The Effects of Anti-insulin Antibodies and Cross-reactivity with Human Recombinant Insulin Analogue in the E170 Insulin Immunometric Assay

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Background: Insulin assays are affected by varying degrees of interference from anti-insulin antibodies (IAs) and by cross-reactivity with recombinant insulin analogues. We evaluated the usefulness of the E170 insulin assay by assessing IA effects and cross-reactivity with 2 analogues. Methods: Sera were obtained from 59 type 2 diabetes patients receiving continuous subcutaneous insulin infusion and 18 healthy controls. Insulin levels were determined using an E170 analyzer. To investigate the effects of IAs, we performed IA radioimmunoassays, and analyzed the differences between directly measured insulin (direct insulin) and polyethylene glycol (PEG)-treated insulins (free, IA-unbound; total, IA-bound and unbound insulin). We performed in-vitro cross-reactivity tests with insulin aspart and insulin glulisine.

Results: In IA-positive patients, E170 free insulin levels measured using the E170 analyzer were significantly lower than the direct insulin levels. The mean value of the direct/free insulin ratio and IA-bound insulin, which were calculated as the difference between total and free insulin, increased significantly as endogenous IA levels increased. The E170 insulin assay showed low cross-reactivities with both analogues (< 0.7%).

Conclusions: IAs interfered with E170 insulin assay, and the extent of interference correlated with the IA levels, which may be attributable to the increase in IA-bound insulin, and not to an error in the assay. The E170 insulin assay may measure only endogenous insulin since cross-reactivity is low. Our results suggest that the measurement of free insulin after PEG pre-treatment could be useful for β cell function assessment in diabetic patients undergoing insulin therapy.

Key Words: Insulin, Insulin antibodies, Insulin immunoassay, Insulin analogues, Cross-reactivity

INTRODUCTION

Insulin assays are used for the diagnosis of hypoglycemia and may be useful in determining the pathogenesis of type 1 and 2 diabetes. However, the previously used radio-immunoassays have limitations, such as varying degrees of cross-reactivity with endogenous insulin precursors and recombinant insulin analogues, and interference from anti-insulin antibodies (IAs) [1]. These limitations have caused significant inter-assay variation and have led to the misinterpretation of laboratory results.

In the recent decades, insulin analogues have been developed through protein engineering, and the use of recombinant human insulin has markedly reduced the incidence of insulin resistance related to IAs, compared to that related to poorly purified animal insulin preparations. Therefore, interference from IAs is no longer thought to be serious. However, technological advancements in the mode of insulin administration, i.e., continuous subcutaneous insulin infusion (CSI), continuous peritoneal insulin infusion (CPII), and insulin inhalation, may increase the risk of the IA formation [2].

As recently reviewed by Sapin [3], commercially available insulin immunoassays still have similar limitations due to IAs. However, the studies themselves also had some limitations, many of which stem from a simple comparison between directly measured insulin (direct insulin) and IA-un-
bound insulin (free insulin) levels, and few studies have determined the exact cause of IA interference in the currently used automated insulin immunoassays.

We evaluated the usefulness of an insulin electro-chemiluminescence immunoassay by assessing the influences of IAs in targeting patients who were receiving intensive insulin therapy and by estimating cross-reactivity with 2 human recombinant insulin analogues. To evaluate the possible cause of influence from IAs, we compared total insulin levels (IAs-bound and unbound insulin) with direct and free insulin levels. We also compared the results of this assay with 2 other insulin immunoassays.

MATERIALS AND METHODS

1. Study subjects

Fasting blood samples from 59 type 2 diabetes patients receiving CSII therapy while attending Konkuk University Hospital diabetes out-patient clinics in Seoul between May and September 2008 were enrolled. To exclude the possible influence of the disease itself on preprocessing for IA precipitation and to determine whether healthy people have IAs, 18 healthy control patients attending the medical health examination center at the same medical institution were enrolled during the identical time. The diabetes group included 36 men and 23 women and the mean age was 57.4 ± 10.0 yr (range, 36-81). The healthy control group included 16 men and 2 women and the mean age was 39.8 ± 9.1 yr (range, 19-56). The patients’ medical records were reviewed, not only for the results of initially requested laboratory tests, such as serum insulin, C-peptide, fasting glucose, and glycated hemoglobin (HbA1c) levels, but also for the insulin therapy type they had used and the duration of CSII treatment. Serum insulin and C-peptide levels were determined using a MODULAR analytics E170 module (Roche Diagnostics, Mannheim, Germany) and fasting glucose and plasma HbA1c were determined using a TBA 200FR Neo system (Toshiba Medical Systems Co., Ltd., Tokyo, Japan), and a HLC-723 G7 analyzer (Tosoh Corporation, Tokyo, Japan), respectively. The Institutional Review Board at the University of Konkuk, Seoul, Korea, approved this study.

2. Direct insulin

To distinguish the measurement of direct insulin from insulin pre-treated with polyethylene glycol (PEG) (free and total insulin), direct insulin was defined as insulin levels determined using the E170 module in fasting blood samples without preprocessing.

3. Free and total insulins

Free insulin (IA-unbound) was determined using the E170 insulin assay after precipitation of immune complexes with a 50% PEG 6000 solution [4]. The PEG solution was mixed with the same amount of serum sample, and then centrifuged at 3,000 g for 15 min at 4°C. Total insulin (bound and unbound) was determined using the procedure described by Kuzuya et al. [5], which includes the addition of HCl solution before PEG precipitation to separate insulin molecules from IAs. Then, only the separated IAs were precipitated by PEG treatment, finally NaOH was added for neutralization. Ultimately, both the free and total insulin levels were calculated using the dilution rates from the measured direct insulin levels of each supernatant.

4. Anti-insulin antibody

To investigate the interference from IAs on insulin level measurement, we determined the free IA levels (not combined with insulin molecules) for all subjects using radio-immunometric assays (anti-insulin IRMA, CIS Bio International, Areva S.A., France). The positive threshold was set at 5.4%, as per the manufacturer’s instructions. IA-positive patients were divided into 3 groups using arbitrarily determined criteria based on IA levels (Group 1, 5.4%<IA≤20.0%, N = 21; Group 2, 20.0%<IA≤40.0%, N = 16; Group 3, IA>40.0%, N = 14).

5. Comparison of three insulin immunoassays

Three insulin concentration measurement methods were compared. We used the E170 analyzer, the Advia Centaur Immunoassay System (Simens, Erlangen, Germany), and a manual INS-IRMA assay (BioSource, Nivelles, Belgium) for 38 samples meeting the condition of sufficient residual volume among the 59 patients in the diabetes group. All the insulin assays were calibrated against the WHO 66/304 reference. After the E170 insulin assay, the remaining sample was stored at 4°C and analyzed using the other methods within 24 hr, without any pre-treatment. Directly measured insulin levels from each method (E170, Advia Centaur, and IRMA) were comparatively analyzed.

6. Analysis of cross-reactivities in E170 insulin assay

We obtained vials of 2 insulin analogues, insulin aspart (NovoRapid, Novo Nordisk, Bagsvaerd, Denmark) and insulin glulisine (Apidra, Sanofi-Aventis, Paris, France), which are suitable for injection and with which our diabetes patients were most frequently treated. We serially diluted the analogues with 7% aqueous bovine serum albumin (BSA) to final concentrations of 30, 100, 300, and 1,000 mIU/L.
The insulin levels of all dilutions were measured by E170 insulin assay, and each sample was analyzed in duplicate. In-vitro cross-reactivity (%) with these 2 insulin analogues was calculated from the ratio of the measured and nominal concentrations.

7. Statistical analysis

Data were analyzed with the Predict Analytics Software program version 17.0 (PASW, formerly Statistical Package for the Social Sciences, SPSS, SPSS Inc., Chicago, IL, USA). The One-Sample Kolmogorov-Smirnov Goodness-of-Fit Test was used to assess the distribution of all variables, which included E170 direct, total and free insulin, IRMA and Advia Centaur direct insulin, C-peptide, glucose, HbA1C, and CSII duration. Differences between E170 direct and free insulin, and differences between E170 direct and total insulin were determined by using Wilcoxon matched-pair signed-rank tests. Furthermore, differences among the E170 direct, total and free insulin levels and differences among the E170, IRMA, and Advia Centaur direct insulin levels within each group were determined by using Friedman two-way ANOVA by ranks. Differences of each variable between healthy control and diabetes mellitus (DM) patients were assessed by using Mann-Whitney U or Student’s t tests. Differences among the 5 groups (healthy control, IA negative DM group and IA positive group 1-3) were determined using Kruskal-Wallis H or one-way ANOVA followed by post hoc test with Bonferroni correction. The Pearson's correlation and stepwise multiple linear regression analysis were used to find a correlation between IA and total insulin with other variables (all, P < 0.005, Table 2). The differences in C-peptide and fasting glucose levels among the 4 DM groups and total the 5 groups were statistically significant (all, P < 0.001 by Kruskal-Wallis H test). The differences in C-peptide and fasting glucose levels among the 5 groups were significant (P = 0.031 and 0.003, respectively by one-way ANOVA), but not among the 4 DM groups (P = 0.053 and 0.183, respectively). Fasting glucose levels were significantly higher in the IA negative group than in all of the IA positive patients (P = 0.046 by Student's t test). The differences in HbA1C and CSII duration were not statistically significant among the 5 groups (P = 0.330 and 0.146, respectively), however, CSII duration was significantly shorter in the IA negative group than in all of the IA positive patients (10.2 ± 16.6 vs. 26.1 ± 15.8, P = 0.027). Differences among E170 direct, total and free insulin levels were assessed within each of the 4 DM groups (Fig. 1), and were significant only

### Table 1. Clinical characteristics of 59 diabetes patients and 18 healthy controls

| Variables       | DM patients (N = 59) | Healthy control (N = 18) | P value* |
|-----------------|----------------------|--------------------------|---------|
| Age (yr)        | 57.4 ± 10.0          | 39.8 ± 9.1               | < 0.001 |
| Sex, male/female| 36 (61.0%) / 23 (39.0%) | 16 (88.9%) / 2 (11.1%)   | 0.027   |
| Fasting insulin* (mIU/L) | 42.3 ± 64.6 | 5.2 ± 2.5               | < 0.001 |
| IA (%)          | 25.5 ± 19.8          | 4.7 ± 0.3                | < 0.001 |
| C-peptide (mg/mL) | 2.5 ± 1.2          | 2.0 ± 0.6                | 0.167   |
| Fasting glucose (mg/dL) | 172.5 ± 52.1 | 92.1 ± 23.5             | < 0.001 |
| HbA1C (%)       | 6.9 ± 1.2            | 5.7 ± 0.8                | < 0.001 |
| CSII duration (month) | 24.1 ± 16.6 |                        |         |

Values are presented as the mean ± standard deviation or number of patients (%). *DM patients are type 2 diabetes mellitus patients on continuous subcutaneous insulin infusion therapy; † values were determined using Mann-Whitney U or Student’s t-test; ‡Fasting insulin was directly measured using the E170 insulin assay without any pre-treatment.

**RESULTS**

1. Comparison of diabetes patients with healthy controls

All of the 18 healthy controls and 8 of the 59 diabetes patients were IA negative (range, 4.25-5.36%). The average of CSII durations in diabetic patients was 24.1 ± 16.6 months (range, 0-84 months). Fasting insulin levels measured using the E170 insulin assay (E170 direct insulin), fasting glucose levels, and the percentage of IA and HbA1C were significantly higher in diabetes patients than in healthy controls (E170 direct insulin and IA, P < 0.001 by Mann-Whitney U-test; fasting glucose and HbA1C: P < 0.001 by Student’s t-test). The difference in C-peptide levels between diabetes patients and healthy controls was not statistically significant (P = 0.167 by Student’s t-test) (Table 1).
Values are presented as mean ± SD.

Table 2. Data from 59 diabetes patients categorized according to anti-insulin antibody levels and 18 healthy controls

| IA negative* | Group 1† (5.4% < IA ≤ 20.0%) | Group 2‡ (20.0% < IA ≤ 40.0%) | Group 3** (IA > 40.0%) | P value†† |
|--------------|-----------------------------|-------------------------------|------------------------|-----------|
| E170 direct insulin (mIU/L) | 5.3 ± 2.5 | 6.3 ± 1.7 | 11.2 ± 4.4 | 25.1 ± 18.9 | 128.9 ± 85.4 | < 0.001 |
| E170 free insulin (mIU/L) | 4.3 ± 2.3 | 2.9 ± 3.0 | 6.4 ± 5.3 | 9.7 ± 7.6 | 28.8 ± 21.0 | < 0.001 |
| IA-bounded insulin‡‡ (mIU/L) | 4.6 ± 2.3 | 4.1 ± 2.9 | 9.3 ± 6.3 | 24.4 ± 21.7 | 114.1 ± 64.7 | < 0.001 |
| Direct/free insulin ratio | 1.3 ± 0.3 | 2.3 ± 3.4 | 2.3 ± 2.4 | 3.0 ± 1.5 | 4.7 ± 1.8 | < 0.001 |
| C-peptide (mg/mL) | 2.0 ± 0.6 | 2.1 ± 1.5 | 2.3 ± 0.8 | 2.3 ± 1.1 | 3.2 ± 1.3 | 0.031 |
| Fasting glucose (mg/dL) | 92.1 ± 23.5 | 206.5 ± 62.9 | 163.7 ± 49.3 | 161.8 ± 31.5 | 178.4 ± 64.0 | 0.003 |
| HbA1C (%) | 5.7 ± 0.6 | 2.1 ± 1.5 | 2.3 ± 0.8 | 2.3 ± 1.1 | 3.2 ± 1.3 | 0.030 |
| CSII duration (month) | 10.2 ± 16.6 | 27.8 ± 19.2 | 24.7 ± 13.5 | 25.1 ± 13.7 | 0.146 |

Values are presented as mean ± SD.

*IA negative is anti-insulin antibody negative (IA ≤ 5.4%); †IA positive is anti-insulin antibody positive (IA > 5.4%); ‡Groups 1–3 were composed of IA positive patients, which were divided into 3 groups according to IA levels, and the criteria for classification were arbitrarily determined; ††P values were determined by using one-way ANOVA followed post hoc test with Bonferroni correction or Kruskal-Wallis H tests, which represents the differences in each variable among the 5 groups (Healthy control, IA negative DM group, and IA positive groups 1-3); ‡‡E170 direct insulin levels were directly measured; ††E170 free insulin levels were measured after PEG precipitation, which denoted IA-unbound insulin; **P values were determined using Wilcoxon signed rank test, which represents the difference between E170 direct and free insulin levels within each group; ††E170 total insulin levels were measured after addition of HCl (to separate insulin molecules from IAs) and PEG precipitation, which included IA-bound and unbound insulin; all of these were measured using the E170 insulin assay; ‡‡IA-bounded insulin levels were calculated from the difference between the E170 total and free insulin levels.

Abbreviations: IA, anti-insulin antibody; DM, diabetes mellitus; CSII, continuous subcutaneous insulin infusion therapy.

Fig. 1. The tendency of E170 direct, total and free insulin levels and fasting glucose level toward the higher anti-insulin antibody level group. The bars are in order of direct, total, and free insulin determined by the E170 insulin assay. The line graph shows the mean level of fasting glucose. Severe insulin resistance appeared in the IA positive DM group 3 (IA > 40.0%).

*IA(−) DM is the anti-insulin antibody negative group of diabetes patients; †groups 1-3 are groups with anti-insulin antibody positive diabetes patients, which were classified according to IA levels; ††P values were determined by using Friedman test.

Abbreviations: IA, anti-insulin antibody; DM, diabetes mellitus.

3. The relationship between IA and other variables and predictors of E170 total insulin

To identify the factors related to IA, for all subjects, Pearson’s correlation was conducted between IA levels and other variables (E170 direct, total, and free insulins, E170 direct/free insulin ratio, IA-bound insulin calculated from the difference between total and free insulin levels, C-peptide, fasting glucose, HbA1C, and the duration of CSII treatment). Among these variables, IA-bound insulin, E170 total, direct, and free insulin, E170 direct/free insulin ratio, C-peptide, and fasting glucose (in order of correlation coefficients) were correlated with IA levels (Table 3).

From the stepwise multiple linear regression analysis, E170 direct insulin (β = 0.825, P < 0.001) and IA level (β = 0.189, P < 0.001) were selected as independent predictive factors among the other variables (E170 total and free insulins, E170 direct/free insulin ration, IA-bound insulin, C-peptide, fasting glucose, HbA1C, CSII duration and IA), which influence E170 total insulin levels (Table 4). E170 direct insulin was
Comparison of three insulin immunoassays  

The differences among the direct insulin levels measured by 3 different methods were significant ($P=0.032$ by Friedman test). The average E170 direct insulin level from the 38 comparable patients was $41.4 \pm 65.7$ mIU/L, and the average of Advia Centaur and IRMA direct insulin levels were $19.9 \pm 27.5$ and $12.2 \pm 6.9$ mIU/L, respectively. E170 direct insulin was more correlated with Advia Centaur insulin than IRMA ($R^2 = 0.763$ vs. $R^2 = 0.415$, $P < 0.001$ by simple linear regression analysis). Advia Centaur direct insulin levels was more correlated with E170 direct insulin than E170 total and free insulin ($R^2 = 0.763$ vs. 0.759 and, 0.663, respectively, $P < 0.001$), while IRMA direct insulin was most correlated with E170 direct insulin and total insulin ($R^2 = 0.520$ vs. 0.415 and 0.382, respectively, $P < 0.001$).

Analysis of cross-reactivity with insulin analogues in the E170 insulin assay  

The measured concentration and calculated cross-reactivity of each stratified diluted insulin analogues is summarized in Table 5. The E170 insulin assay had a low cross-reactivity of <0.7% for both insulin analogues even at the highest concentrations tested. could detect human recombinant insulin analogues, in order to determine the usefulness of the E170 insulin assay.

Previous studies have reported that the measurement of serum insulin concentration in patients treated with insulin may lead to unreliable results because IAs interfere with insulin both in 1 or 2-site radioimmunometric assay, and the recently used automated immunometric assays [3, 6-9]. The free insulin fraction is generally regarded as the biologically active form, and the overestimation of insulin concentration compared to free insulin may lead to the misinterpretation of a patient’s status. Therefore, there was a consensus that endogenous antibodies should be removed before the assay. This has been achieved by precipitation with PEG, or ethanol, and by gel filtration [10]. However, the overestimation of serum insulin concentration has not been determined in all currently available insulin assays; thus the extent and underlying causes of overestimation in each assay are not yet clear. Furthermore, it is generally accepted that human recombinant insulin analogues currently in use result in very little endogenous antibody formation compared to animal insulin, so the importance of preprocessing to eliminate antibodies before insulin measurement has greatly diminished over the last 2 decades.

Large, interventional studies in diabetes patients have shown that tight blood glucose control with intensive insu-
The presence of surfactant or polyethylene glycol [12, 13].

Our study suggests that the E170 insulin assay accompanied by PEG treatment can assess residual β-cell function in diabetic patients receiving insulin therapy. Moreover, directly measured insulin concentrations using the E170 insulin assay may also provide information about immunogenic insulin resistance, a condition in which the body produces insulin but does not use it properly, thus in spite of a high insulin concentration, glycemic control seriously deteriorates. Insulin resistance is an intra-cellular phenomenon in metabolic syndrome or pre-diabetes stage of type 2 diabetes mellitus [19]. However, immunogenic insulin resistance is due to circulating IAs, and a long time ago, it was suggested that the difference between immunogenic insulin resistance and nonresistance in insulin-treated patients depends primarily on the concentration of insulin-binding antibody [20]. High circulating levels of IAs may interfere with insulin pharmacokinetics, leading to worse hyperglycemic control as well as unexplained hypoglycemia, which are attributed to the ‘dumping’ of insulin from its antibody reservoir. In our results, IA levels were correlated with total insulin (Table 3), and E170 direct insulin was nearly concordant with total insulin. Therefore, if the insulin concentration measured using the E170 insulin assay was unreliably high, and if the fasting glucose also high or unexpectedly low, physicians could surmise that it was due to the formation of IAs without determining total insulin.

From our correlation results (Table 3), IA levels were not related to CSII duration, but the mean CSII duration of IA negative DM patients was significantly shorter than that of IA positive DM patients (groups 1-3). Therefore, IAs might be produced during insulin therapy. It has been suggested that immunogenic insulin resistance occurs more frequently in patients with type 2 diabetes [21]. The duration of insulin therapy before the onset of severe insulin resistance has been reported to be from 1 month to 15 yr, and in 50-85% of patients, it occurred after less than 1 yr [22, 23]. Therefore, physicians should perform insulin level and fasting glucose tests more frequently, especially during intensive insulin therapy, because severe insulin resistance is often associated with fasting hypoglycemia and both symptomatic hyperglycemia, including episodes of ketoacidosis and hyperosmolar coma. However, clinicians should be also aware of the
cross-reactivity of various insulin analogues with the assay used in an individual clinical laboratory because, if their insulin assays detect only endogenous insulin, they will not be able to assess patient compliance or titrate insulin dosage.

Our study has a number of important limitations that need to be considered. First, there were significant differences between direct and free insulin levels in IA negative groups (healthy controls and IA negative DM patients). One possible explanation is the minimal precipitation of free insulin by PEG treatment [24]; another is the unavoidable delays in the time between the venipuncture and PEG treatment because we targeted patients from whom we had requested insulin measurement. Previous studies have found that the measurements change over time [10, 24], and it might be due to an insulin-degrading enzyme. The other possibility is low sensitivity caused by sample dilution, especially at low free insulin concentrations [25]. Second, we measured free and total insulin levels only by the E170 insulin assay. Further experimental investigations are needed to understand the characteristics of each method, and it could be helpful in the standardization of insulin assays.

In conclusion, we evaluated the effects of IAs and cross-reactivity with 2 human recombinant insulin analogues in the E170 insulin immunoassay. Directly measured insulin using the E170 assay was overestimated compared to free insulin, thus we confirmed that IAs interfered with the E170 insulin assay. Both E170 direct/free ratio and IA-bound insulin were correlated with IA levels. Therefore interference may result from the increase of IA-bound insulin, not from an assay error from IAs. The E170 insulin assay is thought to measure only human endogenous insulin because of low cross-reactivity with recombinant insulin analogues. It could be made available for diabetes patients receiving insulin therapy to assess β-cell function, via the determination of free insulin with PEG pre-treatment.

Authors’ Disclosures of Potential Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Sapin R. Insulin immunoassays: fast approaching 50 years of existence and still calling for standardization. Clin Chem 2007;53:810-2.
2. Radermecker RP, Renard E, Scheen AJ. Circulating insulin antibodies: influence of continuous subcutaneous or intraperitoneal insulin infusion, and impact on glucose control. Diabetes Metab Res Rev 2009;25:491-501.
3. Sapin R. Insulin assays: previously known and new analytical features. Clin Lab 2003;49:113-21.
4. Walker JM, ed. The protein protocols handbook. 2nd ed. New Jersey: Humana Press, 2002:991-2.
5. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH. Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1977;26:22-9.
6. Gennaro WD and Van Norman JD. Quantification of free, total, and antibody-bound insulin in insulin-treated diabetics. Clin Chem 1975;21:873-9.
7. Armitage M, Wilkin T, Wood P, Casey C, Loveless R. Insulin autoantibodies and insulin assay. Diabetes 1988;37:1392-6.
8. Sapin R. Anti-insulin antibodies in insulin immunometric assays: a still possible pitfall. Eur J Clin Chem Clin Biochem 1997;35:365-7.
9. Sapin R. The interference of insulin antibodies in insulin immunometric assays. Clin Chem Lab Med 2002;40:705-8.
10. Hanning I, Home PD, Alberti KG. Measurement of free insulin concentrations: the influence of the timing of extraction of insulin antibodies. Diabetologia 1985;28:831-5.
11. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
12. Jeandidier N, Bovin S, Sapin R, Rosart-Ortega F, Uring-Lambert B, Réville P, et al. Immunogenicity of intraperitoneal insulin infusion using programmable implantable devices. Diabetologia 1995;38:577-84.
13. Fineberg SE, Kawabata TT, Finco-Kent D, Fountaine RJ, Finch GL, Krasner AS. Immunological responses to exogenous insulin. Endocr Rev 2007;28:625-52.
14. Owen WE and Roberts WL. Cross-reactivity of three recombinant insulin analogues with five commercial insulin immunoassays. Clin Chem 2004;50:257-9.
15. Ibrahim F, Bagnard G, Boudou P. Differences in circulating insulin levels following glargine administration. Clin Biochem 2008;41:429-31.
16. Becker RH. Insulin glulisine complementing basal insulins: a review of structure and activity. Diabetes Technol Ther 2007;9:109-21.
17. De Meyts P. Insulin and its receptor: structure, function and evolution. Bioessays 2004;26:1351-62.
18. Devendra D, Galloway TS, Horton SJ, Evenden A, Keller U, Wilkin TJ. The use of phage display to distinguish insulin autoantibody (IAA) from insulin antibody (IA) idiotypes. Diabetologia 2003;46:802-9.
19. Kim HR, Lee MK, Park AI. The -308 and -238 polymorphisms of the TNF-α promoter gene in type 2 diabetes mellitus. Korean J Lab Med 2006;26:58-63.
20. Berson SA and Yalow RS. Quantitative aspects of the reaction between insulin and insulin-binding antibody. J Clin Invest 1959;38:1996-2016.
21. Kahn CR and Rosenthal AS. Immunologic reactions to insulin: insulin allergy, insulin resistance, and the autoimmune insulin syndrome. Diabetes Care 1979;2:283-95.
22. Grunfeld C. Insulin resistance: pathophysiology, diagnosis, and therapeutic implications. Spec Top Endocrinol Metab 1984;6:193-240.
23. Davidson JK and DeBra DW. Immunologic insulin resistance. Diabetes 1978;27:307-18.
24. Arnqvist H, Olsson PO, von Schenck H. Free and total insulin as determined after precipitation with polyethylene glycol: analytical characteristics and effects of sample handling and storage. Clin Chem 1987;33:93-6.
25. Peluso I, Dean B, Harrison L.C. Separation of free and antibody-bound insulin in plasma using a bench ultracentrifuge (Beckman ‘Airfuge’). Clin Chim Acta 1984;139:317-20.