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L-Arginine enriched biscuits improve endothelial function and glucose metabolism: A pilot study in healthy subjects and a cross-over study in subjects with impaired glucose tolerance and metabolic syndrome

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ABSTRACT

Objective. The aim of this study was to evaluate the effects of a new L-arginine-enriched biscuit on endothelial function, insulin sensitivity/secretion and body composition.

Materials/Methods. The project was composed of two studies. The first study was an acute pilot postprandial study in 7 healthy subjects that evaluated bio-availability and vascular effects of L-arginine-enriched biscuits that contained 6.6 g L-arginine, 21.9 g carbohydrates, 3.6 g protein, 7.5 g fat and 4.3 g dietary fiber compared with placebo biscuits and 6.6 g powdered L-arginine. Subjects underwent the tests in random order, in at least 14-day intervals. The second study was a double-blind crossover study in 15 obese subjects with IGT and MS. These subjects consumed 6.6 g of L-arginine-enriched biscuits or placebo biscuits in a 1600 kcal diet. Each study period lasted 2 weeks with a 2-week washout in between. Endothelial function, glucose tolerance, insulin sensitivity and insulin secretion were evaluated at the end of each intervention period.

Results. In the first study, the groups that received the L-arginine-enriched biscuits and the powdered L-arginine had similarly increased L-arginine, NOx and cGMP levels and post-ischemic blood flow (PI-BF). In both cases, these levels were significantly higher than those in the placebo biscuit recipient group. In the second study, the L-arginine-enriched biscuit recipient group displayed increased L-arginine, NOx, cGMP, PI-BF, and Matsuda index levels, whereas their circulating glucose, proinsulin/insulin ratio and fat mass were decreased compared with the placebo biscuit recipient group.

Conclusions. L-Arginine-enriched biscuits with low sugar and protein content enhance endothelial function and improve glucose metabolism, insulin sensitivity and insulin secretion in subjects with IGT and MS.

Abbreviations: OGTT, oral glucose tolerance test; MS, Metabolic Syndrome; IGT, impaired glucose tolerance; BMI, body mass index; HDL, high-density lipoprotein; NOx, nitrite and nitrate; cGMP, cyclic guanosine monophosphate; PI-BF, post-ischemic blood flow.

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1. Introduction

It is well known that metabolic syndrome is a cluster of metabolic abnormalities, which includes impaired glucose regulation, hypertension, dyslipidemia, obesity and increased cardiovascular disease risk [1]. Metabolic syndrome affects one in five people, and prevalence increases with age. Some studies estimate the prevalence in the USA to be nearly 25% of the population [2].

It is known that endothelium-derived nitric oxide is a potent endogenous vasodilator that plays a major role in vascular tone [3]. L-Arginine is the endogenous precursor of nitric oxide synthesis, and it has been previously demonstrated that L-arginine potentiates insulin-mediated glucose uptake by increasing blood flow [4]. Previously, our group demonstrated that the nitric oxide pathway was an important regulator of hepatic glucose metabolism in rat hepatocytes by modulating glucokinase activity and hepatic glycogen content [5]. Further, previous studies have found that chronic L-arginine therapy, when added to a physical exercise and diet program, may improve glucose metabolism and insulin sensitivity in type 2 diabetic patients with or without metabolic syndrome [6,7]. Furthermore, this therapy improved endothelial function, oxidative status, and adipokine release in these subjects [6]. Supplementing food with relatively large doses of L-arginine has proven to have a salutary effect on cardiovascular diseases in animals and humans, which has been extensively described in the literature [8,9]. L-Arginine was found to be bio-available and effectively prevent glucose metabolism impairment and endothelial dysfunction, thus improving blood flow [10,11]. Although these L-arginine doses were well tolerated by the study subjects, using this amount of L-arginine orally in the form of pills, capsules or vials may not be practical. Therefore, developing a biscuit may be attractive to adults because the biscuits will have low levels of fat, sugar and calories and will contain nutrients that reduce diabetes and coronary heart disease risk, which are highly desirable.

Therefore, we decided to formulate a biscuit with a high content (at least 10%) of L-arginine completely bioavailable (> 99%) with low amount of sugar which was manufactured by a low-temperature technological process. The aim of the present study was to evaluate the benefit of this new L-arginine-enriched biscuit on endothelial function and insulin sensitivity.

The present study was structured into two different studies. The first consisted of a pilot postprandial study in healthy subjects that was designed to evaluate bio-availability and vascular effects of the L-arginine-enriched biscuits compared with placebo biscuits that were prepared with all of the same ingredients except L-arginine and powdered L-arginine. The second study was a double-blind crossover study that evaluated the effects of the L-arginine-enriched biscuits compared with placebo biscuits, which were given for 14 days as morning and afternoon snacks, on endothelial function, glucose tolerance, insulin sensitivity and insulin secretion in obese subjects with impaired glucose tolerance (IGT) and metabolic syndrome (MS).

2. Methods

2.1. Biscuits

L-Arginine-enriched biscuits were manufactured in a single batch by combining 20% L-arginine Kyowa (Eurosup, Castello D'Agogna, PV, Italy) with 26 % mixed cereal flakes (corn, oat, whole wheat), 18% puffed rice, 17% hazelnuts, and 17% candied orange peel. Approximately 8%–10% water was added to support homogenization. Then, a sonication-based aggregation/shaping process, which is currently being patented, was performed; this technology allows for low working temperatures, thus preventing heat-dependent product modifications, including arginine and other amino acid degradation by Maillard reactions. After manufacturing, the products were dried in a continuous oven at less than 60 °C to increase the shelf life. Finally, the biscuits were appropriately packed and stored at room temperature. Each 10 g biscuit contained at least 1 g L-arginine. Placebo biscuits were prepared as described above without L-arginine addition. The biscuits' proximate composition was assessed by AOAC methods [12]; soluble and insoluble dietary fiber was assessed by the enzymatic–gravimetric procedure [13], and carbohydrates were evaluated as simple sugars [14], total and resistant starch [15]. L-Arginine content was assessed by HPLC [16]; approximately 100% of the added L-arginine was recovered. The volunteers thought that the biscuits had a pleasant taste and did not notice differences in the flavor of the L-arginine-enriched or placebo biscuits, as evaluated by appropriate analogic scale.

The two studies were approved by the local Ethical Committee of the San Raffaele Scientific Institute and were conducted in accordance with the International Conference on Harmonisation guidelines on Good Clinical Practice and the principles in the Declaration of Helsinki. All of the participants provided written informed consent.

2.2. Pilot postprandial study in healthy subjects

2.2.1. Subjects

Seven healthy subjects (2 male /5 female) participated in the study. Their clinical and metabolic characteristics are reported in Table 1. A complete medical history and physical examination including height, weight, waist and hip circumferences and blood pressure measurements were taken for each subject.

2.2.2. Diet and Protocol

All of the volunteers were asked to follow a 2000 kcal/day standard diet as described in Supplementary Table 1 in the online appendix according to LARN [17]. Subjects were also asked to complete a 3-day food diary consisting of two workdays and one holiday before each test to evaluate their short-term food intake. Food diaries were processed using dedicated software (Nutritionist Pro 2.5, Axial System, Stafford, Texas) and were modified by introducing the L-arginine content obtained from the INRAN and USDA databases for more than 700 different food items.

Subjects underwent 3 different tests in random order with at least 14 days in between. During a test, the subjects consumed a 60 g, 171 kcal portion of the L-arginine-enriched biscuits