Study on the analytical evaluation of pre-diabetes and its prevention by food compounds

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（かくれ糖尿病の分析化学的評価および食品成分による予防作用に関する研究）

区　　分：甲

論　文　内　容　の　要　旨

Pre-diabetes, defined as impaired glucose tolerance and/or impaired fasting blood glucose, is a high-risk state of developing diabetes. The onset of type 2 diabetes can be prevented or delayed by early interventions such as life-style change and therapeutic treatments at pre-diabetic stage. Given the availability of effective intervention to prevent increased burden of diabetes worldwide, earlier identification of individuals at risk seems to be crucial. Advanced glycation end-products (AGEs), which are generated from the modification of proteins with reactive carbonyl intermediates from reducing sugar, glucose, have been revealed to exert considerable effects in diabetes. Moreover, it was found that the increase of glycation metabolites was associated with the progression of diabetes. Herein, it was hypothesized that altered plasma AGEs levels may be served as diagnostic predictor of pre-diabetes and enable preventive interventions.

First, for high-sensitive detection of plasma AGEs in a free form, a liquid chromatography/tandem mass spectrometry method (LC-MS/MS) in combination with 2,4,6-trinitrobenzene sulfonate (TNBS) derivatization technique was established. Five targets, glyoxal-derived hydroimidasolone (G-H1), MG-derived hydroimidazolone (MG-H1), N^{ε}-carboxymethyl-lysine (CML), N^{ε}-(1-carboxyethyl)-lysine (CEL), and argpyrimidine (AP), were assayed by TNBS-LC-MS/MS method. Owing to successful derivatization of AGE-free adducts with TNBS, MS/MS detection in positive mode of each target was markedly enhanced (e.g., by a factor of >1,000 for MG-H1), due to high ionization efficiency of the derived trinitrophenyl moiety and its hydrophobicity. The advantages of the proposed TNBS derivatization method also include high detection limit of 1.0 pmol/mL-plasma as well as rapid (30 min) and mild (pH 8.5, 30°C) derivatization conditions. Using isotope-labeled standard-guided calibration curves, the method showed high accuracy with >93% of recovery of each AGE from spiked rat plasma and high reproducibility with 2 - 9% RSD.

Secondly, the proposed TNBS-LC-MS/MS method was applied for the determination of plasma levels of AGE-free adducts in young (10-week-old) Wistar rats under an acute hyperglycemia condition (oral glucose tolerance test). The study provided evidence that the plasma levels of diabetes-related metabolites did not change acutely within 120 min, irrespective to increasing blood glucose levels. The proposed method was applied to quantify a comprehensive range of AGE adducts in plasma samples of long-term living animals. In this study, male Spontaneously Diabetic Torii (SDT) rats and age-matched male Sprague-Dawley (SD) rats as control were maintained from the age of 7 to 25 weeks. Five targeting AGE residue of plasma protein and their corresponding free adducts were determined by the TNBS-MS method over the duration of the protocol. As a result, during the pathogenic progress of diabetes a significant increase in MG-H1 residues was observed in SDT rats (8-week-old SDT: 184.1 ± 8.6 pmol/mg-protein versus 16-week-old: 444.2 ± 27.5 pmol/mg-protein, $p < 0.001$), irrespective of unaltered plasma levels of other AGEs. The results strongly demonstrated that MG-H1 residue of plasma protein may behave as a predictor of pre-diabetes.

Thirdly, *in vivo* study on preventive effect of foods against pre-diabetes was performed in SDT rats using the hot-water extract of rose hip (*Rosa canina*). The result showed that the supplement of rose hip extract (100 mg/kg/day, 8-week-old SDT for 12 weeks) could improve glucose tolerance, preserve beta-cell function, and prevent the developing of pre-diabetes, together with the suppression of increasing MG-H1 residue during the stages.

In conclusion, this study must open the potential of functional foods to prevent pre-diabetes by the aid of AGE predictor.