogenism (after exclusion of any other endocrine disease).

During the PCOS development several endocrine deteriorations support each other in an vicious circle. PCOS patients frequently present a shift in the ratio of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). 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 pituitary gland, and the circle is closed. 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. This hyperinsulinemia supports the already existing hyperandrogenemia by directly inducing androgen production in the ovaries and by leading to increased LH secretion in the pituitary gland. In addition, insulin has a direct suppressive effect on SHBG formation in the liver and does on its own induce the additional formation of androgens in the adrenal glands.

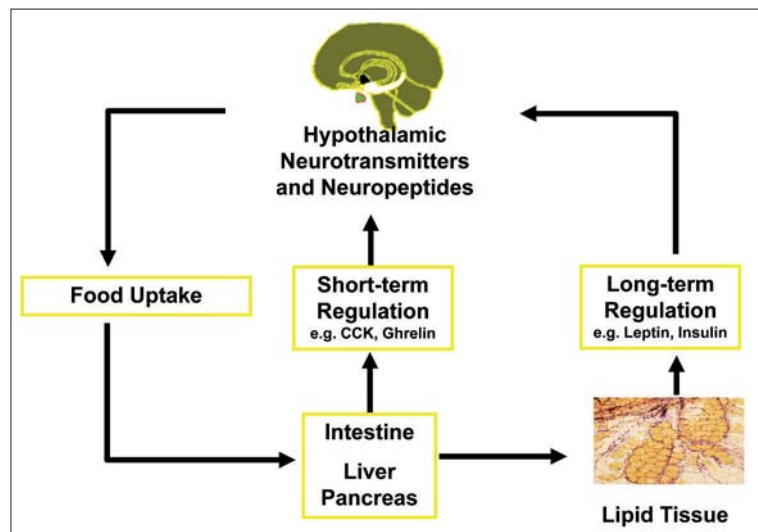
Insulin resistance does not represent the only cause for PCOS development, but the accompanying hyperinsulinemia supports the development by the acceleration of ovarian and adrenal androgen production. This understanding of the pathophysiological disease background has led to the use of insulin sensitizing 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 [20]. Similar effects have been reported for intervention with glitazones (rosiglitazone, pioglitazone). These drugs stimulate PPAR γ receptors and mediate reduction of insulin resistance in lipid, muscle and liver tissue. Both rosiglitazone (4 – 8 mg/day) and pioglitazone (30 mg/day), respectively, led to improvements in insulin resistance, lipid status, menstrual cycle and ovulation in patients with PCOS.

The key parameters for diagnosing insulin resistance in PCOS patients appear to be the HOMA-IR score and adiponectin or intact proinsulin.

1.5 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 [13]. 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 [14]. 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 4, [15]).

Figure 4:
Factor controlling appetite and food uptake



1.6 hsCRP:

Next to the consequences on appetite regulation, an increasing amount of visceral lipid tissue exposes the patient to an increased macrovascular risk. The pro-inflammatory 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. While CRP has been considered to be an unspecific indicator of inflammation of any origin in the past, it could be shown with data derived from the Framingham population study that it provides additional information regarding the prevalence of a systemic vascular inflammation when concentrations are measured within the CRP normal reference range (< 10 mg/l). 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 [16, 17].

The above mentioned laboratory biomarkers intact proinsulin, adiponectin, hsCRP and leptin can be used in combination to analyze and evaluate the metabolic and vascular risk of an individual patient with metabolic syndrome or diabetes mellitus in a manner which goes far beyond the possibilities of traditional diagnostic markers (e. g. glucose, lipids, HbA1c). They can be used for identification and selection of most optimal therapy. Changes of the parameters during and after therapeutic interventions with different drugs indicating improvements or worsening of the metabolic or cardiovascular risk have in the meantime been investigated in numerous controlled and uncontrolled prospective clinical studies.

2 Bone and Diabetes

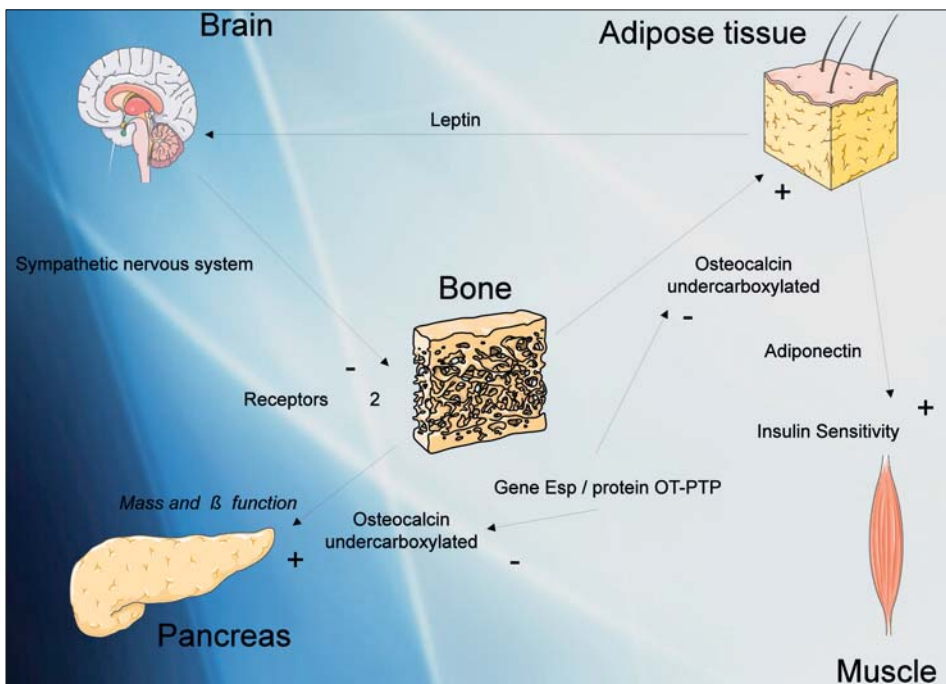
2.1 Cross-Regulation between Bone Metabolism and Energy Expenditure

Bone metabolism is partly regulated by exogenous energy contribution and by digestive hormones. Interferences between leptin, ghrelin and adiponectin with bone metabolism have been discovered and confirmed during the past years. Recently, a direct controlling role of bone on energy expenditure has been discovered. The availability of reliable assays for the molecules involved in these regulatory pathways has led to a refinement of the pathophysiological model of these interactions and introduced the exploration of new promising clinical applications.

2.2 Regulation of bone metabolism by energy contribution and digestive hormones

These metabolic pathways are driven by two main contributors:

a. Leptine, a protein secreted by subcutaneous and visceral adipose tissue on a regular basis, influenced by food intake. This satiety molecule increases bone formation through a direct peripheral action, but has also an osteolytic action through a central beta adrenergic effect. [1].



b. Ghrelin is synthesized in the stomach fundus, endocrin pancreas, and hypothalamus. Its influence on bone is mediated by an increase of growth hormone (GH) and insulin-like-growth-factor I (IGF-1). Ghrelin directly activates differentiation of osteoblasts and increases bone mineralisation [2].

In parallel, insulin and its modulators have an impact on bone metabolism. By increasing insulin sensitivity, adiponectin has a pro-osteoblastic effect. Whether hormones like resistin and visfatin, which are considered to decrease insulin-sensitivity have an influence on bone metabolism has not been shown yet and remains hypothetical.

Clinically, the increased risk of osteoporotic fractures in all kinds of diabetes is a good example for the difficulty to get a consistent pathophysiological model. Type I Diabetes is insulinopenic and subjects have usually a normal body weight and normal bone mass. Type 2 Diabetes is often found in obese patients with a high bone mass [3, 4]. The larger fat mass, as determined by the body composition, is a major factor of cardiovascular risk, while a lean mass is linked to an increased osteoporotic risk [5].

2.3 Energy balance regulation by bone

Osteocalcin is a non-collagenous protein derived from the bone. After carboxylation by action of vitamin K, osteocalcin has a positive influence on the mineralization of the bone matrix. The uncarboxylated osteocalcin increases the insulin secretion and improves the insulin sensitivity through adiponectin secretion by subcutaneous adipose tissue. Uncarboxylated osteocalcin production by bone is inhibited by the OST-PTP protein, which is coded by the Esp gene. These genetic interactions have been detected using a Esp^{-/-} knock-out mouse model in which insulin production, β -cell proliferation in the islets of Langerhans, and adiponectin synthesis by adipose tissue are increased [6].

The scientific background of the link between bone and energy metabolism are still under investigation and in a preliminary stage. The existence of a reverse control loop is still to be proven. The complexity of the hormonal interactions in this relation impose the requirement of careful clinical interpretations and conclusions, taking into account the obviously existing differences between humans and animal models.

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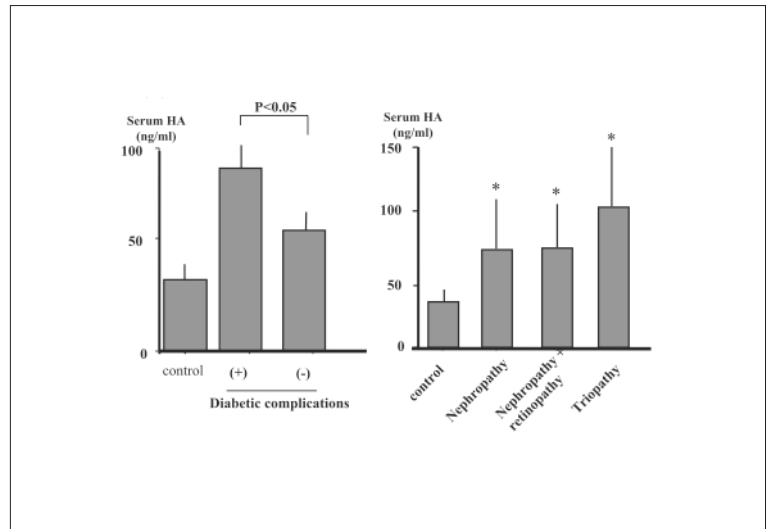
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3 Hyaluronic acid and diabetes

Serum levels of hyaluronic acid were significantly higher in diabetic patients than in normal subjects and correlated with the levels of fasting plasma glucose and CRP as well as the body mass index (BMI). Diabetic complications like retinopathy and nephropathy were associated with higher hyaluronic acid serum levels. The hyaluronic acid level also correlated with the occurrence of diabetic angiopathy. In overweight diabetes patients hypertrophic fat cells showed an increased synthesis of hyaluronic acid causing a chronic inflammation of the adipose tissue.

Correlation between HA levels and diabetic complications



Literature

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4 Intact Proinsulin

Staging of insulin resistance and β -cell dysfunction

Therapy selection

Therapy monitoring

Identification of high risk patients for CAD

Polycystic ovary syndrome

Proinsulin is produced in the pancreatic β -cells and is normally cleaved into insulin and C-peptide. Increased insulin requirement in patients with type 2 diabetes and pronounced insulin resistance leads to increased secretion of intact proinsulin. Proinsulin is considered to be an independent cardiovascular risk factor, as the molecule itself as well as plasma degradation products have an inhibitory effect on fibrinolysis by stimulating plasminogen-activator inhibitor 1 protein production in lipid tissue (PAI-1).

The fasting morning concentration of intact proinsulin can be used in clinical practice as a highly specific marker for clinically relevant insulin resistance. Intact proinsulin information can support the selection of the most suitable therapy (e. g. for insulin resistance) and can be used to monitor β -cell function during therapeutic interventions.

Intact proinsulin is very stable in EDTA-blood specimens (48 hours at room temperature without centrifugation) and can be determined in the same sample that was taken for HbA1c analysis.

- **Timepoint:** Fasting morning blood draw
- **Specimen:** EDTA-plasma, (e. g. HbA1c-tube), heparine plasma, or serum
- High levels indicate advanced β -cell dysfunction, clinically relevant insulin resistance and increased cardiovascular risk.
- Elevated intact proinsulin levels or increased intact proinsulin to insulin ratio are independent predictors of diabetes development in non-diabetic patients.
- A decrease of intact proinsulin levels during therapeutic intervention indicate an improvement in the cardiovascular prognosis.

Reference range:

fasting value ≤ 11 pmol/l

⇒ Mean 3.99 pmol/l \pm 1.58 SD.

⇒ No qualitative β -Cell dysfunction.

⇒ No limitation in the choice of the anti-diabetic therapy.

⇒ In case of therapy with diet and exercise only or sulfonyl urea drugs/glinides, it is recommended to monitor intact proinsulin levels after 12 months (diet) or 6 months (SU), respectively.

Pathologic range:

fasting value > 11 pmol/l

⇒ β -Cell dysfunction and insulin resistance.

⇒ β -Cell-protecting interventions recommended (e. g. exercise, glitazone, insulin).

⇒ The therapeutic effect can be monitored after 3 and/or 6 months by repeating the intact proinsulin determination

Interpretation in non-diabetic patients:

Intact proinsulin > 11 pmol/l (or total proinsulin > 50 mU/l) :

It is highly recommended to search for diabetes mellitus or insulinoma, and to investigate the cardiovascular risk profile.

5 Adiponectin

Adiponectin is a 30kDA protein, and represents 0,01% of all serum proteins. It exists in several different oligomeric versions and is mainly (but not exclusively) produced in adipose tissue.

Low Adiponectin concentrations are closely associated with insulin resistance, metabolic syndrome, increased risk for diabetes development, and increased cardiovascular risk. The current understanding is that Adiponectin acts as an endogenous insulin sensitizer by decreasing glucose levels and inducing the burning of lipid tissue in muscle and liver without increasing insulin levels. Adiponectin appears to be a link between glucose and lipid metabolism. It is considered to have direct anti-atherosclerotic effects and may be involved in inflammation.

Clinical application:

- Obesity
- Atherosclerosis
- Energy metabolism
- Coronary artery disease
- Metabolic syndrome
- Polycystic ovary syndrome

Adiponectin is a stable and robust glycoproteine. As compared to other endocrine proteins it circulates in very high concentrations in human plasma.

- **Timepoint:** Fasting morning blood draw
- **Specimen:** EDTA-plasma (e. g. HbA1c-tube) or serum
- Low levels indicate clinically relevant insulin resistance and increased macrovascular risk. In general, it may be stated: The lower the concentrations, the higher the risk.
- Assessment of adiponectin is particularly useful for monitoring insulin resistance and metabolic risk during therapeutic interventions in patients with metabolic syndrome, diabetes mellitus and atherosclerosis. An increase in adiponectin levels indicates an improvement of the risk profile.

Reference range:

Normal female values: 10 – 12 mg/l

Normal male values: 8 – 10 mg/l

fasting value > 10 mg/l

Greyzone:

Only individual interpretation possible
(e.g. male vs. female)

fasting value 7 – 10 mg/l

Pathologic range:

⇒ Pronounced insulin resistance.

⇒ Increased cardiovascular risk.

fasting value < 7 mg/l

Polycystic Ovary Syndrome:

fasting value < 10 mg/l

6 hsCRP

Besides the chronic inflammation during atherosclerosis, the CRP concentration is also affected by bacterial infections, cancer, inflammatory-rheumatic diseases, postoperative complications (thrombosis, hematomae, necrosis), myocardial infarction or deep leg vein thromboses: the values may rise significantly within a few hours. If the hsCRP level > 10mg/l, one should also look for non-cardiovascular causes.

- **Timepoint:** CRP is independent of the food intake and is not subject to circadian variations. The blood draw may be carried out **fasting or postprandial** any time.
- **Specimen:** Serum (1 ml) (e. g. the sample for determination of lipids)
- At room temperature, the CRP concentration in the serum remains stable for approx. 5 days (better is storage at 2 – 8 °C).
- The hsCRP value reflects the inflammation and atherosclerosis activity in the vascular wall.
- Levels above the reference range mean an increased risk for coronary, cerebral and other cardiovascular events. The cardiovascular risk increases in linear proportion to the hsCRP level.
- Decrease in hsCRP improves the cardiovascular risk prognosis ⇒ measurement for monitoring cardiovascular health and patient motivation (changes in lifestyle may lower the values).
- The **highly sensitive** C-reactive protein (hsCRP) is measured for cardiovascular risk assessment: ultrasensitive CRP-assays are used which also cover low ranges from 1 – 10 mg/l. The normal CRP assay is not suitable for assessment of the cardiovascular risk!

Reference range:

fasting value **0 to 1 mg/l**

⇒ Low cardiovascular risk.

Average cardiovascular risk:

fasting value **> 1 to 3 mg/l**

⇒ The probability of a cardiovascular event in the next 10 years is between 6 % and 20 %.

High cardiovascular risk:

fasting value **> 3 to 10 mg/l**

⇒ The probability of a cardiovascular event in the next 10 years is above 20 %.

No assessment possible:

fasting value **> 10 mg/l**

⇒ Non-specific inflammatory events: also look for cardiovascular induced inflammations!

⇒ The measurement should be repeated after approx. 3 weeks.

7 Leptin

The proteohormone leptin has been identified as a product of the ob gene; it is almost exclusively produced by differentiated fat cells and plays a key role in the regulation of body weight. It acts on the central nervous system, in particular on the hypothalamus, with leptin suppressing the food intake and increasing the energy consumption. Beside the effect on the food intake, leptin affects the reproduction and a number of metabolic and endocrine axes. Since leptin is of major importance for reproductive functions, infertility may be associated with insufficient leptin production. The most important variable that determines the circulating leptin concentration is the body fat mass. The leptin concentration increases exponentially with the fat mass.

Clinical application:

- Metabolic syndrome
- Obesity
- Cachexia and metabolic disorders
- Nutritional disorders

- **Timepoint:** With normal food intake rhythm, samples can be taken until 2 p.m. Leptin shows a circadian variation, with a maximum at 2 a.m.; the values then are approx. 30 % – 100 % higher. Besides the food intake, these variations have to be taken into account when selecting the time point for drawing blood.
- **Specimen:** Serum, heparin plasma, urine, CSF, cell culture.
EDTA and Citrate plasma show 20 % lower values.

Reference range:

The leptin concentration is dependent on age and sex and the values have to be related to the share of body fat (e. g. BMI).

8 Ghrelin

Ghrelin is a 3.5 kDa protein of 28 amino acids and its 3-serin is octanoylated. Bioactivity of the peptide hormone depends on acylation of this serin residue. Ghrelin is mainly synthesized in the stomach, but also in duodenal and heart cells.

Ghrelin exerts influence on several neurological processes and affects the secretion of hormones such as HGH, ACTH, cortisol and prolactin. Ghrelin is also present in pancreatic islets and regulates insulin secretion.

Ghrelin receptors are present in the hypothalamic nucleus arcuatus, a brain area that plays a key role in the hormonal regulation of food intake. Several investigations demonstrate a circadian rhythm of Ghrelin secretion, controlled by food intake.

Shortly before food intake the Ghrelin plasma concentration increases and decreases after finishing the meal.

Obesity results in reduced Ghrelin concentration in blood. In anorexia nervosa, an increase of Ghrelin concentration in the serum can be detected. Ghrelin might act as counterpart to leptin in the regulation of food intake and fat utilization. In patients with primary biliary cirrhosis, Ghrelin serum concentration decreases parallel to increasing leptin levels. Ghrelin also influences the adipogenesis negatively.

- **Timepoint:** Samples can be taken until 2 p.m., food intake should be taken into account.
- **Specimen:** Serum, plasma

Reference range:

Women: Average value 760 pg/ml
Men: Average value 1240 pg/ml

9 Resistin

Resistin (FIZZ3) is an adipokine influencing fat metabolism and inflammation processes. In humans, it is also expressed in bone marrow and transported into the adipose tissue by macrophages. Resistin stimulates pre-adipocyte proliferation and lipolysis activity 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 VCAM-1 and ICAM-1 and thus may influence endothelial inflammatory processes, and thereby atherosclerosis. Moreover, due to its association with Endothelin-1, Resistin also plays a role in cardiovascular diseases.

Clinical application:

- Obesity
 - Insulin resistance, diabetes
 - Atherosclerosis
 - Inflammation
 - Lipolysis
-
- **Timepoint:** Samples can be taken until 2 p.m.
 - **Specimen:** Serum and EDTA / Heparine plasma

Reference range:

Women: 7 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m²)
Men: 6 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m²)

10 Visfatin

Visfatin, an adipocytokine, is strongly accumulated in visceral fat tissue of humans and mice. Its concentration increases as obesity develops. Visfatin corresponds to the Pre-B cell colony enhancing factor (PBEF), a 52-kD Cytokine expressed in lymphocytes.

PBEF, a cytokine involved in inflammation, is necessary for the delayed neutrophil apoptosis in sepsis. In cell cultures, Visfatin shows insulin effects and reduces the glucose level in mice. Surprisingly, Visfatin binds and activates the insulin receptor. New studies show that plasma concentrations in patients with type 2 diabetes mellitus and obesity are increased, suggesting that the determination of Visfatin is a relevant tool for understanding metabolic diseases.

- **Timepoint:** Fasting morning blood draw
- **Specimen:** Serum and EDTA plasma

Reference range:

Healthy control group: 15.8 +/- 16.7 ng/ml
Type 2 diabetes: 31.9 +/- 31.7 ng/ml

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11 Laboratory parameters for the differential diagnosis of type 2 diabetes mellitus

Intact Proinsulin, Adiponectin and hsCRP

Introduction:

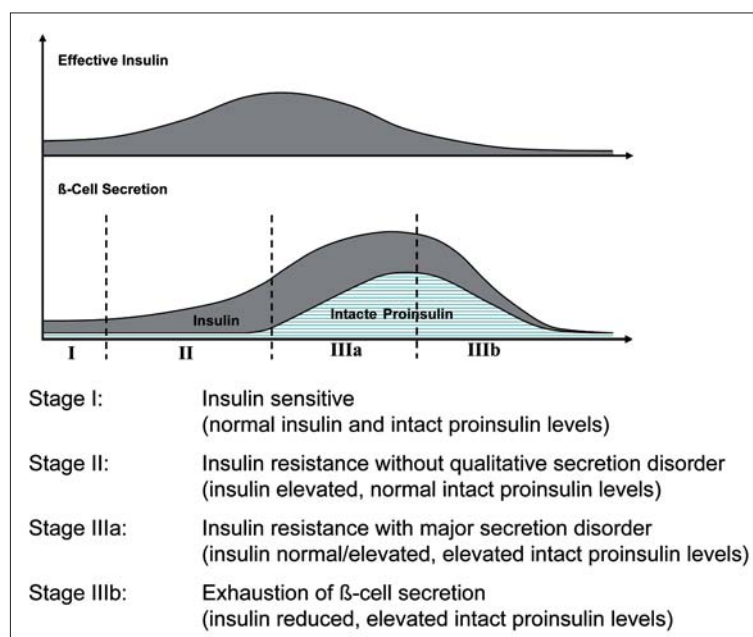
Today, diagnosis and therapy of the type 2 diabetes mellitus is still based on blood sugar values and the associated values for glycosylated hemoglobin (HbA1c). But even with good blood sugar adjustment, patients still have an increased cardiovascular risk. Still, 75 % of patients with type 2 diabetes 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.

Pathophysiologically, type 2 diabetes is characterized by a largely genetically determined insulin resistance and a complex defective secretion of the pancreas. In particular, insulin resistance 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 [1]. In case of insulin resistance, therefore, 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 β -cells of the pancreas then leads to clinically manifest type 2 diabetes in approximately one third of these patients [2]. 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 often are the actual cause of death.

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. If there is a simultaneous β -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 [3 – 5].

Both hormones also increase adipogenesis and lead to intensified differentiation of mesenchymal stem cells into pre-adipocytes and finally into adipocytes [6]. At this stage, adipose tissue, a highly active endocrine organ, secretes hormones directed against insulin, e. g. estrogens, which in turn enhances insulin resistance [7]. 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 [8, 9]. A vicious circle is created within which insulin resistance, β -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 α , free fatty acids, RBP4 or PAI-1. The consequence is the development or enhancement of hypertension, dyslipidemia and increased macrophage activation which ultimately contributes to atherosclerosis [10]. 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 (see Figure 1).

Figure 2:
Staging of β -cell dysfunction on the basis of the fasting level of insulin and proinsulin [3]



11.2 Adiponectin – a blood glucose independent marker for insulin resistance and metabolic risk

The fat and connective tissue hormone adiponectin is regarded as a good marker of insulin resistance and metabolic syndrome. Clinical studies showed that values below 7 mg/l were associated with an increased risk of cardiovascular events [8, 9]. Even though adiponectin appears to be less suitable for the initial diagnosis of insulin resistance than intact proinsulin [16], 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.

11.3 hsCRP – a blood glucose independent marker for inflammation and cardiovascular risk

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. Based on the data of the Framingham trial, Ridker and his colleagues were able to deduce that hsCRP-values, stratified into three risk groups, have their own predictive value for cardiovascular risk in the low measurement range (< 10 mg/l) [17] (see below). In the meantime, the staging proposed by Ridker has been confirmed in numerous studies and meta analyses and has been included in the official diagnosis criteria of the American Heart Association [18]. A reduction of the hsCRP in the course of the observation shows an improvement of the cardiovascular risk profile [19]. Further markers of cardiovascular risk, e. g. matrix metalloproteinase 9 (MMP-9) or monocyte chemoattractant protein 1 (MCP-1), are presently being investigated. The results of further studies have to show whether they have any information value beyond the already discussed possibilities.

12 Assessment of laboratory parameters and influence of therapeutic measures on risk factors and diagnostic markers

Up to now, determination of HbA1c, glucose, cholesterol and triglycerides, and clinical measurement of body mass index and high blood pressure allowed only inadequate assessment of the underlying pathophysiology, consisting of insulin resistance, β -cell dysfunction and atherosclerosis. In addition, the effect of the therapy used on pathophysiological basic components can be checked by means of the new markers.

Using the values for intact proinsulin, adiponectin and hsCRP,

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

can be assessed. 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.

Figure 3:
Assessment of biomarkers

Assessment summary:		
Intact proinsulin	≤ 11 pmol/l = good	○
	> 11 pmol/l = poor	●
Adiponectin	> 10 mg/l = good	○
	7–10 mg/l = greyzone	●
	< 7 mg/l = poor	●
hsCRP	0–1 mg/l = good	○
	> 1 –3 mg/l = average	●
	> 3 –10 mg/l = poor	●
	> 10 mg/l = not assessable	●

Since proinsulin, adiponectin and hsCRP are not only lab markers but also independent risk factors for type 2 diabetes as well as for macrovascular complications, one may ask which therapeutic interventions can improve the biomarker levels. 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. Modifications have been observed especially with glitazones (all three markers), Metformin (intact proinsulin and hsCRP) and preprandial insulin analogs (intact proinsulin). Such positive evidence is not available for other oral antidiabetic drugs, for example, sulfonylurea [11, 13, 14, 19].

The practical aspects of the application of new biomarkers are summarized below:

Figure 4:
Effect of various therapies on biomarkers

Effect on biomarkers for insulin resistance, β -cell dysfunction and cardiovascular risk			
	Intact proinsulin ↓	Adiponectin ↑	hsCRP ↓
“Lifestyle” (physical activity, weight reduction)	+	+	+
Glitazone	++	++	++
Metformin	+(+)	-	+
Sulfonyl urea	-	-	-

Summary:

The discovery of the central role of adipose tissue and its comprehensive endocrine secretion in the pathophysiology of type 2 diabetes has significantly expanded our knowledge of the complex interaction between insulin resistance, β -cell dysfunction and atherosclerosis and its role in the development of dramatically increased cardiovascular mortality. Based on these findings, lab-chemical markers could be identified which provide information on the severity of the respective disorder and which may show intervention-induced changes. They allow a pathophysiologically oriented therapy of the disease. In addition to the determination of blood glucose, HbA1c and lipids, determination of the new lab markers of intact proinsulin, adiponectin and hsCRP allow us to get a detailed picture of the disease components of type 2 diabetes mellitus. Based on these values, therapeutic improvements can be achieved, which ultimately may contribute to a reduction of the cardiovascular risk of patients. Even though there are, presently, no “evidence“-based long-term studies using this concept, one has to demand, based on the existing pathophysiological considerations, that with respect to the therapy of type 2 diabetes one has to discard the idea of pure “blood sugar cosmetics“. The new markers described can help to better identify a patient's risk situation and to monitor the success of therapeutic measures. These parameters that are today determined in the laboratory will in future certainly be available as rapid tests for physicians in their medical practice.

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Human Intact Proinsulin (TECO®)

CE

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 up to 2 years at -20 °C, or up to 4 years at -80 °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)
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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
Split (65,66) – Proinsulin	1000 pmol/l

* not present in Serum and Plasma samples

Adiponectin high sensitive (TECO®)

Total Human Adiponectin

CE

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:	100 µl (diluted)
Sample type:	Serum, heparin plasma (dilution 1:200 to 1:500) breast milk, urine, saliva, CSF (dilution 1:2 to 1:10), Cell culture 1:5 to 1:200
Sample preparation:	Blood collection - fasting is recommended. Whole blood should be refrigerated as soon as possible following collection. Samples are stable for 1-2 days at room temperature and 4-8 days at 2-8 °C. Long-term storage stable for 2 years at -20 °C. Max. 5 freeze and thaw cycles.
Reference values:	Men Adult 8-10 mg/l Female Adult 10-12 mg/l Cut off: 10 mg/l Comprehensive clinical reference data related to age and gender are available for this test.
Species:	Human

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 (1:10000 pre-diluted)
Sample type:	Serum and plasma
Sample preparation:	Generally, samples should be refrigerated as soon as possible following collection. Samples are stable for 1-2 days at room temperature and 4-8 days at 2-8 °C. Long-term storage up to 2 years at -20 °C or below. Avoid repeated freezing/thawing of specimens.
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 (1:5000 pre-diluted)
Sample type:	Serum and plasma
Sample preparation:	Generally, samples should be refrigerated as soon as possible following collection. Samples are stable for 1-2 days at room temperature and 4-8 days at 2-8 °C. Long-term storage up to 2 years at -20 °C or below. Avoid repeated freezing/thawing of specimens.
Species:	Rat

Leptin, Human (TECO®)

CE

Cat. No.:	TE1015
Tests:	96
Method:	ELISA
Range:	1 - 100 ng/ml, recombinant Leptin WHO NIBSC 97/594
Sensitivity:	0.2 ng/ml
Incubation time:	2 hours
Sample volume:	20 µl
Sample type:	Serum, heparin plasma, urine, CSF, cell culture EDTA and Citrat-Plasma will show 20 % lower results.
Sample preparation:	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 1-2 days at room temperature, 4 days at 2-8 °C. Long-term storage stable for 2 years at -20 °C. Max. 5 freeze and thaw cycles.
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

Leptin, Mouse/Rat

Cat. No.:	E06
Tests:	96
Method:	ELISA
Range:	25 - 1600 pg/ml
Sensitivity:	10 pg/ml
Incubation time:	3.5 hours
Sample volume:	100 µl (1:5 pre-diluted)
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

GLP-1, Total

Total Glucagon-like peptide 1

Cat. No.:	KT-876
Tests:	96
Method:	ELISA
Range:	1.8 - 55 pmol/l
Sensitivity:	0.91 pmol/l
Incubation time:	20 - 24 hours
Sample volume:	100 µl
Sample type:	EDTA plasma
Sample preparation:	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 BD™ 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.
Reference values:	Depending on blood collection fasting or none fasting the values are different.
Species:	Human
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

Active Glucagon-like peptide 1 (7-36)

Cat. No.:	KT-871
Tests:	96
Methode:	ELISA
Range:	6 - 180 pg/ml = 1.8 - 55 pmol/l Sensitivity: 1 pg/ml = 0.3 pmol/l
Incubation time:	20 - 24 hours
Sample volume:	100 µl
Sample type:	EDTA plasma
Sample preparation:	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 BD™ 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.
Reference values:	Depending on blood collection fasting or none fasting the values are different.
Species:	Human
Specificity:	GLP-1 (7-36) 100% GLP-1 (9-36) < 0.1 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

Resistin

CE

Cat. No.:	E50
Tests:	96
Method:	ELISA
Range:	20 – 1000 pg/ml
Sensitivity:	6 pg/ml
Incubation time:	4 hours
Sample volume:	100 µl (1:20 pre-diluted)
Sample type:	Serum, EDTA and heparin plasma, cell culture
Sample preparation:	Hemolytic samples may show falsely high Resistin levels. Collect samples until 14 o'clock. Whole blood should be chilled as soon as possible following collection. Serum and plasma samples are stable for 1–2 days at room temperature and 4 days at 2–8 °C. Long-term storage should be carried out at -20 °C or below. Avoid repeated freezing/thawing of specimens.
Reference values:	Women: 7 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m ²) Men: 6 ng/ml +/- 2.5 SD (referred to BMI ~ 25 kg/m ²)
Species.:	Human, rat

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

Ghrelin

CE

Cat. No.:	R90
Tests:	100
Method:	RIA
Range:	0 – 6400 pg/ml
Sensitivity:	40 pg/ml
Incubation time:	2 x overnight
Sample volume:	100 µl
Sample type:	Serum, plasma
Sample preparation:	Collect a morning or early afternoon sample until 14 o'clock (in special cases: food-intake should be taken in consideration). Samples should be stored at 2-8 °C. Long-term storage at -20 °C for several years.
Reference values:	Women: Average value 760 pg/ml Men: Average value 1240 pg/ml
Species:	Human

Intended use:

Ghrelin is a 3.5 kDa protein, consisting of 28 amino-acids, octanoylated at the serine residue three. The biological activity of the peptide hormone depends on the acidification of this serine residue. Ghrelin is mainly synthesized in the stomach, but also in the duodenum and heart-cells. Ghrelin stimulates many neurological processes and the secretion of hormones as HGH, ACTH, cortisol and prolactin. Ghrelin can also be shown in the Islets of Langerhans and influence the regulation of the insulin-secretion. Ghrelin receptors are located in the hypothalamic nucleus arcuatus, a brainregion, which has a key function in the hormone regulation of food-intake. Several studies showed a circadian rhythm of the ghrelinsecretion, controlled by food-intake. The ghrelin-plasma-concentrations increase shortly before food-intake and decrease after the meal. Plasma ghrelin-levels are decreased in chronic obesity, but elevated in anorexia nervosa.

For the regulation of food-intake and fat utilisation, ghrelin has possibly an opposite effect to that of leptin. In patients with primary billiary cirrhosis the serum-ghrelin concentration decreases parallel to increasing leptin-levels - ghrelin negatively effects the adipo-genesis.

Fetuin-A, Human

CE

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:	25 µl (prediluted 1:10,000)
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: 0.35 - 0.95 g/L
Mean 0.57 g/L - SD 0.13 g/L

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.

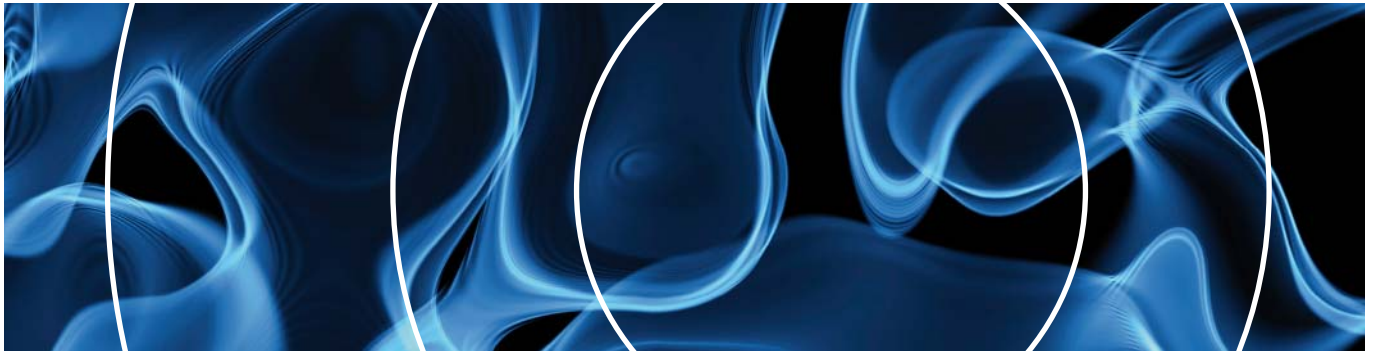
- Fetuin-A level (< 0.35 g/l) indicates a higher risk of cardiovascular calcification and increase mortality in ESRD-patients.
- Fetuin-A level (> 1.00 g/l) in elderly population, an independent risk factor of type II diabetes.
- Fetuin-A is an important predictor of death in acute myocardial infarction.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

Fetuin-A, Rat

Cat-No.:	KT-873
Tests:	96
Method:	ELISA
Range:	320 - 8600 ng/mL
Incubation time:	2.5 hours
Sample volume:	25 µl (1:300 prediluted)
Sample type:	Serum, plasma
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: 0.5 - 1.0 g/L
Mean 0.7 g/L - SD 0.178 g/L

Species: Rat



The Specialist for Biochemical Markers

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