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High fasting serum insulin level due to autoantibody interference in insulin immunoassay discloses autoimmune insulin syndrome: a case report

Une interférence dans le dosage de l'insuline révèle un syndrome auto-immun anti-insuline

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Abstract. Insulin-antibodies are a cause of misleading results in insulin immunoassays. They may also mediate deleterious blood glucose variations. A patient presented with overtiredness, recurrent episodes of sweating, dizziness and fainting fits. A fasting serum insulin assay performed on a Modular platform (Modular analytic E170, Roche Diagnostic, Meylan, France) showed a highly elevated value of 194.7 mIU/L, whereas on the same sample glucose and C-peptide levels were normal. Other immunometric insulin assays were performed, as well as antibodies anti-insulin radiobinding assay (RBA) and gel filtration chromatography (GFC). While complementary insulin assays yielded closer to normal fasting levels, the free insulin concentration assessed after PEG precipitation was 14.0 mIU/L and the RBA was positive. GFC revealed that most of the insulin was complexed with a 150 kDa molecule, corresponding to an immunoglobulin G (IgG). A high fasting serum insulin level in a patient with neuroglucopenic symptoms was related to a high insulin-antibody level, suggesting an insulin autoimmune syndrome.

Key words: *interference, insulin antibody, insulin immunoassay, insulin autoimmune syndrome, autoimmunity*

Résumé. Les anticorps anti-insuline représentent une cause de résultats erronés dans les dosages immunologiques de l'insuline. Ils peuvent également intervenir sur l'action de l'insuline et entraîner des variations délétères de la glycémie. Une de nos patientes présentait une fatigue excessive, des épisodes récurrents de sueurs, des étourdissements et des évanouissements. Un dosage sérique à jeun de l'insuline exécuté sur une plate-forme modulaire (Modular E170 analytique, Roche Diagnostic, Meylan, France) a montré une valeur très élevée de 194,7 mUI/L, tandis que sur le même échantillon la glycémie et les taux de peptide C étaient normaux. D'autres dosages immunométriques de l'insuline ont été effectués, ainsi que le dosage des Ac anti-insuline par radio-ligand et une chromatographie par filtration sur gel. Les dosages d'insuline complémentaires ont montré des niveaux proches de la normale à jeun, la concentration d'insuline libre évaluée après précipitation au PEG était de 14,0 mUI/L d'insuline et les Ac anti-insuline étaient positifs. La chromatographie par filtration sur gel a révélé que la plupart de l'insuline était complexée avec une molécule de 150 kDa, correspondant à une immunoglobuline G (IgG). Le taux d'insuline sérique à jeun élevé chez la patiente présentant des symptômes neurologiques d'hypoglycémie était lié à un niveau d'Ac anti-insuline élevé. Ce cas suggère donc un diagnostic de syndrome auto-immun anti-insuline.

Mots clés : *interférences, anticorps anti-insuline, dosages immunologiques de de l'insuline, syndrome auto-immun anti-insuline, auto-immunité*

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Presentation of the case

A 59-y old Caucasian woman attended her general practitioner for overtiredness, recurrent episodes of sweating, dizziness and fainting fits (lipothymia). Considering her body weight (1.66 m, 90 kg), an impairment of glucose metabolism was suspected, and she was prescribed measurements of fasting blood glucose and serum insulin levels. The fasting blood glucose level was 5.7 mmol/L (Cobas C501, Roche Diagnostic, Meylan, France; reference range: 3.88-6.10 mmol/L). The serum insulin assay performed on a Modular platform (Modular analytic E170, Roche Diagnostic, Meylan, France) showed an elevated concentration of 194.7 mIU/L (reference range: 2.6-24.9 mIU/L). The serum C-peptide concentration (Modular E170, Roche Diagnostics, Meylan, France) was normal: 4.26 µg/L (reference range: 1.15-4.50 µg/L). The discrepancy between the high fasting serum insulin and normal plasma C-peptide levels, in a context of normal fasting blood glucose level, prompted complementary assessments, after the hypotheses of dissimulated insulin injection or abnormal excess of insulin secretion were ruled out. All biological data are resumed in *table 1*. The assay was retested on second serum and provided a similar value. The fasting blood glucose level was confirmed to lie within the normal range: 5.0 mmol/L. On a third sample the postprandial blood glucose level was also 5.0 mmol/L. Because assay interference was suspected, serum insulin measurements was further assessed with other immunometric assays (IMAs), namely, the DxI assay (UniCel® DxI 800, Beckman Coulter) and the bi-Insulin IRMA assay (CIS bio International, Gif-sur-Yvette, France). They yielded values of 17.8 mIU/L and 38.7 mIU/L, respectively. In order to quantify free insulin, a bi-Insulin IRMA CIS bio test was performed after PEG precipitation, yielding a result of 14.0 mIU/L. To confirm suspicions of interaction with anti-insulin auto-antibodies (auto-Abs), the serum was further tested with an anti-insulin radiobinding assay (CIS bio International, Gif-sur-Yvette, France) and showed a value of 52% (positivity cut-off = 2.5%). When a heterophilic blocking tube (HBT, Scantibodies Laboratory Inc, Santee, CA, USA) was used, the Modular value obtained after 1 hour of serum incubation at room temperature was not different from the value obtained without serum pre-treatment. On a second serum sample obtained 2 months later, serum insulin measurements performed on Modular and DxI platforms read 205.1 mIU/L and 20.8 mIU/L, respectively. After PEG precipitation, the DxI insulin measurement read 9.7 mIU/L. Gel filtration chromatography (GFC) (Superdex 10/30 200 pre-packed column, Pharmacia, Sweden) calibrated with Gel Filtration Standard (Bio-Rad N 151-1901, England) was then performed to determine different fractions of circulating insulin. The chromatogram presented 2 peaks around 158 000 and 6 000 daltons, corresponding to an insulin complex and free insulin respectively (*figure 1*). The disclosure of high anti-insulin antibody levels in a context of unexplained recurrent episodes of unconsciousness lead to the diagnosis of an insulin auto-immune syndrome.

The clinical chemist comment

A highly elevated Modular fasting serum insulin value measured in a context of normal blood glucose and plasma C-peptide levels, as reported in the present case, raises some concern.

Different kind of interferences in immunoanalysis could lead to a false elevated value as interference with metabolites, pharmacological insulin analogs or and circulating anti-insulin auto-Abs.

Insulin (51 amino acids, 5.808 kDa), produced by β-cells in the pancreatic islets of Langherans, is secreted into the bloodstream after proteolysis of the precursor proinsulin leading also to proteolysis fragments des 31,32 proinsulin, des 64,65 proinsulin). These fragments also display insulin

Table 1. Patient laboratory data.

Assay	Reference	Reference value	Serum 1	Serum 2
Serum insulin	Modular analytic E170 Roche Diagnostics	2.6-24.9 mIU/L	-194.4* -194.1‡	205.1*
	Unicel DxI 800 Beckman-Coulter	2-17 mIU/L	-17.8*	-20.8* -9.7†
	Bi-Insulin IRMA CIS bio	2-17 mIU/L	-38.7* -14†	
Anti-insulin antibodies	Anti insulin RBA CIS bio	Positivity >2.5%	52*	
C-Peptide	Modular analytic E170 Roche Diagnostics	1.15-4.50 µg/L	4.26*	
Anti GAD/Anti IA2	CentAK antiGAD 65 /IRMA anti IA2 Immunotech	Positivity > 1 kUI/L	<1*	
Pro-insulin	Human Intact Proinsulin AbCys ELISA	obese 2.0-6.0 pmol/L		3.2*

* No pre-treatment; ‡ After pre-treatment by HBT (Scantibodies); † After precipitation by PEG.

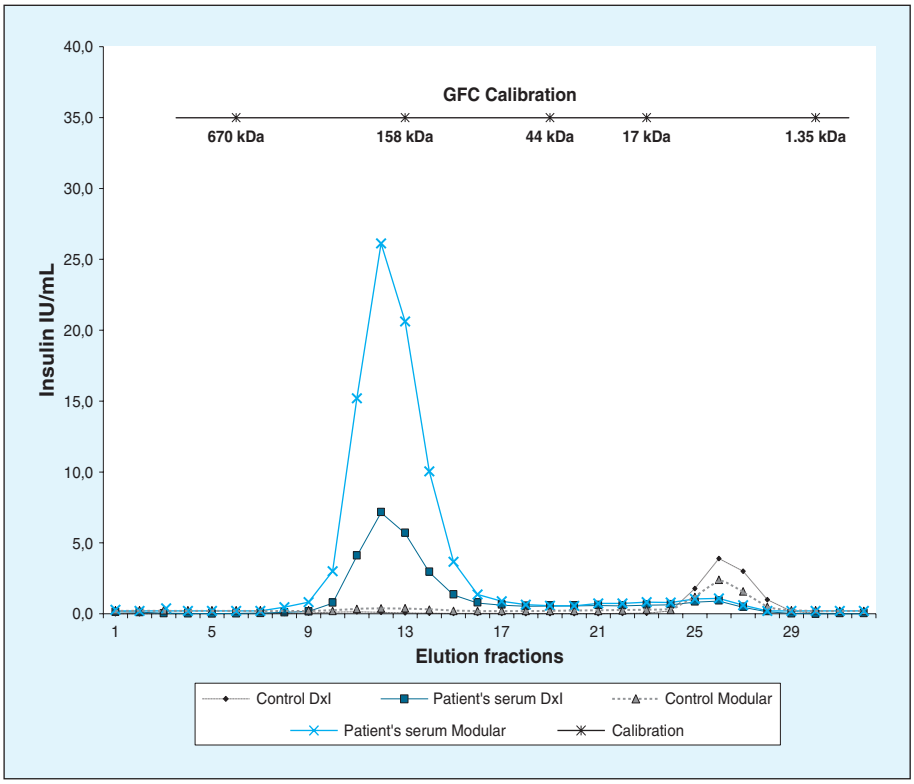

Figure 1. Gel filtration chromatography. The serum #2 of the patient and a control serum without anti-insulin antibodies were analyzed by GFC on a Superdex 10/30 200 pre-packed column (Pharmacia, Sweden) eluted in pH 7.2 phosphate buffered saline (PBS) at 0.5 mL/min. Two hundred µL of serum were injected in each run. 50 µL of bovine serum albumin (BSA) at 10 g/L in phosphate buffer (10mM; pH7) were dispensed in plastic tubes before the collection of 0.5 mL elution fractions. The GFC column was calibrated with Gel Filtration Standard 750, 670, 158, 44, 17, 1.35 kDa (Bio-Rad N 151-1901, England). Elution fractions were then tested for the presence of insulin on the Beckman Coulter DxI and the Roche Modular platforms in order to establish an insulin immunoreactivity profile (Insulin concentrations of the patient and control serums read respectively 20.8 and 22.9 mIU/L on the DxI platform and 205.1 and 18 mIU/L on the Modular platform).

epitopes. The latter could be recognized as circulating insulin by antibodies used in serum insulin assays. Since the development of anti-insulin monoclonal antibodies and the introduction of 2-site IMAs, tests are now more specific [1]. Despite this advance, Modular Insulin assay presents a 24.7% cross reactivity rate for des 31,32 proinsulin [2]. However, such interference could not fully explain the value we obtained with the Modular Insulin assay. Besides, this test was described to be just as specific as 10 other assays, in a study performed by ADA [3]. Proinsulin was then measured in serum, yielding a normal value.

Analytical pitfalls in serum insulin measurement are more often reported in relation reactivity of pharmacological insulin analogs [2]. This point could be easily eliminated from the knowledge that the patient received no insulin treatment or dissimulated insulin injections.

Interferences due to anti-insulin Abs have been described both with competitive radioimmunoassays and IMAs. In our case, elevated values were found with the Modular assay and, albeit to a lesser extent, with the CIS bio insulin assay, whereas the value obtained with the DxI test was close to the upper reference value. In order to rule out a possible interference from heterophilic antibodies, the test was repeated, after pre-treatment using an Heterophilic Blocking Tube (tube containing murine immunoglobulin [Ig] directed against human IgM), but the same Modular insulin value was obtained with or without pre-treatment. We then screened for specific anti-insulin auto-Abs. They were present in the serum with a high positive value, explaining the importance of the interference with the Modular insulin test. As shown by GFC, most of the insulin was complexed with a 150 kDa molecule, corresponding to an immunoglobulin G (IgG). Elution fractions were then tested for the presence of insulin on the Beckman Coulter DxI and the Roche Modular platforms. The 150 kDa complex was detected with both methods, but the Modular insulin assay yielded a higher value. This was in accordance with values obtained on the whole serum with the Modular and DxI assays. It has been previously shown that anti-insulin Abs interference was more marked in the Modular assay than in the Bio-Rad assay (Bi-Insulin IRMA; Bio-Rad) [4], the former name of the current Bi-insulin CIS

bio assay. It could be explained by the mapping of Roche anti-insulin Abs [one, MAK-Bi, recognizes the A7–A10 portion of the A-chain; and the other, Fab-Ru recognizes the C-terminal part of the B-chain] that could recognize an anti-insulin/insulin complex made up by antibodies targeting the B-chain (*figure 2*).

From our data, we can confirm high levels of antibody-bound serum insulin in a fasting normoglycemic woman. As underlined by Ismail [5], insulin-Abs that are a cause of misleading results in immunoassay, may also generate deleterious glucose fluctuations. Anti-insulin autoimmunity is a common condition. Fineberg *et al.* [6] have shown that 88% of blood samples taken from healthy non-diabetic adults present anti-insulin antibodies. But, less frequently, the presence of large amounts of antibodies in patients who are not exposed to exogenous insulin can induce an insulin autoimmune syndrome (IAS), also called Hirata's disease [7]. In this syndrome, hypoglycemia can be intermittent and blood glucose levels frequently swing from hyper- to hypoglycemia.

We then requalified the patient's complaints as neuroglucopenic symptoms caused by insulin release from anti-insulin Abs. Indeed, insulin bound to auto-Abs in blood represents a stock that can release enough free/unbound active insulin to cause hypoglycemia. Its severity depends on the Ab affinity/avidity. The differential diagnosis with insulinoma can be made because of the high plasma insulin level associated with disproportionately low/normal C-peptide level. Circulating anti-insulin Abs delay insulin clearance and reverse the relative half-lives of plasma insulin and C-peptide. Of note, screenings for anti-GAD and anti-IA2 were negative, as it is described for IAS [8].

There are different specificities between IAS in Asians and Caucasians. IAS is the third leading cause of spontaneous hypoglycemia in Japan, while only rare cases have been reported in Europe and in the United States. Polyclonal anti-insulin Abs are more frequently found in East-Asians, whereas most of the previously described Caucasian IAS patients presented monoclonal Abs. Our patient presents a more likely Asian phenotype with polyclonal IgG anti-insulin. Most cases of IAS have been described in Asians with specific human leukocyte antigens class II. Japanese patients are more likely associated with human leukocyte antigen class II DRB1*0406, DQA1*0301 or DQB1*0302. Our patient presents a DRB1*0411 HLA class II genotype. This genotype has never been described for this pathology, but corresponds to a DR4 phenotype as shown for Caucasian and Japanese patients presenting polyclonal antibodies [9].

The physician comment

A 10-day pre- and postprandial glycemia follow-up was performed in our patient with a blood glucose meter but no hypoglycemia was detected. However, as shown previously, glycemia frequently did not rise after eating, and at the same time the patient felt a sensation of weakness and leg tremor. Brun *et al.* [10] have previously described a postprandial adrenergic syndrome, evocative of neurosis that may be difficult to diagnose because fasting glycemia, postprandial glycemia, and even oral glucose tolerance tests could be normal. Despite normal concentrations of blood glucose, such patients face non-specific symptoms caused by autonomic adrenergic counter-regulation. Finally, our patient was advised to fractionate her meals and to choose high-fiber foods and food with a moderate-to-low glycemic index. At the time of this report, the patient is doing well and always under regular medical follow-up. Although no formal evidence of the release of insulin from insulin-antibodies could be brought in concordance with hypoglycemia and the neurological symptoms in this case, IAS remains a very likely diagnosis. Indeed, most hypoglycemic episodes in IAS occur at night when insulin secretion and free insulin levels decrease, promoting the dissociation of insulin-antibody complexes. Continuous glucose recording could help identifying occult nocturnal episodes in such cases.

As a conclusion, unexpectedly high fasting serum insulin measurements deserve further specific investigations. In the present case, a rare IAS with polyclonal IgG anti-insulin Abs was disclosed owing to the interference that they caused in the Modular insulin assay.

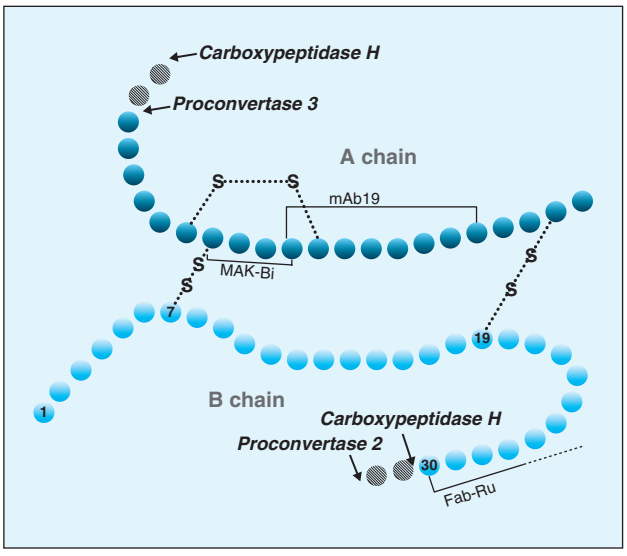

Figure 2. Insulin and mapping of Abs anti-insulin used in IMA's. Roche anti-insulin Abs: MAK-Bi, recognizes the A7-A10 portion of the A-chain; Fab-Ru recognizes the C-terminal part of the B-chain. Bi-Insulin IRMA Bio-Rad and insulin IRMA CIS bio assays use mAb19 targeting the A10-A17 portion of the A-chain.

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