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Synergistic association of elevated serum free fatty acid and glucose levels with large arterial stiffness in a general population: The Nagahama Study

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ABSTRACT

Background. Previous studies have reported that artificial increases in circulating free fatty acid (FFA) levels might have adverse effects on the vasculature. However, whether or not this effect can be extrapolated to physiological variations in FFA levels has not been clarified. Given that FFAs exert a lipotoxic effect on pancreatic β-cells and might directly damage the arterial endothelium, we hypothesized that these adverse effects might synergize with hyperglycemia.

Methods. A total of 9396 Japanese subjects were included in the study. Serum FFA levels were measured at baseline examination. Brachial-to-ankle pulse wave velocity (baPWV) was measured as an index of arterial stiffness.

Results. As serum levels of FFA were markedly lower in subjects with higher insulin level, a significant association between FFA levels and baPWV was observed only in subjects with blood samples taken under fasting (≥12 h, $P < 0.001$) or near-fasting (5–11 h, $P < 0.001$) conditions, and not in those taken under non-fasting (<5 h, $P = 0.307$) conditions. Although type 2 diabetes and HbA1c showed a strong association with baPWV, the association between FFA level and baPWV remained significant ($\beta = 0.052$, $P < 0.001$) after adjustment for glycemic levels. In addition to their direct relationship, FFA and glucose levels were synergistically associated with baPWV (FFA*glucose; $\beta = 0.036$, $P < 0.001$). Differences in baPWV between the lowest and highest subgroups divided by a combination of FFA and glucose reached approximately 300 cm/s.

Conclusions. Physiological variations in FFA concentrations might be a risk factor for large arterial stiffness. FFA and hyperglycemia exert a synergistic adverse effect on the vasculature.

Abbreviations: baPWV, brachial-to-ankle pulse wave velocity; DBP, diastolic blood pressure; FFA, free fatty acid; hsCRP, high sensitive C-reactive protein; SBP, systolic blood pressure.

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1. Introduction

Circulating free fatty acid (FFA) is released mainly by adipose tissue and is used as a major energy source by cardiac and skeletal muscles [1]. However, excessive FFA exposure induces a lipotoxic effect on pancreatic β-cells, which might reduce insulin secretion [2] and increase β-cell apoptosis [3]. These adverse effects of FFA were first observed *in vitro* and subsequently confirmed *in vivo* in both animal models [4] and human studies [5,6], which reported an association of chronically high FFA levels with reduced insulin sensitivity and impaired compensatory increases in insulin secretion.

In addition to lipotoxic effects on the insulin pathway that might increase the risk of type 2 diabetes [7], elevated circulating levels of FFA might exert a direct adverse effect on large arteries via impaired insulin-mediated vasodilation [8]. FFA might also exert adverse effects via impaired endothelium-dependent vasodilation [9]. However, nearly all human studies investigating the adverse effects of FFA on insulin signaling [5] and vascular function [8,9] have used lipid infusion to increase circulating FFA levels. Although one small-scale study (n = 105) [10] reported an inverse association between serum FFA levels and abdominal aortic distensibility, we are unaware of any data from large-scale populations on the potential effects of physiological variations in FFA levels on arterial properties. Longitudinal studies in a general population [11] and in patients undergoing coronary angiography [12] reported positive associations between the elevation of FFA levels and incidence of ischemic heart disease, as well as cardiovascular mortality. Therefore, even physiological variations in FFA might result in adverse effects on arteriosclerotic vasculature change. Further, as FFA might exert adverse effects on arteries by bidirectional pathways [13], namely direct effects on vascular endothelium [14] and indirect effects via lipotoxicity, we hypothesized that higher circulating FFA and glucose levels have a synergistic association with arteriosclerosis.

Here, to further clarify the direct and synergistic adverse effects of FFA on arterial stiffness, we conducted a cross-sectional study by analyzing a dataset of the Nagahama Prospective Cohort for Comprehensive Human Bioscience (the Nagahama Study), which is a large-scale population-based cohort study in Japan. We also investigated factors that are potentially associated with circulating FFA levels to elucidate the descriptive and epidemiological characteristics of FFA in a general population.

2. Methods

2.1. Study Participants

Study participants consisted of 9396 apparently healthy middle-aged to elderly citizens who were participants of the Nagahama Study. The study cohort was recruited from 2008 to 2010 from the general population living in Nagahama City, a largely rural city of 125,000 inhabitants located in central Japan. Residents aged 30 to 74 years living independently in the community and with no physical impairment or dysfunction were recruited for the Nagahama cohort. Of a total of 9804 participants, those meeting any of the following conditions were excluded: history of cardiovascular diseases (n = 266), presently taking insulin therapy (n = 22), pregnant (n = 43), or no data for or outlying clinical parameters required for this study (n = 77).

All study procedures were approved by the ethics committee of Kyoto University Graduate School of Medicine and the Nagahama Municipal Review Board. Written informed consent was obtained from all participants.

2.2. Clinical Characteristics of Study Subjects

Basic clinical parameters, including plasma markers, were measured at baseline examination. Each participant was asked the time of their last meal, and fasting conditions were defined as follows: fasting, 12 h or more; near-fasting, 5 to 11 h; and non-fasting, less than 5 h. Serum FFA levels were quantified using an enzymatic assay (NEFA-HR; Wako Pure Chemical Industries, Osaka, Japan). In FFA measurements, the intra-assay coefficient of variation was 1.42% and inter-assay coefficient of variation was 1.79%. Smoking status and medication use were evaluated using a structured, self-administered questionnaire.

2.3. Evaluation of Arterial Stiffness

Brachial-to-ankle pulse wave velocity (baPWV) was used as an index of arterial stiffness. baPWV was measured by applying cuffs to both brachia and ankles, and blood pressure was simultaneously measured using a cuff-oscillometric device (Vasera-1500; Fukuda Denshi, Tokyo, Japan). Pulse volume waveforms were also simultaneously recorded using a plethysmographic sensor connected to the cuffs. baPWV was calculated from the time interval between the wave fronts of the brachial and ankle waveforms and the path length from the brachia to ankle (0.597 × height + 14.4014) [15]. Co-linearity of baPWV with a carotid-to-femoral PWV, a standard measure of arterial stiffness, has been reported [16].

2.4. Assessment of Other Risk Factors

Hypertension was defined as any or all of brachial systolic blood pressure (SBP) ≥140 mmHg, diastolic BP (DBP) ≥90 mmHg, or taking antihypertensive medication. Type 2 diabetes was defined as any or all of fasting plasma glucose ≥126 mg/dl, occasional plasma glucose ≥200 mg/dl, HbA1c ≥6.5%, and use of hypoglycemic treatment.

2.5. Statistical Analysis

Group differences in numeric and categorical variables were assessed by analysis of variance (ANOVA) or a chi-squared test. Quartiles of numeric variables were calculated within each sub-group divided by fasting condition. Factors independently associated with FFA and baPWV were analyzed by multiple linear regression analysis. Statistical analysis was performed using JMP 9.0.3 software (SAS, Cary, NC, USA). *P*-values less than 0.05 were considered significant.