Evaluation of Ischemia-Modified Albumin and C-Reactive Protein in Type 2 Diabetics With and Without Ketosis

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Abstract

Overview: To investigate whether serum ischemia-modified albumin or C-reactive protein is reliable for predicting type 2 diabetic patients with ketosis.

Approach: One hundred and four diabetic patients, 48 with diabetic ketosis, and 33 controls were enrolled in the study. Serum ischemia-modified albumin and C-reactive protein were measured and evaluated for their ability to distinguish diabetic ketosis.

Results: Compared to the controls, the ischemia-modified albumin and C-reactive protein levels were higher in patients with diabetic ketosis and type 2 diabetes at the baseline. The levels of ischemia-modified albumin were higher in patients with type 2 diabetes than in the controls. C-reactive protein and ischemia-modified albumin levels were reduced after insulin treatment. The level of ischemia-modified albumin was an independent risk marker for diabetic ketosis ($\text{OR} = 1.085$, $P = 0.008$, 95% CI: 1.022–1.152). Receiver operating characteristic curves revealed that the areas under the curve were 0.917 for the modified albumin and 0.357 for C-reactive protein.

Conclusion: This study indicates that ischemia-modified albumin was significantly associated with diabetic ketosis and was more sensitive than C-reactive protein in reflecting diabetic ketosis.

Keywords: diabetic ketosis, ischemia-modified albumin, C-reactive protein, biomarker
Introduction

Ischemia-modified albumin (IMA) is a promising biomarker for evaluating patients with ischemic events. In many diseases that are accompanied by ischemia, the serum levels of IMA rise.\(^1,^2\) The precise mechanism of how IMA is produced is not known but appears to be related to the production of reactive oxygen species that modify the metal binding sites.\(^2,^3\) Data exist suggesting that IMA is not specific for cardiac ischemia and is also elevated in patients with liver cirrhosis,\(^4\) pulmonary embolism,\(^5\) end-stage renal disease,\(^6\) and cerebrovascular diseases.\(^7,^8\)

Oxidative stress has been implicated in the development of chronic complications related to diabetes mellitus.\(^9\) Some previous studies have reported that compared to healthy controls, patients with type 2 diabetes (T2D) and chronic microvascular complications have higher serum levels of IMA.\(^10–^12\)

To our knowledge, there are no data regarding the levels of IMA in patients with type 2 diabetic ketosis (T2DK) without ketoacidosis (ie, diabetic ketonuria). T2DK, as an acute complication of diabetes mellitus, was accompanied by significant hyperglycemia and most likely reflects relative insulinopenia. Only 23% of the episodes of ketonuria were acknowledged in the progress notes.\(^13\) Ketonuria occurs more frequently than ketoacidosis in patients with T2D and may be portentous of serious future events.\(^14\)

C-reactive protein (CRP) is a type I acute phase response protein that is synthesized in the liver.\(^15\) CRP has been established as a marker for diabetic ketoacidosis.\(^16\) Both chronic and acute complications related to diabetes can lead to the potent production of free radicals and pro-inflammatory cytokines.\(^9,^17\) However, it is unknown if there are differences regarding IMA or CRP in patients with T2DK and T2D. Importantly, is the IMA or CRP better at predicting T2DK?

In the present study, we examined IMA and CRP levels in T2D subjects with and without ketosis. We also assessed the association between the serum IMA and several metabolic parameters affecting the subjects.

Methods

Study design and exclusion criteria

This controlled study was performed at the Department of Endocrinology and Metabolism, the affiliated Huai’an hospital of Xuzhou Medical College from April 2010 to July 2011. The study was approved by the ethics committee of the hospital. Informed consent was obtained from all of the participants.

Fifty-six consecutive adult subjects with T2D, 48 T2DK subjects with diabetic ketosis and 33 control subjects (Table 1) participated in the study. Patients with T2DK, who were negative for the glutamic acid decarboxylase autoantibody, were treated with oral hypoglycemic agents, insulin, or diet control. Diabetic ketosis was defined as having a blood glucose > 11.1 mmol/L, moderate to large (++++) urine ketones, and a normal blood pH range (7.35–7.45). The exclusion criteria included: pregnancy, acute alcohol intoxication, type 1 diabetes, or T2D with a history of ischemic events. Patients with hepatic, renal, or cardiac insufficiency were also excluded. Also excluded were patients with signs of secondary inflammatory conditions, electrocardiogram abnormalities at the time of admission or those who had a previous corticosteroid treatment. The control subjects, who had no clinical evidence of a major disease, were recruited from an unselected population that underwent a routine medical check-up.

All T2DK subjects received medical nutrition therapy and 4 times daily insulin injections of human insulin. The outcome was followed up until the ketonuria was eliminated.

Biochemical analyses

The body mass index was calculated as the body weight in kilograms divided by height in meters squared. Blood samples were drawn from T2D and control subjects and dispensed into vacutainer tubes by venipuncture in a fasting state (10 hours). In patients with T2DK, the blood samples were drawn (a) at the time of presentation, before the initial therapy and (b) 120 hours after the administration of fluids and insulin.

The blood samples were centrifuged and stored at −20 °C for a maximum of four weeks before measuring for IMA. The levels of serum IMA were measured using a commercial kit (Co-Health Laboratories Co., Ltd., Beijing, China) on an Olympus AU 2700 autoanalyzer (Olympus, Tokyo, Japan). The levels of serum CRP was assayed with the immunonephelometric method (Dade Behring Marburg, Marburg, Germany). The reference concentrations for IMA and CRP were <77.6 U/mL.
Ischemia-modified albumin in diabetic ketosis

Table 1. Clinical and biochemical characteristics of the study participants.

| Variable                                      | Control (n = 33) | T2D (n = 56) | T2DK (n = 48) |
|-----------------------------------------------|------------------|--------------|---------------|
| Female/Male (n)                               | 10/23            | 20/36        | 12/36         |
| Age (years)                                   | 52.45 ± 11.66    | 52.78 ± 9.72 | 53.98 ± 11.67 |
| Systolic blood pressure (mmHg)                | 144 ± 15         | 145 ± 18     | 145 ± 15      |
| Diastolic blood pressure (mmHg)               | 89 ± 11          | 88 ± 10      | 89 ± 10       |
| Body mass index (kg/m²)                       | 24.23 ± 2.91     | 25.58 ± 2.10 | 25.78 ± 3.06  |
| Fasting plasma glucose (mmol/L)               | 5.07 ± 0.74      | 9.45 ± 2.19** | 16.36 ± 3.21** |§§ |
| 2-hour post plasma glucose (mmol/L)           | 6.12 ± 1.02      | 14.23 ± 3.51** | 19.22 ± 5.62** |§§ |
| Glycated hemoglobin A1c (%)                   | 5.94 ± 0.29      | 9.64 ± 2.55** | 10.76 ± 3.40** |§§ |
| Serum uric acid (μmol/L)                      | 307.23 ± 76.92   | 280.93 ± 72.08 | 285.85 ± 61.09 |
| Serum creatinine (μmol/L)                     | 73.50 ± 14.06    | 75.56 ± 11.42 | 74.92 ± 13.36 |
| Serum albumin (g/L)                           | 42.02 ± 3.14     | 42.08 ± 3.96  | 40.51 ± 4.56  |
| Total cholesterol (mmol/L)                    | 4.47 ± 1.01      | 4.86 ± 1.13  | 4.80 ± 1.47   |
| Triglycerides (mmol/L)                        | 1.32             | 2.33*         | 1.92          |
| High-density lipoprotein cholesterol (mmol/L) | 1.19 ± 0.32      | 1.21 ± 0.35  | 1.08 ± 0.24   |
| Low-density lipoprotein cholesterol (mmol/L)  | 2.84 ± 0.86      | 2.81 ± 0.64  | 2.78 ± 0.66   |
| C-reactive protein (mg/L)                     | 2.31 ± 0.65      | 4.21 ± 1.21*  | 6.15 ± 1.81** |§§ |
| Ischemia-modified albumin (U/mL)              | 46.31 ± 11.42    | 61.47 ± 10.93** | 78.15 ± 15.39** |§§ |

Notes: Comparison to control group: *P < 0.05, **P < 0.001; comparison to T2D group: †P < 0.05, ‡P < 0.001. All values in the table are given as the mean ± standard deviation, except for the triglyceride values, which are given as the median and the range (min–max).

Statistical analyses

The quantitative data were presented as the mean ± standard deviation. Statistical analyses were conducted with the SPSS 11.0 package (SPSS Inc., Chicago, IL) for Windows. A comparative analysis among the three groups was carried out using the Student Newman-Keuls ANOVA. The Chi-squared test was used to compare other clinical features. The paired t-test was used to determine the significance of the changes in the patients with T2DK. The correlation between the IMA and CRP levels and other parameters was examined in all diabetic patients using Pearson correlation analysis. Multiple linear regression analyses were used to estimate the factors affecting the IMA levels. The risk markers for T2DK were examined by multiple logistic analyses. To determine the optimal cutoff values and the diagnostic performance of these variables, a receiver operating characteristic (ROC) curve analysis was performed. The area under the curve (AUC) was also used to determine the ability of IMA levels to diagnose T2DK. A two-tailed P < 0.05 was considered statistically significant.

Results

Clinical characteristics

The baseline clinical characteristics of the subjects are shown in Table 1. A total of 137 cases were included in the study. The fasting plasma glucose (FPG), 2-hour post plasma glucose (2hPG), and HbA1c were significantly higher in the subjects with T2D and T2DK than in the control group (P < 0.001 or P < 0.05). There were no significant differences among the three groups with respect to age, gender, systolic blood pressure, diastolic blood pressure, body mass index, serum albumin, serum uric acid, serum creatinine, and lipids profile, except for triglycerides.

IMA and CRP levels

The mean CRP levels were 2.31 ± 0.65 mg/L in the control group, 4.21 ± 1.21 mg/L in the T2D group, and 6.15 ± 1.81 mg/L in the T2DK group. The differences in CRP levels among the three groups were statistically significant (P < 0.05). In the group comparisons, there was a statistical difference between the T2DK and T2D groups (P < 0.001), the T2DK and control
groups \((P < 0.001)\), and the T2D and control groups \((P < 0.05)\). These results are shown in Figure 1.

The mean levels of serum IMA were \(46.31 \pm 11.42\) U/mL in the control group, \(61.47 \pm 10.93\) U/mL in the T2D group, and \(78.15 \pm 15.39\) U/mL in the T2DK group. The differences among the three groups were statistically significant \((P < 0.001)\). As shown in Figure 2, serum IMA levels were significantly increased in patients with T2D and T2DK compared to the controls \((P < 0.001)\) and were higher in patients with T2DK than T2D \((P < 0.001)\). One hundred and twenty hours after the treatment, the levels of CRP, FPG, 2hPG, and IMA were reduced in the T2DK subjects \((4.56 \pm 1.28 mg/L, 7.56 \pm 2.30 mmol/L, 9.75 \pm 4.12 mmol/L,\) and \(56.87 \pm 12.44 U/mL,\) respectively) compared to the baseline \((P = 0.035, P < 0.001, P < 0.001, P = 0.01,\) respectively).

Correlation and regression analyses

Bivariate correlation analyses were performed to assess the relationships between the baseline serum IMA concentrations and the metabolic parameters. There was no significant correlation between the levels of IMA and CRP \((P = 0.805)\) or between the level of IMA and other variables \((all P > 0.05)\). In addition, there was no correlation between the levels of CRP and all the metabolic variables \((all P > 0.05)\) for all the diabetic cases. A multiple regression analysis showed that diabetic ketosis was independent of the factors influencing the levels of serum IMA and CRP \((\beta = 0.743, P < 0.001; \beta = 0.453, P < 0.05,\) respectively) at the baseline. To examine the risk markers for T2DK, a multiple logistic regression analysis of the three significant clinical variables \((CRP, FPG, 2hPG,\) and IMA\) was performed. The odds ratios and 95% confidence intervals (CI) were calculated. The differences for CRP and IMA were statistically significant \((OR = 0.860, P = 0.017, 95% CI 0.760–0.973; OR = 1.085, P = 0.008, 95% CI 1.022–1.152,\) respectively) \((Table 2)\). Our analysis shows that the IMA may be more appropriate as a risk marker for T2DK.

ROC analyses

ROC analyses were used to identify the optimal serum IMA and CRP cutoff values for predicting T2DK \((Fig. 3)\). We observed a statistically significantly higher AUC for the IMA \((0.917 \pm 0.031)\) in comparison with CRP \((0.357 \pm 0.060)\) \((P < 0.05)\). The optimum diagnostic cutoff for IMA that maximally increased sensitivity and specificity in the estimation of T2DK was \(72.4\) U/L \((87.0\% and 85.7\%,\) respectively). This point was calculated as \(5.81\) mg/L for CRP \((57.0\% and 65.7\%,\) respectively).

Figure 1. The mean CRP levels in the control, T2D, and T2DK groups.

Notes: The statistical significances between the groups are indicated as follows: comparison to control group: \(*P < 0.05, **P < 0.001;\) comparison to T2D group: \(§§P < 0.001).\)
This indicates that compared to CRP, IMA has a higher diagnostic potential.

**Discussion**

The major finding of this study is that compared to patients with T2D and control subjects, the levels of IMA were significantly elevated in patients with T2DK. Diabetic ketosis was the significant factor influencing serum IMA levels in the participants. Insulin treatment had a favorable effect on glycemic control, IMA and CRP levels. IMA was a risk marker for T2DK and more sensitive than CRP in distinguishing T2DK.

In recent years, researchers have focused their attention on the pathological role of chronic inflammatory and free radicals in T2D. Existing data that show that before the complications of diabetes become clinically evident, hyperglycemia-induced oxidative stress occurs. IMA was a biochemical evaluation based on serum albumin binding to cobalt. IMA is not tissue specific and is elevated in subjects who undergo oxidative stress other than cardiac ischemia. There are concerns about tissue-specificity of IMA, as it has been suggested that IMA is a biomarker for other oxidative stress or ischemia-related diseases. The possible role of IMA was confirmed in previous studies. In the present study, the T2DK patients had significantly poorer glycemic control, which was associated with an increase in inflammatory and oxidative stress biomarkers. Higher levels of IMA and CRP were detected in patients with T2D and T2DK. Hyperglycemia and inflammation reduces the

**Table 2.** Multiple logistic regression analysis using T2DK as a dependent variable with all diabetic subjects (n = 104).

| Variable                          | Multiple logistic regression analysis |
|-----------------------------------|--------------------------------------|
| Fasting plasma glucose (mmol/L)   | Wald: 2.948, OR: 1.115, P: 0.086, 95% CI: 0.985–1.263 |
| 2-hour post plasma glucose (mmol/L)| Wald: 2.102, OR: 1.012, P: 0.121, 95% CI: 0.863–1.220 |
| C-reactive protein (mg/L)         | Wald: 5.716, OR: 0.860, P: 0.017, 95% CI: 0.760–0.973 |
| Ischemia-modified albumin (U/mL)  | Wald: 7.100, OR: 1.085, P: 0.008, 95% CI: 1.022–1.152 |
capacity of albumin to bind cobalt, resulting in higher IMA levels. Based on the above data, the IMA is a relatively new biomarker that may be a valuable signal for oxidative stress in patients with T2DK.

Increased levels of oxidative stress, pro-inflammatory markers, and downstream effector adhesion molecules occur in patients with T2D. T2D is intimately linked to hypertriglyceridaemia. The level of CRP is increased in diabetic patients with severe DKA, even in the absence of an infection, and may serve as a marker for systemic inflammatory response syndrome. Diabetic ketonuria is common among newly diagnosed or untreated patients with T2D and has been used to predict pathologic features or metabolic acidosis. Among Japanese patients with acute onset diabetic ketosis, there was a preponderance of males. The present study obtained similar results: T2DK was poorly controlled and male in the majority. An increase in serum IMA was associated with hyperketonemia in patients. However, there was no correlation between the IMA and CRP. CRP is regulated by the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α. The mechanisms of CRP and IMA production are apparently different. The results of the ROC analysis revealed that IMA could reflect T2DK and was superior to CRP. In contrast, plasma CRP levels were not sensitive or specific enough to reflect T2DK. In agreement with the present study, the IMA could be a risk biomarker for T2DK. The sensitivity and specificity of the IMA depend on the clinical characteristics of the patients under investigation. Further studies are required to investigate the role of IMA in diabetic crises.

Diabetic ketoacidosis and nonketotic hyperglycemia are two acute hyperglycemic emergencies. The plasma levels of pro-inflammatory cytokines, markers of oxidative stress, and lipid peroxidation are elevated on admission in patients with hyperglycemic crises. The elevated levels of ketone body acetoacetate can generate oxygen radicals and cause lipid peroxidation in endothelial cells. In diabetic ketotic patients, the serum IMA and CRP concentrations were high at
the beginning of hospitalization. T2D patients with ketonuria were more likely to be treated with insulin than those without ketonuria. In this study, the CRP and IMA levels were reduced in the T2DK patients, and control over plasma glucose was improved. T2D eventually leads to absolute or relative insulin deficiency. T2DK is the cause of pancreatic β-cells aggravation. As evident by a reduction in CRP, during T2D, insulin may have a modest anti-inflammatory effect. Insulin suppresses pro-inflammatory cytokines, not only by preventing hyperglycemia but also by modulating key inflammatory molecules.27

In summary, the markers CRP and IMA are higher in patients with T2DK but decreased following the treatments. Increasing levels of IMA were independently and significantly associated with T2DK. The present results suggest that IMA may be an interesting biomarker for predicting T2DK. The status of the proinflammatory cytokines and markers of oxidative stress in hyperglycemic crises of diabetic ketosis should be explored.

Author Contributions
Conceived and designed the experiments: MSG, JY. Analysed the data: HW, BF, XW. Wrote the first draft of the manuscript: MSG. Contributed to the writing of the manuscript: BF, XW, YWN. Agree with manuscript results and conclusions: JY, HW, BF, XW, YWN. Jointly developed the structure and arguments for the paper: MSG, JY, HW BF, XW, YWN. Made critical revisions and approved final version: MSG, XW, YWN. All authors reviewed and approved of the final manuscript.

Author Disclosures
We certify that all authors have no financial or other conflicts of interest connected to the submitted article.

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References
1. Bar-Or D, Lau E, Winkler JV. Novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. J Emerg Med. 2000;19:311–5. [Pubmed: 11074321].
2. Bar-Or D, Curtis G, Rao N, Bampson N, Lau E. Characterization of the Co(II) and Ni(II) binding amino-acid residues of the N-terminus of human albumin. Eur J Biochem. 2001;268:42–7. doi: 10.1046/j.1432-3727.2001.01846.x.
3. Gidienne S, Cepa F, Fontan E, Perrier F, Burnet P. Analytical performance of the Albumin Cobalt Binding (ACB) test on the Cobas MIRA Plus analyzer. Clin Chim Lab Med. 2004;42:455–61. [Pubmed: 15147158].
4. Chen CY, Tsai WL, Lin PJ, Shieh SC. The value of serum ischemia-modified albumin for assessing liver function in patients with chronic liver disease. Clin Chim Lab Med. 2011;49:1817–21. [Pubmed: 21851314].
5. Turedi S, Patan T, Gunduz A, Mentese A, Tekinbas C, Tophas M, et al. Ischemia-modified albumin in the diagnosis of pulmonary embolism: an experimental study. Am J Emerg Med. 2009;27:635–40. doi:10.1016/j.ajem.2008.05.002.
6. Turedi S, Cinar O, Yavuz I, Mentese A, Gunduz A, Karahan SC, et al. Differences in ischemia-modified albumin levels between end stage renal disease patients and the normal population. J Nephrol. 2010;23:335–40. [Pubmed: 20349416].
7. Gunduz A, Turedi S, Mentese A, Altnayayoglu V, Turan I, Karahan SC, et al. Ischemia-modified albumin levels in cerebrovascular accidents. Am J Emerg Med. 2008;26:874–8. doi:10.1016/j.ajem.2007.11.023.
8. Ma SG, Xu W, Wei CL, et al. Role of ischemia-modified albumin and total homocysteine in estimating symptomatic lacunar infarction in type 2 diabetic patients. Clin Biochem. 2011;44:1299–03. doi:10.1016/j.clinbiochem.2011.08.1136.
9. Wei W, Liu Q, Tan Y, Liu L, Li X, Cai L. Oxidative stress, diabetes, and diabetic complications. Hemoglobin. 2009;33:370–7. [Pubmed: 19821780].
10. Ukinc K, Eminagaoglu S, Erozu HO, Erem C, Karahan C, Hachisanoglu AB, et al. A novel indicator of widespread endothelial damage and ischemia in diabetic patients: ischemia-modified albumin. Endocrine. 2009;36:425–32. doi:10.1007/s12020-009-9236-5.
11. Piwowar A, Knapik-Kordecka M, Warwas M. Comparison of the usefulness of plasma levels of oxidatively modified forms of albumin in estimating kidney dysfunction in diabetic patients. Clin Invest Med. 2010;33:E109.
12. Turk A, Nuhoğlu I, Mentese A, Karahan SC, Erdol H, Erem C. The relationship between diabetic retinopathy and serum levels of ischemia-modified albumin and malondialdehyde. Retina. 2011;31:602–8.
13. Papadakis M, Grunfeld C. Ketonuria in hospitalized patients with non-insulin- dependent diabetes mellitus. Diabetes Care. 1986;9:596–600. [Pubmed: 3542454].
14. Kazlauskaite R, Evans AT, Mazzone T, Fogelfeld LA. Ethnic differences predicting ketonuria in patients with Type 2 diabetes. J Diabetes Complications. 2005;19:284–90.
15. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448–54.
16. Dalton RR, Hoffman WH, Passmore GG, Martin SL. Plasma C-reactive protein levels in severe diabetic ketoacidosis. Ann Clin Lab Sci. 2003;33: 435–42.
17. Vantyghem MC, Balduyck M, Zerimech F, Martin A, Douillard C, Bans S, et al. Oxidative markers in diabetic ketoacidosis. J Endocrinol Invest. 2000; 23:732–6.

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18. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes*. 2003;52:1–8.

19. Apple FS, Wu AH, Mair J, Ravkilde J, Panteghini M, Tate J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. *Clin Chem*. 2005;51:810–24.

20. Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem*. 2010;43:450–4.

21. Lu B, Yang Y, Yang Z, Feng X, Wang X, Zhang Z, et al. Insulin resistance in Chinese patients with type 2 diabetes is associated with C-reactive protein independent of abdominal obesity. *Cardiovasc Diabetol*. 2010;9:92.

22. el-Mesallamy H, Suwailem S, Hamdy N. Evaluation of C-reactive protein, endothelin-1, adhesion molecule(s), and lipids as inflammatory markers in type 2 diabetes mellitus patients. *Mediators Inflamm*. 2007;2007:73635.

23. Dalton RR, Hoffman WH, Passmore GG, Martin SL. Plasma C-reactive protein levels in severe diabetic ketoacidosis. *Ann Clin Lab Sci*. 2003;33:435–42.

24. Iwasaki Y, Hamamoto Y, Kawasaki Y, Ikeda H, Honjo S, Wada Y, et al. Japanese cases of acute onset diabetic ketoacidosis without acidosis in the absence of glutamic acid decarboxylase autoantibody. *Endocrine*. 2010;37:286–8.

25. Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. *Diabetes*. 2004;53:2079–86.

26. Jain SK, Kannan K, Lim G. Ketosis (acetoacetate) can generate oxygen radicals and cause increased lipid peroxidation and growth inhibition in human endothelial cells. *Free Radic Biol Med*. 1998;25:1083–8.

27. Hyun E, Ramachandran R, Hollenberg MD, Vergnolle N. Mechanisms behind the anti-inflammatory actions of insulin. *Crit Rev Immunol*. 2011;31:307–40.