Journal of Cardiology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Diurnal glycemic fluctuation is associated with severity of coronary artery disease in prediabetic patients: Possible role of nitrotyrosine and glyceraldehyde-derived advanced glycation end products

Makoto Watanabe (MD)[a], Yasuyuki Kawai (MD)[a,*], Michihiko Kitayama (MD)[a], Hironubu Akao (MD)[a], Atsushi Motoyama (MD)[a], Minoru Wakasa (MD)[a], Ryuhei Saito (MD)[a], Hirofumi Aoki (MD)[a], Kousuke Fujibayashi (MD)[a], Taketsugu Tsuchiya (MD)[a], Hiroaki Nakanishi (PhD)[b], Kazuyuki Saito (MD)[b], Masayoshi Takeuchi (PhD)[c], Kouji Kajinami (MD, FJCC)[a]

[a] Department of Cardiology, Kanazawa Medical University, Ishikawa, Japan
[b] Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan
[c] Department of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Ishikawa, Japan

ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form 10 June 2016
Accepted 4 July 2016
Available online xxx

Keywords:
Glucose fluctuation
Coronary artery disease
Nitrotyrosine
Glyceraldehyde-derived advanced glycation end-products

ABSTRACT

Background: Glucose fluctuation (GF) is a risk factor for coronary artery disease (CAD). However, it remains unknown whether specific indices of GF are risk factors for CAD. Therefore, we evaluated the relationship between GF, as determined by a continuous glucose monitoring system (CGMS) or the glucose level at 2 h after a 75-g oral glucose tolerance test (75 g OGTT 120), and the severity of CAD in prediabetic patients. We also evaluated whether nitrotyrosine (NT) and glyceraldehyde-derived advanced glycation end-products (Glycer-AGE) were induced by GF.

Methods: Twenty-eight prediabetic patients underwent coronary angiography (CAG), and the Gensini score and the SYNTAX score were evaluated as the severity of CAD, while the mean amplitude of glycemic excursions (MAGE) by CGMS and 75 g OGTT 120 were evaluated. Serum NT and Glycer-AGE were measured.

Results: The MAGE was closely associated with the Gensini score ($r = 0.742$, $p < 0.001$) and the SYNTAX score ($r = 0.776$, $p < 0.001$), respectively. The 75 g OGTT 120 was not associated with the Gensini score ($r = 0.36$, $p = 0.06$), but it was significantly associated with the SYNTAX score ($r = 0.413$, $p = 0.036$). Multiple linear regression analysis showed that the MAGE was the only independent determinant for the severity of CAD. The levels of NT and Glycer-AGE were significantly higher in the high MAGE group than in the low MAGE group.

Conclusions: Diurnal GF is associated with the severity of CAD, even in prediabetic patients. GF, NT, and Glycer-AGE may play a pathological role in the progression of CAD.

Introduction

Diabetic hyperglycemia is associated with an increased risk of coronary artery disease (CAD) [1]. Recent studies have reported that post-challenge blood glucose level is a risk factor for CAD among apparently healthy individuals without diabetes [2] and daily glucose fluctuation (GF) affects coronary plaque vulnerability in patients with CAD [3]. Postprandial hyperglycemia, a component of GF, can be evaluated by a 2-h oral glucose tolerance test (OGTT) [2]. While it is difficult to evaluate the precise daily glucose variability, development of continuous glucose monitoring devices allows assessment of glucose variability on an ambulatory basis [4]. Several indices for GF have been proposed. However, it remains unknown whether specific indices of GF are risk factors for CAD in prediabetic patients. Therefore, we evaluated the relationship between GF, as determined by a continuous glucose

* Corresponding author at: Department of Cardiology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku, Ishikawa 920-0293, Japan.
Fax: +81 76 286 3780.
E-mail address: kawai@kanazawa-med.ac.jp (Y. Kawai).

http://dx.doi.org/10.1016/j.jjcc.2016.07.001
0914-5087/

monitoring system (CGMS) as diurnal GF or the glucose level at 2 h after the 75-g OGTT (75 g OGTT 120) as postprandial GF, and the severity of CAD in prediabetic patients.

Several possible mechanisms by which glucose variability promotes atherogenesis have been proposed. Among them, we focused on nitrotyrosine (NT) and glyceraldehyde-derived advanced glycation end-products (Glycer-AGE). NT is a foot-print of oxidative stress produced by the modification of protein tyrosine residues by peroxynitrite generated from the reaction of nitric oxide (NO) and superoxide [5]. Postprandial hyperglycemia is accompanied by NT generation in patients with diabetes mellitus (DM) and in healthy subjects [6]. Further, the formation of advanced glycation end-products (AGEs) is enhanced by continuous hyperglycemia. Glycer-AGE, among six immunochemically different classes of AGEs, have the strongest binding affinity for the receptor for AGEs (RAGE) and subsequently elicit oxidative stress and vascular inflammation leading to the progressive atherosclerosis in DM patients [7–9]. Therefore, we also evaluated whether NT and Glycer-AGE were induced by GF and assessed the relationship between GF, NT, and Glycer-AGE in prediabetic patients with angina pectoris (AP).

Methods

Subjects

Sixty one consecutive Japanese patients suspected of having stable AP due to typical chest pain on exertion were admitted to our hospital between January 2011 and September 2012. They were subjected to stress testing in the outpatient department to assess for myocardial ischemia. Forty-two patients were positive for single or double two-step exercise tests, 13 patients had positive stress myocardial perfusion scintigraphy and 6 patients had an electrocardiogram (ECG) abnormality such as T-wave inversion or left bundle branch block. All patients underwent diagnostic coronary angiography (CAG) for the first time. Inclusion criteria were no history of DM, glucose level on admission $<$200 mg/dL, and hemoglobin A_{1C} (HbA_{1C}) level $\leq$6.2%. Twenty-seven patients with admission glucose level $\geq$200 mg/dL and/or HbA_{1C} $\geq$6.3%, and two patients requiring hemodialysis were excluded from this study. In addition, four patients declined to take part in the continuous glucose monitoring study. Ultimately, 28 patients were enrolled in this study. All patients received optimal meals (25 kcal/kg/day) during the hospitalization.

The study protocol conformed to the Declaration of Helsinki and was approved by the Human Ethics Committee of Kanazawa Medical University, and all patients provided written informed consent.

Catheterization procedure and angiographic analysis

CAG was performed through a femoral or a radial approach using the standard Judkins technique after administration of 2000 U of heparin. During CAG, 0.25 mg of nitroglycerin was injected routinely into each coronary artery in all patients. Angiographic analysis was carried out by two experienced cardiologists who were blinded to this study. The Gensini score and the SYNTAX score were used to assess the severity of coronary atherosclerosis. The Gensini score was defined as narrowing of the lumen of coronary arteries according to a previous report [10]. The SYNTAX score was defined according to coronary anatomy and lesion characteristics as described in a previous report [11]. These scores represent the severity and extent of coronary atherosclerosis.

75 g OGTT and blood sampling

After an overnight fast, a standard 75 g OGTT was performed at least 2 days after the CAG. Serial venous blood samples for determination of glucose and plasma insulin concentrations were collected before and at 90, 120, and 180 min after the ingestion of a 75 g oral glucose and also for evaluation of serum NT and Glycer-AGE levels. Patients were classified as having: (1) DM when the 2-h plasma glucose measurement was $>$200 mg/dL, (2) impaired glucose tolerance (IGT) when the fasting plasma glucose was $<$126 mg/dL and the 2-h plasma glucose 140–200 mg/dL, and (3) normal glucose tolerance (NGT) when the fasting plasma glucose was $<$110 mg/dL and the 2-h plasma glucose was $<$140 mg/dL [12,13]. Insulin resistance was expressed as the homeostasis model assessment for insulin resistance (HOMA-IR), and the homeostasis model of assessment for β cell function (HOMA-β) was calculated according to a previous report [14].

Continuous glucose monitoring

All patients were equipped with a CGMS (MiniMed CGMS-Gold, Medtronic, Minneapolis, MN, USA) to measure fluctuations in blood glucose levels and were monitored for 72 consecutive hours at least 2 days after the 75 g OGTT. A CGMS sensor was inserted into the subcutaneous abdominal fat tissue and was calibrated according to the Medtronic MiniMed standard operating guidelines. Analysis was limited to the data obtained from the intermediate 48 h of recording to avoid bias due to insertion and removal of CGMS or insufficient stability of the monitoring system. After monitoring for 48 h, the recorded data were downloaded onto a personal computer for analysis of glucose profile and glucose excursion parameters with MiniMed Solutions software [15]. The mean amplitude of glycemic excursions (MAGE) was calculated using these data. The MAGE represents fluctuations in blood glucose levels over a 24-h period and was calculated by measuring the arithmetic mean of the differences between consecutive peaks and nadirs, provided that the differences were $>$1 standard deviation (SD) of the mean glucose value [16].

Biochemical, NT, and Glycer-AGE measurements

Blood samples were collected from an antecubital vein at admission and in the morning following an overnight fast. Serum and plasma samples were prepared from each blood sample through centrifugation and were stored at -80 °C until assay. Serum creatinine, plasma glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured by automatic biochemical analyzer (Hitachi, Tokyo, Japan). Serum concentration of HbA_{1C} was determined by high-performance liquid chromatographic method using an automatic HbA_{1C} analyzer (Tosoh, Tokyo, Japan).

Based on a previous report [17], NT levels were measured by competitive time-resolved fluoroimmunoassay using dissociation-enhanced lanthanide fluorescence immunoassay reagents (Perkin-Elmer Life Sciences, Boston, MA, USA). The fluorescence intensity was measured at 340 nm excitation and 610 nm emissions, using an Arvo SX multilabel counter (Perkin-Elmer Life Sciences). The intra-assay coefficients of variation (CV) were 1.2–6.7% for NT measurements by the time-resolved fluoroimmunoassay, and the inter-assay CV at seven concentrations was 9.2%.

The Glycer-AGE levels were measured using a competitive enzyme-linked immunosorbent assay (ELISA) with immunopurified Glycer-AGE antibodies with reliable intra-assay and inter-assay CV [18].

Please cite this article in press as: Watanabe M, et al. Diurnal glycemic fluctuation is associated with severity of coronary artery disease in prediabetic patients: Possible role of nitrotyrosine and glyceraldehyde-derived advanced glycation end products. J Cardiol (2016), http://dx.doi.org/10.1016/j.jjcc.2016.07.001