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Effect of resistant and digestible rice starches on human cytokine and lactate metabolic networks in serum

Huisong Wang, Guangchang Pang*

Tianjin Key Laboratory of Food Biotechnology, College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China

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ABSTRACT

Resistant starch generated after treating ordinary starch is of great significance to human health in the countries with overnutrition. However, its functional evaluation in the human body has been rarely reported. By determining the lactate metabolic flux, 12 serum enzymes expression level and 38 serum cytokines in healthy volunteers, the variation in cytokine network and lactate metabolic network in serum were investigated to compare the mechanism of the physiological effects between the two starches. The results indicated that compared with digestible starch, resistant starch had anti-inflammatory effects, increased anabolism, and decreased catabolism. Further, the intercellular communication networks including cytokine and lactate metabolic networks were mapped out. The relationship suggested that resistant starch might affect and control the secretion of cytokines to regulate lactate metabolic network in the body, promoting the development of immunometabolism.

1. Introduction

The continual spread of overnutrition-induced obesity, diabetes, and other metabolic syndrome has greatly contributed in recent years to the research on processing digestible starch into starches that are resistant to digestion and absorption [1]. A large number of reports have indicated the health-protective functions of resistant starch [2]. Only a few of the studies were performed in humans, yielding significantly different and even mutually conflicting results [3]. Mathers et al. [4] found that the long-term administration of resistant starch could not significantly prevent the occurrence and development of colon cancer. It is obvious that the functions of resistant starch in the human body cannot be inferred depending only on the results of experiments performed *in vitro* and in animals. Studies conducted in the last 3 years have reported that resistant starch has beneficial effects on the immunity and metabolism of humans. It has anti-inflammatory effects in the gastrointestinal tract [5], increases insulin sensitivity [6], and so forth. Therefore, it is necessary to evaluate physiological functions of resistant starch in the human body.

Both nutrients and nonnutrients in food play important physiological roles [7]. A number of recent studies have shown that functional foods play active roles in human health by signal transduction and information exchange system within the body [8]. These signal exchanges can be mediated by gut microbes and intestinal receptors. On the other hand, a long-term intake of the same food would result in a decrease in its regulating effect because of the adaptive adjustment of the human body. A commonly known fact is that the effect of laxative medicines and health care products is reduced or even lost with the long-term intake. Therefore, it is believed that the functional attributes of the initial effects of functional foods via signaling pathways should be the focus in evaluating their functions.

Functional foods can regulate immunity, endocrine functions, and metabolism via signaling pathways to exert their effects. It is more realistic to provide a health guide after having a good knowledge about the function of food in regulating immunity and metabolic network of the human body. The cytokine and lactate metabolic networks were explored based on previous studies on young healthy volunteers [8–12], to evaluate and compare the physiological actions of rice

Abbreviations: Ac-CoA, acetyl coenzyme A; ALD, aldolase; EGF, epidermal growth factor; F6P, frucose-6-phosphate; EMP, Embden–Meyerhof–Parnas; FGF, fibroblast growth factor; Fit-3L, Fit-3 ligand GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage-colony-stimulating factor; GRO, growth-related oncogene HK, hexokinase; ICDH, isocitrate dehydrogenase; INF, interferon; IL, interleukin α-KGDHC, α-ketoglutarate dehydrogenase complex; LDH, lactate dehydrogenase; IP-10, interferon-inducible protein-10; M-CSF, macrophage-colony-stimulating factor; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein PDHC, pyruvate dehydrogenase complex PFK, phosphofructokinase; PGK, phosphoglyceric kinase; PK, pyruvate kinase; PPP, pentose phosphate pathway; PYR, pyruvic acid; Ru5P, ribulose-5-phosphate; sCD40L, soluble CD40 ligand; TCA cycle, tricarboxylic acid cycle; TGF, transforming growth factor; TNF, tumor necrosis factor; TPI, triose-phosphate isomerase; VEGF, vascular endothelial cell growth factor

* Corresponding author.
E-mail address: pgc@tjcu.edu.cn (G. Pang).

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digestible and resistant starches on immunity and metabolism. The study of these two networks may reveal the difference in physiological functions (immunity and metabolism) of digestible and resistant starch in the human body. Meanwhile, the present study may aid in understanding whether resistant starch plays a role in preventing and treating some inflammatory diseases and metabolic syndrome.

Blood circulation is considered as the channel that transports material, energy, and information between different organized spaces to realize cytokine and lactate metabolic networks throughout the body rather than only in organelles or between cells. Cytokines are substances secreted by specific cells of the immune system, which carry signals between cells [10]. With very complex physiological functions, cytokines can not only act on the immune and endocrine systems but also regulate a variety of other cell metabolisms via blood circulation, thus constituting intercellular cytokine and metabolic networks in the body. Many enzymes are found in intracellular organelles and also distributed in body fluid (e.g., blood). Blood lactate, a signaling molecule, plays a regulatory role in the intracellular and systemic metabolic processes [13]. Coupled with nicotinamide adenine dinucleotide ($NADH^+$), lactate can shuttle among organelles, cells, and tissues [14,15]; regulate cell respiration, oxidative phosphorylation, redox state, anabolism, catabolism, and synthesis of the relevant enzymes; and constitute the network for matter and energy transport among organelles, cells, and tissues [16]. The lactate flux has become an important indicator of catabolism and oxidative phosphorylation level in multicellular organisms. Namely, an increase in lactate flux indicates an increase in catabolism [17]. Hence, the mechanisms underlying the physiological actions of digestible and resistant starch through the changes in anabolic and catabolic flux and cytokine levels can be further explored.

2. Materials and methods

2.1. Retrogradation of rice starches

The rice-resistant starches (RS3) were prepared as described previously [18,19]. Rice starches (100 g; amylose content 25%, median granularity 5.43 μm; Jinnong Biological Technology Corporation, Jiangxi, China). Rice (*Oryza sativa* L. subsp. *indica S. Kato*) blended with 400 mL of distilled water were gelatinized by continuous stirring at 95 °C for 3 h and then autoclaved at 120 °C for 40 min. After natural cooling, the gelatinized starches were retrograded at 4 °C for 72 h. Later 0.6 mL of α-amylase (12,000 U/mL; Nuoao, China) was added at 90 °C for 6 h. Crude retrograded starches were precipitated at 3000*g* for 5 min, and the supernatant fractions were discarded. The retrograded starches were washed with distilled water three times and dried at 50 °C in an oven until constant weight was attained.

2.2. Physicochemical characterization

The resistant and digestible starches were analyzed using a microscope to observe the physical form and particle size (×1000).

2.3. Volunteers

Sixteen healthy volunteers (age 22–26 years, 50% men and 50% women who were not menstruating) were enrolled from the Tianjin University of Commerce, China. These healthy volunteers mainly ate wheat flour (such as steamed bread and noodles) every day and rice occasionally. All the volunteers signed informed consent forms and liability agreement, and the study protocol was approved by the local ethics committee (Ethics Committee of Tianjin University of Commerce). Throughout the trial period, all the volunteers were offered opportunities to study and rest according to a normal daily schedule including 13 diets (5 days) without any medicine or functional food. Additionally, the volunteers were encouraged to maintain a calm mood [9,11].

2.4. Doses and treatments

Samples (40 g) of resistant and digestible rice starches [4] were mixed with boiling water and stirred into a paste [20]. The total trial period lasted 5 days (Friday + first weekend + second weekend; 5 days during this interval): 2 days for regulating effect and 3 days for collecting blood samples. A total of 16 volunteers who ate unbiasedness food were used as control to avoid individual differences in genotype. During 5-day interval, the volunteers maintained normal diets. On the first day (Friday), the volunteers were asked to eat steamed bread + unbiasedness food, and no serum was collected (regulating effect); On the second day (first Saturday), the volunteers were asked to eat steamed bread + unbiasedness food, and blood was collected as control. On the third day (first Sunday), in addition to steamed bread + unbiasedness food, lunch (12:00 p.m.) was supplemented with 40 g of digestible rice starch (stirred into a paste), and blood was collected. On the fourth day (second Saturday), the volunteers were asked to maintain a regulated diet (steamed bread + unbiasedness food), and no serum was collected (regulating effect). On the fifth day (second Sunday), in addition to the regulated diet, lunch (12:00 p.m.) was supplemented with 40 g of resistant rice starch (stirred into a paste), and blood was collected. Blood was collected from each subject at 2:30, 3:00, and 3:30 p.m. on each day of the trial [around this time, food (nutrients or nonnutrients) had relatively significant effects on the human body through the signaling pathways, e.g., cytokine level] [9–12], incubated at 4 °C for 4 h, and centrifuged at 1500*g* for 10 min at 4 °C. The serum was harvested and stored at −70 °C until analysis.

2.5. Cytokine network

2.5.1. Determination of cytokine profiles

Procarta cytokine profiling kits (Millipore) and the Luminex 200 instrument (Millipore) were used to measure the concentrations of 38 types of cytokines in human serum obtained at 3:00 p.m. each day according to the manufacturer instructions. These 38 cytokines were chosen [9,11,12,21] because of relatively mature studies on them and their more explicit functions; these cytokines played an important role in immunity, endocrine function, and metabolism in humans. Most importantly they can be determined (have monoclonal antibody). The system for detecting cytokines was based on antibody-coupled microbial populations, in which each was specifically bound to a certain cytokine [22]. Different cytokines had different minimum detectable concentrations (MinDCs) [11,23]. The standards, controls, and samples were distributed into 96-well plates. The levels of cytokines were determined from the standard curve and levels under control.

2.5.2. Cytokine network diagram

Based on the changes in cytokines in serum after the administration of resistant or digestible rice starch, the rate of change in the content of cytokines *i* (one of 38 cytokines) was expressed through the following equation:

$$\xi_i/\% = \frac{1}{16}\sum_{j=1}^{n=16}\frac{c_{ij}-c_{ij0}}{c_{ij0}} \times 100 \quad (1)$$

where c_{ij} represented the concentration of cytokine *i* after the administration of rice-resistant or digestible starch and c_{ij0} represented the concentration of cytokine *i* in the control test.

This study aimed to map a virtual cytokine network *in vivo* according to the rate of change in the content of 38 cytokines to describe the effect of resistant starch compared with digestible starch in rice. Microsoft Visio 2007 mapping software was used to draw the intercellular cytokine network diagram among the secretory and target

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