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An integrative approach to investigate the association among high-sensitive C-reactive protein, body fat mass distribution, and other cardiometabolic risk factors in young healthy women

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ABSTRACT

Prior research has indicated that as an important biomarker of chronic low-grade inflammation, high-sensitivity C-reactive protein (hs-CRP) can play important roles on the onset of metabolic syndrome and cardiovascular diseases (CVD). We conducted an integrative approach, which combines biological wet-lab experiments, statistical analysis, and semantics-oriented bioinformatics & computational analysis, to investigate the association among hs-CRP, body fat mass (FM) distribution, and other cardiometabolic risk factors in young healthy women. Research outcomes in this study resulted in two novel discoveries. Discovery 1: There are four primary determinants for hs-CRP, i.e., central/abdominal FM (a.k.a. trunk FM) accumulation, leptin, high density lipoprotein cholesterol (HDL-C), and plasminogen activator inhibitior-1 (PAI-1). Discovery 2: Chronic inflammation may involve in adipocyte-cytokine interaction underlying the metabolic derangement in healthy young women.

1. Introduction

Prior research [1] has demonstrated that chronic low-grade inflammation is one of the important characteristics of obesity-related metabolic syndrome. Adipose tissue is known [2,3] to be a source of cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), where the latter stimulates hepatocytes to produce a variety of acute phase reactants including C-reactive protein (CRP) [4]. As a representative biomarker of inflammation in the body, long-term elevation of CRP may have prognostic value in predicting individuals with increased risk of developing cardiovascular diseases (CVD) [5]. In fact, prospective studies [5–7] have discovered that CRP may add moderate forecasting power to predict the development of CVD in middle-aged or elderly persons who have already been frequently accompanied with typical CVD risk conditions such as dyslipidemia, insulin resistance, or cigarettes smoking. In particular, according to the study in [8], CRP was shown to be a strong risk predictor for myocardial infraction even 20 years after the initial blood samples were collected. However, little research to date has investigated the association between high-sensitivity CRP (hs-CRP) and obesity-related metabolic phenotypes (including atherosclerosis) in young people without classical CVD risk factors. Such association study is of particular importance for us to better prevent and intervene the onset of metabolic syndrome and CVD. Towards this end, we conducted an integrative study in young healthy women to investigate the association between hs-CRP and body fat mass (FM) distribution, as well as the association between hs-CRP and other cardiometabolic risk factors. Our approach effectively combines biological wet-lab experiments, statistical analysis, and semantics-oriented bioinformatics & computational analysis.

The rest of this paper is organized as follows. Section 2 describes in detail our methods and materials; Section 3 reports experimental results along with discussion; and finally, Section 4 concludes with future work.

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2. Methods and materials

2.1. Overview of our integrative methods

Our methods consist of three steps

Step I: Biological experiments. We recruited a cohort of healthy young women without conventional cardiovascular risk factors and then performed a series of wet-lab experiments on these subjects. Our experiments were organized into five categories: (1) anthropometry, body composition, and FM distribution; (2) insulin, glucose, and insulin resistance; (3) plasma lipids, lipoprotein, and Apo measurements; (4) inflammation markers, oxidative stress marker, and adipocytokines; and (5) arterial properties.

Step II: Statistical analysis. After obtaining the results from biological experiments, we conducted statistical analysis including univariate analysis and multiple regression analysis. These analysis results provided us with evidence to either support or discourage possible association among hs-CRP, FM distribution, and other cardiometabolic risk factors.

Step III: Semantics-oriented bioinformatics and computational analysis. We utilized an ontology-based analytical software tool to obtain a set of computationally putative microRNA (miR) molecules that may regulate hs-CRP. Besides, rich, additional data for each candidate miR were also provided to us through the software user interface. Such semantically federated knowledge further assisted us in better analyzing results from the first two steps.

2.2. Biological wet-lab experiment design

2.2.1. Subjects

A total of 308 female students at Mukogawa Women's University (MWU) were recruited in this study. All subjects were Japanese and aged between 18 and 22 years old. We excluded any subjects with clinically diagnosed CVD, acute or chronic inflammatory diseases, endocrine diseases, hepatic diseases, and renal diseases. Subjects with hormonal contraception, regular cigarette smoking and alcohol drinking, and unusual dietary habits were also excluded. In addition, no subject was receiving any medications during the study period. Our study was approved by the Ethics Committees at MWU, and written informed consents were obtained from all participants.

2.2.2. Anthropometry, body composition, and FM distribution

Body weight, height, waist circumferences (WC) were measured following standard procedures, and body mass index (BMI) was then calculated. Dual-energy X-ray absorptiometry (DXA)-derived trunk FM was demonstrated [9] to have strong association with total abdominal adiposity measured by computer tomography (CT). Moreover, when combined with anthropometry, DXA offers a good alternative to CT for the prediction of abdominal adiposity. DXA was thus recommended for the early detection of central/abdominal obesity. In this study, DXA with a scanner (Hologic QDR-2000, Waltham, MA) was utilized to measure body FM distribution. A scanned image of the whole body was divided into six sub-regions: head, trunk, and upper/lower limbs on both sides. The dividing borders between those subregions were differentiated by a line underneath the chin, a line between the humerus head and the glenoid fossa, and a line at the femoral neck. The trunk region included the chest and abdomen, excluding the pelvis. The low body region included the entire hip, thigh, and leg. To better express regional fat deposition, we introduced four body fat ratio parameters denoted as %X FM, where X stands for Total, Trunk, Arm, or Lower-body, measuring the percentage of corresponding fat tissue weight over body weight. That is, %Total FM, %Trunk FM, %Arm FM, and %Lower-body FM were calculated as $\frac{total\ fat\ tissue\ weight}{body\ weight}$, $\frac{trunk\ fat\ tissue\ weight}{body\ weight}$, $\frac{arm\ fat\ tissue\ weight}{body\ weight}$, and $\frac{lower\text{-}body\ fat\ tissue\ weight}{body\ weight}$, respectively.

2.2.3. Insulin, glucose, and insulin resistance

Blood samples were obtained in the morning after 12-h overnight fast. Insulin resistance determined by homeostasis model assessment (HOMA-IR) was calculated using fasting plasma glucose and insulin levels [10].

2.2.4. Plasma lipids, lipoprotein, and Apo measurements

Serum lipids, including triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and free fatty acid (FFA), were measured with an autoanalyzer (AU5232, Olympus, Tokyo, Japan). Apolipoprotein A-1 (ApoA1) and apolipoprotein B-100 (ApoB) were measured by an Olympus autoanalyzer (AU600, Mitsubishi Chemicals, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was determined using the Friedewald formula [11]. Small dense LDL-C (sd-LDL) was measured with a precipitation method described in [12]. Remnant-like particle-cholesterol (RLP-C) was measured by an immunoaffinity separation method (RLP-C assay, Otsuka, Japan). Lipoprotein lipase (LPL) was determined by enzyme-linked immunosorbent assay using an assay system (Dai-ichi Pure Chemicals, Tokyo, Japan).

2.2.5. Inflammation markers, oxidative stress marker, and adipocytokines

Hs-CRP was measured by an immunoturbidometric assay with the use of reagents and calibrators from Dade Behring Marbura GmbH (Marburg, Germany; interassay CV < 5.0%,CV: coefficients of variance). TNF-α was measured by immunoassays (R&D Systems, Inc., Minneapolis, MN; interassay CV = 6.0%). PAI-1 was measured by an ELISA method (Mitsubishi Chemicals; interassay CV = 8.1%). For the purpose of statistical analysis, serum concentrations of hs-CRP and TNF-α below the limit of detection were assigned a value of 0.05 mg/L and 0.50 pg/mL (the lowest limit of detection), respectively. Systemic oxidative stress was evaluated by urinary creatinine-indexed 8-epi-prostagland in F-2α (8-epi-PG2α), a validated biomarker of oxidative stress [13]. Urinary 8-epi-PGF2α was measured in the first-voided morning urine sample with an enzyme-liked immunosorbent assay (8-Isoprostane EIA kit, Cayman, Ann Arbor, MI). Intra- and inter-assay CV were 7.5% and 9.2%, respectively. Urinary 8-epi-PG2α was indexed to creatinine as pictograms per millimole creatitine. Adiponectin was assayed by a sandwich enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co. Ltd., Tokushima City, Japan). Intra- and inter-assay CV were 3.3% and 7.5%, respectively. Leptin was assessed by an RIA kit (LINCO research, St. Charles, MO; interassay CV = 4.9%).

2.2.6. Arterial properties

Arterial stiffness was indicated by cardio-ankle vascular index (CAVI) measured by VaSera device (VS-1000, Fukuda Denshi, Tokyo, Japan). CAVI is a recently developed index that reflects stiffness of the aorta, femoral, and tibial artery [14]. CAVI involves the measurement of pulse valve velocity (PWV) but the effects of blood pressure are minimized. CAVI was thus proved to be a reliable screening tool for atherosclerosis [14]. Because carotid intima-media thickness (IMT) was clinically used as an indicator of generalized atherosclerosis [15], arterial thickness was evaluated by carotid artery IMT measured with an ultrasonic device (SDU-1100, Shimadzu, Tokyo, Japan). The maximal IMT was assessed at the far wall as the distance between the lumen-intima interface and media-adventitia interface. The maximal IMT of two measurements conducted at each of the four segments vessels was recorded on both sides and then averaged for both sides. The mean IMT (Avg IMT) and maximal IMT (Max IMT) of the four IMT values were used for analysis.

2.3. Statistical analysis

All data were presented as mean ± standard deviation (SD). Due to deviation from normal distribution, hs-CRP was logarithmic transformed for analysis. (1) First of all, univariate correlations between hs-CRP and regional FM distribution, as well as between hs-CRP and other

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