Whitepaper

"BelDIA PRE"

Prediction of risk for Type 2 Diabetes development.

Prepared as:

Background Information

By: Prof. A. Pfuetzner, MD, PhD; G. Burgard, MD

BelDIA PRE Point-Of-Care-Testing

Elevated Intact Proinsulin Levels Are Indicative of Beta-Cell Dysfunction, Insulin Resistance, and Cardiovascular Risk

Summary:

Proinsulin is the prohormone precursor to insulin made in the beta cells of the islets of Langerhans, which are histologically distinct cell agglomerations of the endocrine pancreas. In humans, proinsulin is encoded by the INS gene. Insulin resistance (IR) and deterioration of beta-cell secretion are main disturbances in the development of type 2 diabetes. This is reflected by increasing serum intact proinsulin levels in later disease stage. Introduction of stable assays that are able to distinguish between intact proinsulin and its specific and unspecific cleavage products has resulted in the finding that serum intact proinsulin values can serve as a direct marker for beta-cell dysfunction. Elevated intact proinsulin is a highly specific indirect indicator of IR, can predict diabetes development in non-diabetic subjects, and can predict cardiovascular risk in diabetic and non-diabetic subjects.

Measurement of fasting intact proinsulin may be used to determine the stage of ß-cell dysfunction and to monitor and optimize antidiabetic treatment approaches. Our study group has been involved in a variety of clinical studies investigating drug effects on beta-cell secretory capacity, IR, and intact proinsulin levels.

Treatment with drugs interacting with the pathophysiology of type 2 diabetes, such as insulin, GLP-1 analogs, glitazones, and SGLT-2 inhibitors resulted in significant decreases in elevated proinsulin levels in type 2 diabetes patients. This effect was independent from glycemic control.

Measurement of fasting intact proinsulin values allows a staging of beta-cell dysfunction and evaluation of IR, thus providing an interesting diagnostic tool for both selection of appropriate therapy and monitoring of treatment success.

Rev: 1.03. Dez. 2019

Overview of Measurement of Fasting Intact proinsulin Values

Type 2 diabetes mellitus (T2DM) is characterized initially by a metabolic insulin resistance (IR) and a genetically determined dysfunction of the insulin-secreting pancreatic beta cells. In clinical practice, patients are classified by laboratory markers and symptoms such as hemoglobin A1c (HbA1c), glucose, lipids, blood pressure, and body mass index. However, this specification does not provide insight into the underlying pathophysiological disorders.

There has been a rapidly growing interest in new methods for the evaluation of pancreatic beta-cell dysfunction to optimize therapeutic intervention strategies.

Conventional means for assessing insulin secretion impairment include homeostasis model assessment (HOMA) score and meal-related functional parameters.

Next to this, a current focus is on the defective islet beta-cell processing of the proinsulin molecule that directly reflects the degree of beta-cell dysfunction.

Impaired beta-cell secretory capacity induces disproportionately elevated serum proinsulin levels, such as found in subjects with T2DM and impaired glucose tolerance.

Beyond its role as a direct biomarker for beta-cell dysfunction, measurement of intact proinsulin values has also emerged to be an important indirect predictor for IR5 and individual cardiovascular risk (**Figure1**). In addition, elevated intact proinsulin is indicative for future type 2 diabetes development in non-diabetic subjects for up to 5-7 years in advance [1-6].

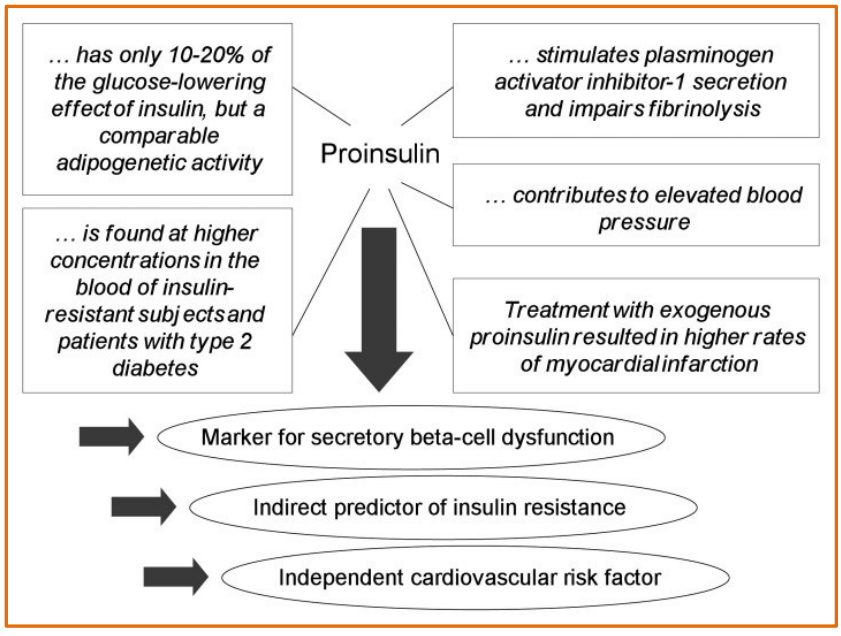

Figure 1: **Characteristics of proinsulin: Its role as a biomarker.**

As a result, determination of fasting intact proinsulin or the proinsulin-to-insulin ratio has become a popular method to describe insulin-resistance-associated beta-cell impairment and the impact of therapeutic interventions on insulin-secreting cells.

In the cross-sectional epidemiological SETT2D study (Study for the Evaluation of Treatment Preference in Type 2 Diabetes), the biochemical and demographic characteristics of 532 individuals with diet or orally treated but insufficiently controlled T2DM were investigated[3].

Both IR, as assessed by HOMA-IR score, and disproportionately increased intact proinsulin levels were prevalent in the vast number of patients. These findings underline the close association between beta-cell function and insulin sensitivity [4].

Routine assessment of intact proinsulin therefore provides a better understanding of the underlying disease conditions and may allow optimization of antidiabetic therapy beyond simple glucose control.

A variety of clinical studies was conducted that investigated changes in intact proinsulin levels as a biomarker of beta-cell dysfunction and IR. It is noticeable that patients with T2DM may show a very early increase in fasting intact proinsulin secretion, independent from disease duration or other clinical feature[2,5].

Proinsulin: A Pathophysiological Background

Proinsulin is synthesized by the beta cell of the pancreas as a precursor molecule for insulin. Physiologically, virtually all proinsulin molecules are intracellularly cleaved by carboxypeptidases into insulin and C-peptide.

In healthy subjects, only a minor percentage of uncleaved intact proinsulin is (postprandially) released into the circulation.

Progressive IR leads to an increased demand for insulin. Thus, the cleavage capacity of the processing enzymes may be exhausted, and the intact precursor or partially processed proinsulin is secreted in addition to insulin and C-peptide[2,5]. Intact proinsulin binds to the insulin receptor. However, it has only 10–20% of the glucose-lowering effect of insulin but comparable adipogenesis activity[6].

In the past, conventional nonspecific assays showed a high cross reactivity with various fractions of proinsulin-like molecules. This has led to only partial and sometimes incorrect conclusions about the role of proinsulin in the prediction and diagnosis of beta-cell dysfunction and T2DM progression[7,8].

New stable assays have been developed that can distinguish between intact proinsulin and its specific and unspecific cleavage products[7-9]. Use of these assays in epidemiological and interventional studies has helped to get a better understanding about beta-cell dysfunction and its relation to IR and cardio-vascular risk. Noteworthy is a new specific intact proinsulin enzyme-

linked immunosorbent assay (ELISA) that can be easily introduced into routine laboratories and does not require any further specific instrumentation[9]. In addition, the new BelDIAPRE point-of-care-test has been developed to enable rapid and economic (semi-quantitative or quantitative) intact proinsulin testing without need of any extra laboratory equipment[10].

Practical Aspects for the Use of Fasting Intact proinsulin Values in Daily Therapeutic Practice

In later stages of T2DM, proinsulin and proinsulin-like molecules are secreted in increasing amounts with insulin. Based on very specific antibodies, assays are now able to specifically differentiate intact proinsulin from other degradation products, thus allowing a reliable staging of beta-cell dysfunction and IR evaluation[2,5].

Intact proinsulin is stable in ethylenediaminetetraacetic acid (EDTA) whole blood samples, which can be obtained from the routine sample for HbA1c measurement.

However, the point-of-care proinsulin test from PharmACT AG enables people to measure their proinsulin level with capillary blood obtained from a finger-prick every time and everywhere, without expensive laboratory equipment.

Time point:

1.0 - 1.5 hours after the meal (time-point of largest ß-cell stress), or

Fasting morning state (time-point of lowest ß-cell stress)

Specimen:

2 drops of capillary whole blood obtained from the fingertip or ear lobe

Result:

Fasting intact proinsulin <7 pmol/liter at manifestation or therapy control = normal value:

- No qualitative beta-cell dysfunction
- Start or continue therapy to reach glucose target
- Repetition after 6 months (sulfonylurea treatment) or 12 months (diet) recommended

Fasting intact proinsulin >7 pmol/liter = elevated value:

- Beta-cell dysfunction and IR
- Beta-cell protective therapy recommended (exercise, pharmacological insulin-sensitizing strategies, glucagon-like peptide-1 analog, insulin)
- Further diagnosis of cardiovascular risk recommended
  (e.g. lipids or high-sensitivity C-reactive protein)
- Control measurement after 3 months recommended
- A positive value in case of healthy subjects indicates type 2 diabetes development for up to 5-7 years

Rev: 1.03. Dez. 2019

Elevated Intact proinsulin Values Reveal Beta-Cell Dysfunction

Fasting measures of intact proinsulin values allow for a pathophysiological staging of T2DM based on islet beta-cell processing[2,5].

Beta-cell dysfunction is comprised of three components: (1) secretion timing disorder, (2) quantitative disorder, and (3) qualitative disorder. A loss of first phase insulin response, an important inhibitory signal for hepatic glucose release, portrays the secretion timing disorder in early stages of T2DM.

Disease progression may then lead to a decline in pulsatile insulin release, reflecting an additional secretion timing failure. The quantitative disorder starts when beta cells increase the volume of insulin release based on the progressive external demand.

In later stages, exhaustion of the production capacity may result in an almost complete loss of insulin secretion. The quantitative increase in proinsulin secretion will finally induce a deterioration of secretion product composition[2,5] (**Figure 2**).

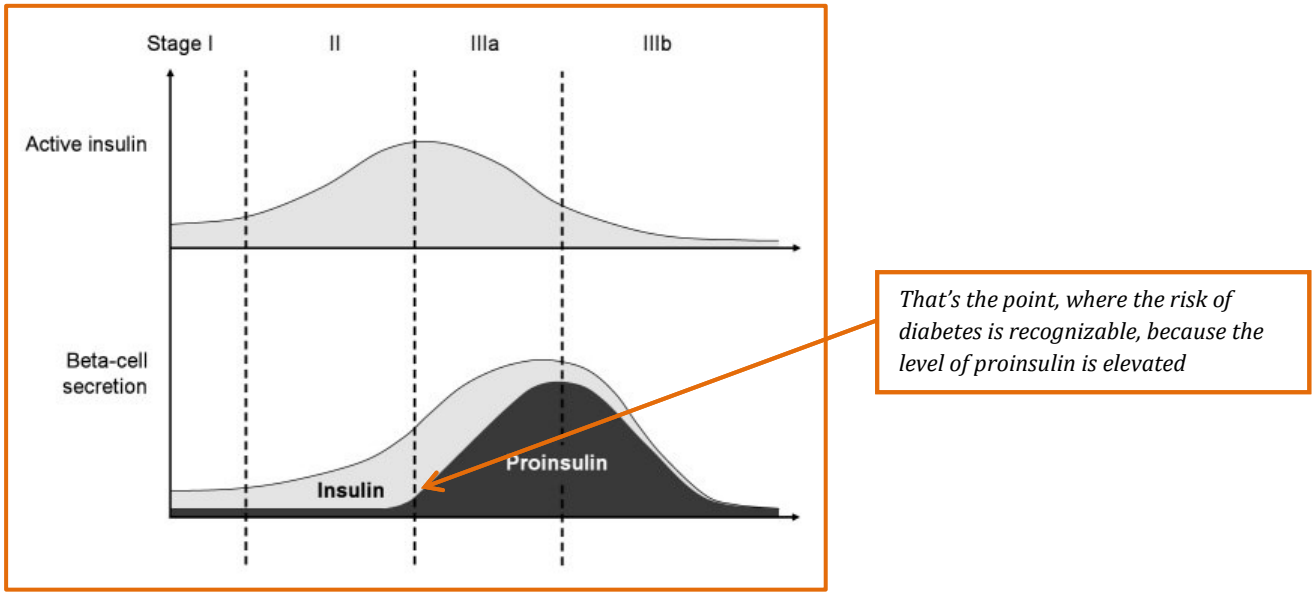

Figure 2: **Classification of type 2 diabetes based on the pathophysiology of beta-cell secretion.**

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3192645/#b16

Insulin-sensitive patients with normal quantitative insulin secretion but lack of the first-phase insulin response would be classified as stage I (timing disorder). **…**

In the beginning of "stage IIIa", the elevated proinsulin may be recognizable long before blood glucose is elevated. The Point-of-Care Test "BelDIA PRE" can therefore be used for very early detection self-test for diabetes development and prevention of diabetes by changing lifestyle and nutrition may still be possible[11].

Because of the minor but evident glucose-lowering effect[6], patients with severe beta-cell dysfunction and high proinsulin output may still have sufficient glucose-lowering capacity to avoid the diagnosis of diabetes mellitus in an oral glucose tolerance test experiment[11].

In fact, beta-cell dysfunction and proinsulin secretion are not consequently correlated with diabetes duration and proinsulin secretion may precede the onset of clinically overt T2DM[2,5,11-13].

Intact Proinsulin Is an Independent Cardiovascular Risk Factor

Current evidence suggests that proinsulin contributes to the excess incidence of cardiovascular disease in T2DM by stimulating plasminogen activator inhibitor-1 secretion and the consecutive inhibition of fibrinolysis (**Figure1**)[2,5,14]. Elevated proinsulin concentrations predicted a 200% increased risk for cardiovascular death and morbidity over a 27-year period, independent of other major cardiovascular risk factors in male patients without diabetes[14].

In the Hoorn Study, fasting proinsulin levels proved to be significantly associated with all-cause and cardiovascular mortality, independent of glucose tolerance status and IR and largely independent of other cardiovascular risk factors[15,16].

Numerous other clinical trials provide similar results, supporting an independent association between the increase of proinsulin levels and cardiovascular disease[14-19].

Intact Proinsulin Predicts Progression of Insulin Resistance

Insulin resistance is a hallmark of T2DM and has been proposed as the common link between glucose metabolism disorder and cardiovascular disease[21].

Progression of IR in the course of T2DM leads to increased insulin demands and finally to an impairment of beta-cell function in later stages of the disease. Disproportionately elevated intact proinsulin levels in the peripheral blood serve as an appropriate laboratory marker for this phenomenon by disclosing the exhausted cleavage capacity of intra-cellular processing enzymes[22].

Haffner and colleagues4 examined the relation between the fasting proinsulin-to-insulin ratio with a number of metabolic disorders believed to be associated with the IR syndrome. In 423 subjects without diabetes, an increased ratio was significantly associated with hypertension, low high-density lipoprotein cholesterol, high triglyceride levels, and impaired glucose tolerance[23].

These results suggest that even nondiabetic individuals with the IR syndrome not only exhibit hyperinsulinemia as a marker of IR, but also show elevated proinsulin values, which may reflect relative beta-cell failure or malfunction. Our group conducted further research including T2DM patients. Data from IRIS-II (study on Insulin Resistance and Insulin Sensitivity – II)] a large epidemiology study with 4270 people with diabetes revealed a calculated specificity of 93.2% (sensitivity 46.9%) for elevated intact proinsulin levels (>10 pmol/liter in the chemiluminescence assay) as an indirect marker of IR. Moreover, patients who presented with elevated proinsulin values demonstrated a higher prevalence of micro- and macro-vascular disease[1].

The disproportionate increase in fasting intact proinsulin concentrations even appeared to be a more specific marker for IR and increased cardiovascular risk than suppression of fasting adiponectin[24].

Another investigation explored the predictive value of intact proinsulin in 48 T2DM patients as a highly specific indicator for clinically relevant insulin resistance (**Figure 3**).

Again, there was a significant correlation between intact proinsulin values and gold standard IR measurements (i.v. glucose challenge test with minimal model analysis, $p < .05$; HOMA,$p < .01$). Elevation of intact proinsulin values above the reference range (>10 pmol/liter in the chemiluminescence assay) showed a very high specificity (minimal model analysis, 100%; HOMA, 92.9%) and a moderate sensitivity (minimal model analysis, 48.6%; HOMA, 47.1%) as marker for IR[25].

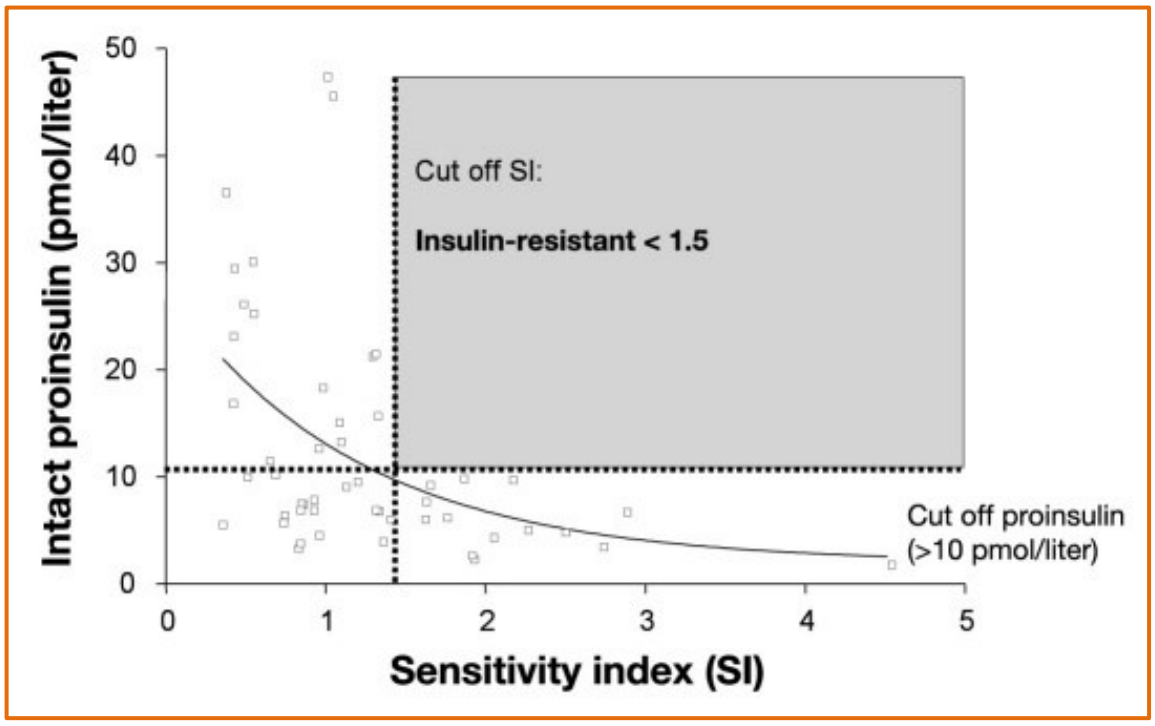

Figure 3: **Intact proinsulin is a highly specific marker for IR**

Conclusion

In conclusion, assessment of intact proinsulin by means of a (semi)quantitative point-of-care rapid test may be a useful tool to identify otherwise healthy subject, which are in the process to develop type 2 diabetes. In diabetes patients, this test can be used to identify subjects with particularly increased cardiovascular risk. A positive test result should lead to further clinical and laboratory assessment of these subjects and introduction of appropriate preventive measures once the screening tests are confirmed.

Publications

1. Pfützner A, Standl E, Hohberg C, Konrad T, Strotmann HJ, Lübben G, Langenfeld MR, Schulze J, Forst T. IRIS II study: intact proinsulin is confirmed as a highly specific indicator for insulin resistance in a large cross-sectional study design. Diabetes Technol Ther. 2005;7(3):478–486.
2. Pfützner A, Pfützner AH, Larbig M, Forst T. Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus. Diabetes Technol Ther.2004;6(3):405–412.
3. Pfützner A, Pfützner AH, Stridde E, Huppertz E, Reimer T, Derwahl M, Forst T, Petrak F. Insulin resistance and b-cell-dysfunction in insufficiently controlled type 2 diabetes: the SETT2D Trial. Diabetes Stoffwechsel Herz. 2007;2:91–97.
4. Pfützner A, Kunt T, Hohberg C, Mondok A, Pahler S, Konrad T, Lübben G, Forst T. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. Diabetes Care. 2004;27(3):682–687.
5. Pfützner A, Kann PH, Pfützner AH, Kunt T, Larbig M, Weber MM, Forst T. Intact and total proinsulin - new aspects for diagnosis and treatment of type 2 diabetes mellitus and insulin resistance. Clin Lab. 2004;50(9-10):567–573.
6. Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH, Bowsher RR, Anderson JH. Biosynthetic human proinsulin. Review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. Diabetes Care. 1992;15(5):666–692.
7. Pfützner A, Kunt T, Langenfeld M, Löbig M, Knesovic M, Forst T. Clinical and laboratory evaluation of specific chemiluminescence assays for intact and total proinsulin. Clin Chem Lab Med. 2003;41(9):1234–1238.
8. Pfützner A, Pfützner AH, Kann PH, Stute R, Löbig M, Yang JW, Mistry J, Forst T. Clinical and laboratory evaluation of a new specific ELISA for intact proinsulin. Clin Lab. 2005;51(5-6):243–249.
9. Siebenhaar R, Weise A, Safinowski M, Reisinger K, Musholt PB, Reimer T, Pfützner A, Forst T. Clinical and laboratory evaluation of a new specific ELISA for intact proinsulin. Diabetes Stoffwechsel Herz. 2008; 2:275–281.
10. Pfützner A., Pfützner AH., Kann PH, Burgard G. Clinical and laboratory evaluation of a new specific point-of-care test for intact proinsulin. J. Diabetes Sci. Technol. 2017;11(2):278-283
11. Pfützner A, Hermanns I, Ramljak S, Demircik F, Pfützner AH, Kann PH, Weber MM. Elevated intact proinsulin during an oral glucose challenge indicate progressive ß-cell dysfunction and may be predictive for development of type 2 diabetes. J. Diabetes Sci. technol. 2015; 9(6):1307-1312
12. Nijpels G, Popp-Snijders C, Kostense PJ, Bouter LM, Heine RJ. Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn Study. Diabetologia.1996;39(1):113–118.
13. Alssema M, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ; Hoorn Study. Proinsulin concentration is an independent predictor of all-cause and cardiovascular mortality: an 11-year follow-up of the Hoorn Study. Diabetes Care.2005;28(4):860–865.
14. Nordt TK, Bode C, Sobel BE. Stimulation in vivo of expression of intra-abdominal adipose tissue plasminogen activator inhibitor type I by proinsulin. *Diabetologia*. 2001;44:1121-1124
15. Zethelius B, Byberg L, Hales CN, Lithell H, Berne C. Proinsulin is an independent predictor of coronary heart disease: report from a 27-year follow-up study. *Circulation*. 2002;105:2153-2158
16. Bavenholm P, Proudler A, Tornvall P, Godsland I, Landou C, de Faire U, Hamsten A. Insulin, intact and split proinsulin, and coronary artery disease in young men. Circulation. 1995;92(6):1422–1429
17. Wareham NJ, Byrne CD, Hales CN. Role of insulin and proinsulin in diabetic vascular disease. Metabolism. 1995;44(10 Suppl 4):76–82.
18. Lindahl B, Dinesen B, Eliasson M, Røder M, Jansson JH, Huhtasaari F, Hallmans G. High proinsulin concentration precedes acute myocardial infarction in a nondiabetic population. Metabolism. 1999;48(9):1197–1202.
19. Lindahl B, Dinesen B, Eliasson M, Røder M, Hallmans G, Stegmayr B. High proinsulin levels precede first-ever stroke in a nondiabetic population. Stroke.2000;31(12):2936–2941.
20. Yudkin JS, May M, Elwood P, Yarnell JW, Greenwood R, Davey Smith G; Caerphilly Study. Concentrations of proinsulin like molecules predict coronary heart disease risk independently of insulin - prospective data from the Caerphilly Study. Diabetologia. 2002;45(3):327–336.
21. Katz RJ, Ratner RE, Cohen RM, Eisenhower E, Verme D. Are insulin and proinsulin independent risk markers for premature coronary artery disease Diabetes. 1996;45(6):736–741.

Rev: 1.03. Dez. 2019

22. Røder ME, Porte D, Schwartz RS, Kahn SE. Disproportionately elevated proinsulin levels reflect the degree of impaired ß-cell secretory capacity in patients with non-insulin dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1998;83:604-608.
23. Haffner SM, Mykkänen L, Stern MP, Valdez RA, Heisserman JA, Bowsher RR. Relationship of proinsulin and insulin to cardiovascular risk factors in nondiabetic subjects. *Diabetes*. 1993;42:1297-1302.
24. Langenfeld MR, Forst T, Standl E, Strotmann HJ, Lübben G, Pahler S, Kann P, Pfützner A; IRIS II study. IRIS II Study: sensitivity and specificity of intact proinsulin, adiponectin, and the proinsulin/adiponectin ratio as markers for insulin resistance. Diabetes Technol Ther. 2004;6(6):836–843. [PubMed]
25. Pfützner A, Kunt T, Mondok A, Pahler S, Konrad T, Lübben G, Forst T. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. Diabetes Care. 2004 ;27:682-687.

Contact
PharmACT AG for BelDIA PRE
Prof. Dr. Dr. med. Andreas Pfützner, PHSI Institute, Mainz, Germany.
Mailto: customer@pharmact.eu

//END//