TECOmedical Group

Intact Proinsulin

Biomarker for insulin resistance associated with β-cell dysfunction

- Biomarker for ß-cell dysfunction in patients with Type 2 Diabetes mellitus
- Important secretion marker for the different stages of β-cell dysfunction
- Early and fast prediction of the development of Type 2 Diabetes (T2DM)
- Progression marker to monitor therapy results

INTACT PROINSULIN

Insulin resistance is closely correlated with macrovascular complications and increased mortality rate. While many patients are able to compensate the increased insulin demand over long periods, nearly a third of patients will suffer from an additional β-cell dysfunction and will develop clinically T2DM.

Different stages of β-cell dysfunction based on secretory pattern

The development stages of β-cell dysfunction (fig.1) and therefore T2DM can be described by the dynamic of secretory patterns in morning fasting samples [1,2]. Stages I and II show increasing insulin resistance and therefore an increased insulin secretion - the glucose level are between normal and elevated (Tab.1). During these stages insulin resistance can be detected by using the HOMA score (relation between insulin and glucose level) [1, 2].

Intact Proinsulin is a marker for impaired β-cell-secretion

In conjunction with genetical predisposition increasing insulin resistance will result into disturbances in secretory activity of the β-cells (Stage IIIa) inducing a rise of intact proinsulin levels in fasting samples. As intact proinsulin itself show glucose lowering capacity of about 10-20% compared to insulin, the glucose stay between normal and elevated levels. At the end of the final stage IIIb a total breakdown of β-cell function will occur. The insulin -intact proinsulin secretion will shift more and more towards intact proinsulin and finally at the end of this stage, the intact proinsulin secretion will also stop. Latest at this stage the balance to keep the glucose level within physiological range will collaps and constant elevated glucose levels will be observed. Starting at stage II the cardiovascular risk is increasing significantly.

Diagnostic value in determination of intact proinsulin

Intact proinsulin is synthesized in the β-cells and is enzymatically cleaved within the cells into insulin and C-peptid and released into circulation. During the qualitative secretory dysfunction the enzymes responsible for cleavage of proinsulin become exhausted and therefore more unprocessed, intact proinsulin is secreted into circulation. The diagnostic value of intact proinsulin is based on the secretory profile demonstrated in table 1, which shows a clear rise of fastening intact proinsulin when β-cell dysfunction occur in stage IIIa .

Intact proinsulin is a biomarker for the β-cell dysfunction and therefore also for the clinically relevant insulin resistance.

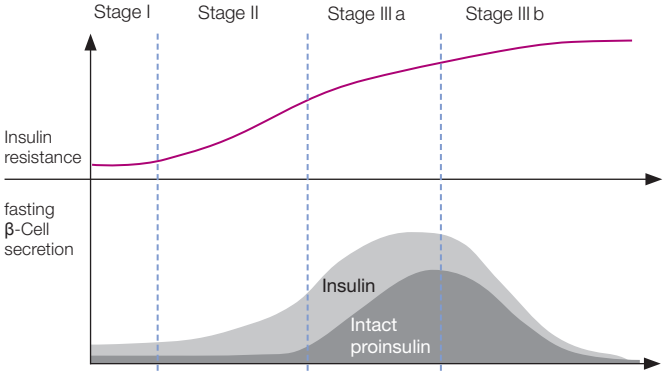

Figure 1: Staging of β-cell dysfunction on the basis of insulin resistance and composition of secretion product (insulin and intact proinsulin) independently from the glucose value (adopted by [2]).

For the diagnosis the following consequences (regardless of the glucose levels) and clinical conclusions are possible:

1. Detection of advanced β-cell dysfunction, clinically relevant insulin resistance and thus a (pre) type 2 diabetes

A fasting intact proinsulin value >11 pmol/l is pointing to an insulin resistance with impaired β-cell secretion; specificity for the presence of insulin resistance is in this case 100%, regardless of the glucose values [1,3].

Recognition of a significantly increased risk of cardiovasculaire events

In patients with normal glucose and HbA1c values increased intact proinsulin levels in the morning fasting samples are often the only indication of a progressively developping diabetes.

Due to the damages insulin resistance can cause to the vessels at this stage atherosclerotic changes may become macrovascular events before diabetes becomes visible according to classical criteria of increased blood glucose levels. Prospective studies have shown the value of intact proinsulin as a predictive markers for infarction and stroke risk [4,5].

Stage	Description	Insulin	Intact Proinsulin	Glucose	Cardiovasculaire risk
I	Insulins sensitive, but lack of acute insulin response	Normal	Normal	Normal	Low
II	Insulin resistance without qualitative dysorder of secretion	Elevated	Normal	Normal or elevated	Elevated
IIIa	Insulin resistance with severe dysorder of β-cell secretion	Normal to elevated	Elevated	Normal or elevated	High
IIIb	collapse of β-cell secretion	Low	Elevated to low (in final stage)	Elevated	High

Table 1: β-cell-dysfunktion: Description of glucose independent staging based on the secretion pattern (insulin, Intact proinsulin) [2].

2. Importance of intact proinsulin determination in oGTT

The oral glucose tolerance test (oGTT) is used to determine the ability of the body to control blood glucose levels under glucose stress - normal subjects patients with pre-diabetes and patients with type 2 diabetes show different courses in the glucose and intact proinsulin response [6].

Significant increase of intact proinsulin in patients with pre-diabetes type 2

The glucose profiles in normal and diabetic subjects differ dramatically from one another, whereas patients with impaired glucose tolerance show an intermediate, rather indistinct effect (Figure 2). In contrast to that the intact proinsulin response in prediabetic and diabetic patients show an almost concurrent 2.5-fold increase to values above 15 pmol/l. (Figure 2). These nearly identical curves reveal even at prediabetics already the existence of a disturbed β-cell function - 5 years after oGTT all prediabetics had developed a diabetes type 2 [6]).

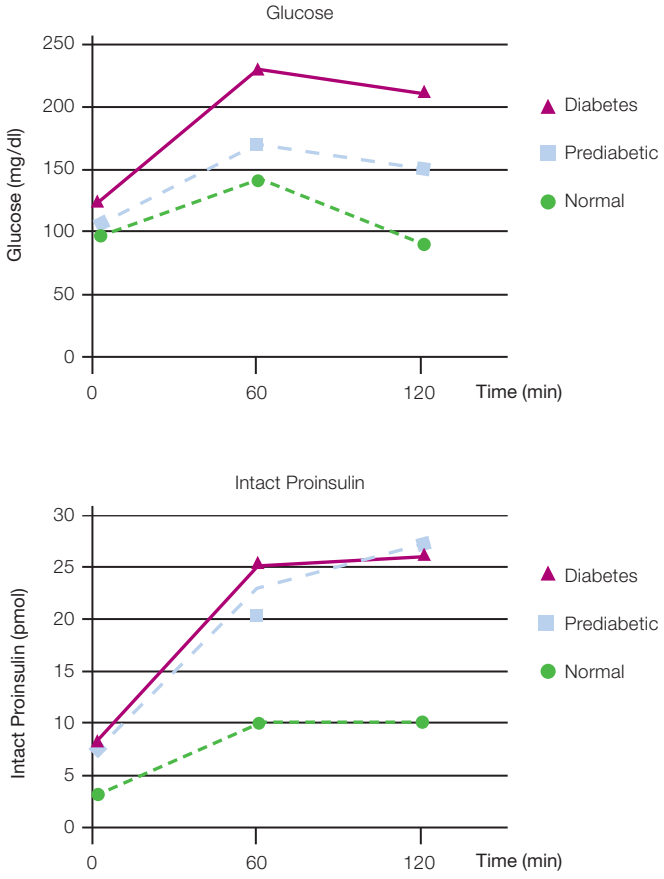

Figure 2: Response curves of glucose and intact proinsulin levels in the oGTT

Predictive value of intact proinsulin for the development of diabetes in high risk patients

Figure 3 shows the concentration profiles in glucose and intact proinsulin levels in normal subjects without (n = 7) and with (n = 4) later development of diabetes (confirmation of these findings 5 years after the oGTT (6)). While glucose levels in both groups follow the typical pattern without indications of any disorder, the intact proinsulin level show a clear distinction of the two groups. Normal subjects with later development of diabetes show a steep rise in concentration over three times the initial values (> 15 pmol / L) and therefore an already impaired ß-cell function in oGTT.

During the oGTT, the beta cells are put under „glucose stress“ and pushed depending on the pathophysiological situation to the capacity limits of the insulin synthesis. This stress situation is reflected in the intact proinsulin response levels after 2 hours and allows even in patients with normal or slightly elevated glucose levels the timely detection of impaired ß-cell function as an indicator of future deterioration of hyperglycemia and the development of type 2 diabetes.

An elevated 2-hour value for intact proinsulin > 15 pmol/l in the oral glucose tolerance test (oGTT) indicates a progressive β-cell dysfunction and thus an increased risk for the development of:

- *a progressive type 2 diabetes and*
- *a cardiovascular disease before deterioration of glucose tolerance .*

The 2-hour measurement of intact proinsulin during oGTT may predict the subsequent development of diabetes and will allow therefore an early intervention.

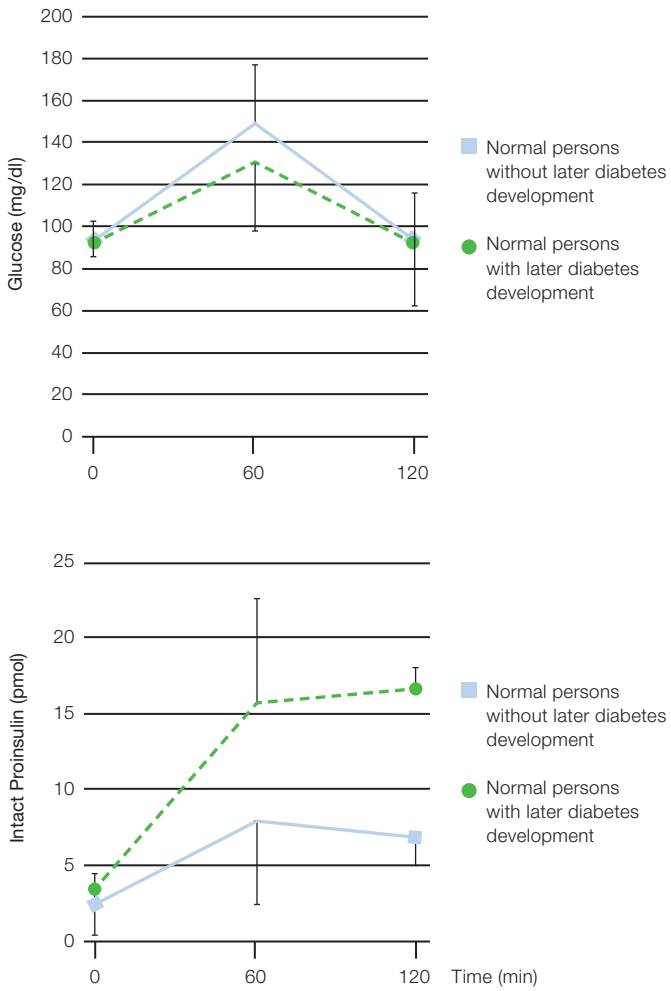

Figure 3: Glucose and intact proinsulin in OGTT in normal subjects with and without diabetes later development

3. The following tests are recommended:

- oGTT with measurement of fasting glucose and glucose after 1 and 2 hours as well as the determination of fasting and the 2-hour intact proinsulin values:
  - A fasting value for intact proinsulin of > 11 pmol/l suggests a β-cell dysfunction in the late stage and insulin resistance
  - A 2-hour value for intact proinsulin > 15 pmol/l indicates an increased risk for developping both a progressive diabetes and a cardiovascular disease.
- The achieved therapeutic success for normalisation of intact proinsulin levels and the β-cell function by change in lifestyle and by therapeutic intervention, can already be assessed after one month using the same test scheme.

Intact proinsulin as monitoring marker during therapy

Prospective studies have shown that the rather pathophysiologically-oriented intervention based on exercise or/and treatment with Metformin, thiazolidinediones, SGLT-II inhibitors, GLP-1 agonists or insulin contributes to conserve the β-cells and consequently leads to a drop in fasting intact proinsulin levels. Under purely glucocentric therapy using sulfonylurea no such effect was observed [7-10].

Test method for measuring total proinsulin and intact proinsulin

In patients suffering from a dysfunction of β-cells, both intact proinsulin and proinsulin des31,32-fragments appear in blood. Intact proinsulin has a half-life of 15 minutes while the des31,32 - fragment persists in the blood for hours. Due to these differences in half-life the specific diagnostic measurement of intact proinsulin allows a better functional assessment of the β-cell activity/dynamic and leads thus to a better diagnostic selectivity [11-12].

Optimal diagnosis of diabetes type II, prediabetes and assessment of cardiovascular risk based on glucose and intact proinsulin values

Determination of HbA1c

Based on the HbA1c value, a first distinction between diabetic and non-diabetic patients can be made.

HbA1c	≥ 6.5% ≥ 48 mmol/mol	5.7 - 6.4% 39 - 47 mmol/mol	< 5.7% < 39 mmol/mol
Diagnosis	Diabetes	Further clarification required	No diabetes
Therapy	According to guideline	Fasting glucose or oral glucose tolerance test (oGTT)	None

In patients with HbA1c values in the gray area, a fasting glucose test or an oral glucose tolerance test (oGTT) is recommended.

Determination of fasting glucose

Further differentiation of diabetic and non-diabetic subjects is possible and patients with elevated glucose levels are detected.

Fastening Glucose	≥ 126 mg/dl	≥ 100 - 125 mg/dl	< 100 mg/dl
Diagnosis	Diabetes	Elevated glucose level	Kein Diabetes
Therapy	According to guideline	Education about diabetes risk, Lifestyle intervention, Treatment of risk factors, New risk assessment and HbA1c determination after 1 year	None

Determination of fastening glucose and intact proinsulin for the purpose of detection of ß-cell status

The additional determination of intact proinsulin provides valuable information on the functional beta cell status and thus allows a more sophisticated diagnostic. The fasting value for intact proinsulin > 11 pmol/l indicates an existing ß-cell dysfunction (stage III) regardless of the glucose status and allows among others the detection of prediabetic status of the patient. Even in glucose normal patients the intact proinsulin value indicates a possible later occurrence of diabetes or the presence of a macrovascular disease. Based on the ß-cell staging the cardiovascular risk can be estimated (see also table 1).

Fasting Glucose	≥ 126 mg/dl			≥ 100-125 mg/dl			< 100 mg/dl		
Fasting Intact Proinsulin	< 11 pmol/l	< 11 pmol/l	> 11 pmol/l	< 11 pmol/l	< 11 pmol/l	> 11 pmol/l	< 11 pmol/l	< 11 pmol/l	> 11 pmol/l
Diagnosis	Diabetes		Diabetes Typ II	Elevated glucose level		Prediabetes	No diabetes		later manifestation of diabetes or macrovasculaire disease possible
ß-Cell function	No qualitative disturbance of ß-cell secretion		Dysfunctional ß-cells	No qualitative disturbance of ß-cell secretion		Dysfunctional ß-cells	No qualitative disturbance of ß-cell secretion		Dysfunctional ß-cells
Stage of ß-Cell dysfunction	Normal or increased		Stadium III	Normal or increased		Stadium III	Normal or increased		Stadium III
Cardiovascular risk	Stage I or II		High	Stage I or II		High	Stage I or II		High
Therapy	According to guideline		According to guideline, preferably TZD in combination with insulin / GLP 1; without sulfonylureas	Education about diabetes risk, Lifestyle intervention, Treatment of risk factors, New risk assessment and HbA1c determination after 1 year		If necessary temporary low-dose treatment with combination of antidiabetics for ß-cell protection (no sulfonylureas)	None		If necessary temporary low-dose treatment with combination of antidiabetics for ß-cell protection (no sulfonylureas)

Oral glucose tolerance test (oGTT) based on glucose response

A further differentiation between diabetics and non-diabetics is possible and patients with impaired glucose tolerance are recognized.

Fastening Glucose	**≥ 126 mg/dl**	**≥ 100-125 mg/dl**	**< 100 mg/dl**
Glucose in 2h-oGTT	**≥ 200 mg/dl**	**140-199 mg/dl**	**< 140 mg/dl**
Diagnosis	Diabetes	Impaired glucose tolerance	No diabetes
Therapy	According to guideline	Education about diabetes risk, Lifestyle intervention, Treatment of risk factors New risk assessment and HbA1c determination after 1 year	None

Oral Glucose tolerance test (oGTT) based on glucose response and intact proinsulin allow the detection of pathophysiological processes

The additional 2 hour value of > 15 pmol/l allows a further recognition of prediabetics and also in glucose-normal-appearing patients subsequent pre-/diabetes or indicates macrovascular diseases. Based on the ß-cell staging the cardiovascular risk can be estimated (see also table 1).

fasting glucose	**≥ 126 mg/dl**			**≥ 100-125 mg/dl**			**< 100 mg/dl**		
Glucose in 2h-oGTT	**≥ 200 mg/dl**			**140-199 mg/dl**			**< 140 mg/dl**		
Fasting Intact Proinsulin	**< 11 pmol/l**	**< 11 pmol/l**	**> 11 pmol/l**	**< 11 pmol/l**	**< 11 pmol/l**	**> 11 pmol/l**	**< 11 pmol/l**	**< 11 pmol/l**	**> 11 pmol/l**
Intact Proinsulin in 2h-oGTT	**< 15 pmol/l**	**> 15 pmol/l**	**> 15 pmol/l**	**< 15 pmol/l**	**> 15 pmol/l**	**> 15 pmol/l**	**< 15 pmol/l**	**> 15 pmol/l**	**> 15 pmol/l**
Diagnosis	Diabetes		Diabetes Typ II	Impaired glucose tolerance	Prediabetes		No diabetes	Later manifestation of prediabetes/diabetes possible	Later manifestation of diabetes or macrovasculaire disease possible
ß-Cell function	No qualitative disturbance of ß-cell secretion	Impaired ß-cell function	Dysfunctional ß-cells	No qualitative disturbance of ß-cell secretion	Impaired ß-cell function	Dysfunctional ß-cells	No qualitative disturbance of ß-cell secretion	Impaired ß-cell function	Dysfunctional ß-cells
Stage of ß-Cell dysfunction	Stage I or II		Stage III	Stage I or II		Stage III	Stage I or II		Stage III
Cardiovascular risk	Normal or increased		High	Normal or increased		High	Normal or increased		High
Therapy	According to guideline	According to guideline, preferably TZD in combination with insulin /GLP 1; without sulfonylureas		Education about diabetes risk, Lifestyle intervention,Treatment of risk factors, New risk assessment and HbA1c determination after 1 year		If necessary temporary low-dose treatment with combination of antidiabetics for ß-cell protection (no sulfonylureas)	None	Education about diabetes risk, Lifestyle intervention, Treatment of risk factors, New risk assessment and HbA1c determination after 1 year	If necessary temporary low-dose treatment with combination of antidiabetics for ß-cell protection (no sulfonylureas)

The oral glucose tolerance test with the additional determination of intact proinsulin, fasting and after 2 hours, allows the best diagnostic information with regard to the detection of diabetes type II and prediabetes as well as an assessment of cardiovascular risk. In addition ß-cell dysfunction is recognized by intact proinsulin values even in normal glucose patients providing evidence of macrovascular disease and the risk of subsequent pre-/diabetes. Knowing the functional ß-cell status allows a treatment protecting the ß-cell and is thus based on the pathophysiological processes of diabetes.

Intact Proinsulin ELISA - Cat. No.: TE1012 (CE) / TE1011 (RUO)

Sample type	Serum, EDTA / Heparin Plasma, cell culture
Sample preparation	blood sampling - fastening. Due to better sample stability EDTA plasma and Heparin plasma are the preferred sample typs.
Reference ranges	fastening: Average 3.99 pmol/l +/- 1.58 SD < 11 pmol/l (normal secretion) > 11 pmol/l (impaired secretion) 2h-Value in oGTT: >15 pmol/l (progressive β-cell dysfunction)

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