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Developing diagnostics

Technical Note No: 34-0115

The cross reaction of Proinsulin metabolites in Mercodia Proinsulin ELISA (10-1118-01)

Specificity

Insulin	<0.03 %
C-peptide	<0.006 %
Proinsulin Des(64–65)	84 %
Proinsulin Split (65–66)	90 %
Proinsulin Des(31–32)	95 %
Proinsulin Split (32–33)	95 %

Processing of human Proinsulin

Human Proinsulin (hPI) is believed to be processed by endoproteolysis at the junctions of aa (32,33) and (65,66) to give split (32,33) hPI and split (65,66) hPI. The exposed, C-terminal, basic amino acids in the split metabolites are removed by carboxypeptidase to yield des (31,32) and des (64,65).

Concentrations of different proinsulin derivatives in serum

Sobey *et al*. constructed specific two-site assays for intact hPI, insulin, split (65-66) hPI and split (32-33) hPI. By using the different assays they found < 1.0 pM of split (65-66) hPI after overnight fasting in eight normal male subjects and the maximum individual concentration reached in plasma taken during an oral glucose tolerance test was 3.8 pM. Intact proinsulin was 2.3±0.3 pM and split (32-33) proinsulin was 2.1±0.7 pM in the fasting state of the eight normal subjects and 9.9±1.4 pM and 19.7±6.0 pM respectively, during the oral glucose tolerance test. The authors conclude that the very low concentration of split (65-66) hPI meant that this derivative did not interfere significantly with the specificity of the assays of intact proinsulin and insulin used in the study. The authors claim that "in no system was any difference detected between the behavior of (65-66) split and des (64-65) human proinsulin, nor between (32-33) split and des (31-32) human proinsulin".

In studies examining intact and split proinsulin in human subjects only intact and split (32,33) hPI is quantified. The circulating concentrations of split (32-33) hPI is usually found to be as high or higher than intact hPI (Wareham *et al.*, Zethelius *et al.*)

Address Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden
Phone +46 18 57 00 70 | **Fax** +46 18 57 00 80 | **Vat No** SE-556157510001
E-mail info@mercodia.com | **Website** www.mercodia.com

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Clinical implication

It is generally believed that increased proinsulin concentrations are associated with risk of coronary heart disease, insulin resistance and development of type 2 diabetes. However, results are contradictive regarding the importance of split (32-33) hPI and intact hPI versus total proinsulin in prediction of disease.

The cross reactivity of the metabolites des (64,65) and split (65-66) in the Mercodia Proinsulin ELISA has little clinical or analytical relevance as these circulate at low concentrations relative to other forms of proinsulin.

References

Beckman JA, Creager MC and Libby P (2002) Diabetes and atherosclerosis; Epidemiology, pathosphysiology, and management. Review *JAMA* 287:2570-2581.

Given BD, Cohen RM, Shoelson SE, Frank BH, Rubenstein AH and Tager HS (1985) Biochemical and clinical implications of proinsulin conversion intermediates. *J Clin Invest* 76:1398 – 1405.

Haffner SM, Mykkanen L, Valdez RA, Stern MP, Holloway DL, Monterrosa A and Bowsher RR (1994) Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome. J *Clin Endocrinol Metab* 79:1806 –1810.

Ostrega D, Polonsky K, Nagi D, Yudkin J, Cox LJ, Clark PMS and Hales CN (1995) Measurement of proinsulin and intermediates: Validation of immunoassay methods by HPLC. *Diabetes* 44:437– 440.

Wareham N, Byrne CD, Williams NJ, Day NE and Hales CN (1999) Fasting proinsulin concentrations predict the development of type 2 diabetes. *Diabetes Care* 22:262-270

Sobey WJ, Beer SF, Carrington CA, Clark PMS, Frank BH, Gray P, Luzio SD, Owens DR, Schneider AE, Siddle K, Temple RC and Hales N (1989). Sensitive and specific two-site immunoradiometric assay for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. *Biochem J* 260, 535-541.

Zethelius B, Byberg L, Hales CN, Lithel H, Berne C (2002) Proinsulin is an independent predictor of coronary heart disease. *Circulation* 105:2153-2158.