Nutrition 29 (2013) 81–88

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Increased plasma availability of L-arginine in the postprandial period decreases the postprandial lipemia in older adults

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ARTICLE INFO

Article history:
Received 18 November 2011
Accepted 10 April 2012

Keywords:
Elderly
Fatty acids
Triacylglycerols
Stable isotope tracers
Fat meal

ABSTRACT

Objective: Older adults have exaggerated postprandial lipemia, which increases their risk for cardiovascular disease. We sought to determine the effects of increased plasma L-arginine (L-ARG) availability on the oxidation of ingested fat (enriched with [1,1,1-^{13}C]-triolein) and plasma triacylglycerol (TG) concentrations during the postprandial period in older subjects.
Methods: On one day, eight healthy subjects (67.8 ± 1.3 y old) received an intravenous infusion of L-ARG during the first hour of the postprandial period (L-ARG trial), while on a separate day, and in a randomized order, they received saline (control trial).
Results: The 8-h area under the plasma concentration–time curve describing the postprandial plasma TG concentrations was considerably lower in the L-ARG trial than in the control trial (−4 ± 21 versus 104 ± 21 mg · dL^{-1} · h^{-1}, $P < 0.01$). The rate of the postprandial oxidation of the ingested lipid was not different between the trials, but the average contribution of the ingested oleate to the oleate of the TG of the plasma small TG-rich lipoproteins (Svedberg flotation index 20–400) was lower in the L-ARG trial (11 ± 1 versus 18 ± 2%, $P < 0.01$). L-ARG infusion also decreased the 8-h area under the plasma concentration–time curve of the plasma free fatty acid concentrations derived from the ingested fat compared with the saline infusion (0.77 ± 0.09 versus 1.11 ± 0.08; mmol · L^{-1} · h^{-1}, $P < 0.01$).
Conclusion: Increasing the plasma L-ARG availability during the postprandial period decreases the postprandial lipemia in older adults, in association with a decrease in the postprandial contribution of ingested lipids into TGs of the plasma small TG-rich lipoproteins.

Introduction

Cardiovascular disease (CVD) secondary to atherosclerosis is the main cause of death and a major cause of disability in developed societies [1]. Current evidence suggests that impaired lipid metabolism documented as increased fed-state plasma triacylglycerol (TG) concentrations is an independent predictor for atherosclerosis, and that exaggerated postprandial plasma TG response is positively associated with an increased risk for CVD [2–5]. The magnitude and duration of postprandial lipemia (PPL) are increased in older adults [6–9], which can contribute to the increased risk for CVD in the older population. Approaches that effectively decrease the magnitude of PPL are thus particularly important for older individuals in an effort to prevent or retard metabolic processes that increase the risk for CVD in this segment of the population.

Recent studies have suggested that increasing the amino acids in plasma, specifically L-arginine (L-ARG), may be such an approach. In young healthy subjects, increasing the amino acids in plasma by protein ingestion attenuates the postprandial increase in plasma TG concentrations [10]. In older subjects, Borsheim et al. [11] recently showed that supplementation with essential amino acids and L-ARG decreases the plasma TG concentrations in the postabsorptive state. However, such evidence, related to the metabolism of endogenous lipid in the fasting state, cannot be directly translated into responses

This project has been funded in part by Arizona State University start-up funds (C.S.K.) and by grant R21DK082820 from the National Institutes of Health and grant 1-09-CR-39 from the American Diabetes Association (C.M.).
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doi:10.1016/j.nut.2012.04.010

associated with the metabolism of exogenous (i.e., dietary) lipid in the postprandial state. Nevertheless, this overall evidence provides intriguing support for a role of the plasma L-ARG as a unique amino acid in improving the postprandial plasma lipid metabolism in older individuals.

We previously showed that the postprandial oxidation of the ingested fat at the whole-body level is impaired in older adults [9]. Muscle contributes considerably to whole-body lipid oxidation, and a decreased capacity for substrate oxidation in muscle mitochondria with aging [12] may impair the postprandial oxidation of the ingested fat and contribute to the increase in PPL in these individuals. Indeed, decreased lipid oxidation during the postprandial period has been shown to contribute to increases in PPL [13]. In vitro experiments have documented an effect of L-ARG on increasing the fatty acid oxidation at the level of mitochondria isolated from skeletal muscle [14]. Based on such evidence, increasing the plasma L-ARG availability may provide the means to improve the whole-body oxidative disposal of ingested lipid, particularly in a metabolic circumstance associated with the accumulation of lipid in plasma, such as the postprandial period, and thus attenuate the PPL in older adults.

The present study therefore was undertaken to investigate the effects of an acute increase in plasma L-ARG availability on PPL in apparently healthy older subjects, with a special focus on the effects of L-ARG on increasing the postprandial oxidative disposal of the ingested fat during the 8-h postprandial period. We used a standardized protocol of an intravenous bolus infusion of L-ARG that has been traditionally used to study the effects of L-ARG on various physiologic and metabolic parameters [15–18]. This 1-h bolus infusion of L-ARG rapidly increases (~45-fold) the plasma L-ARG concentrations [15]. After the end of the L-ARG infusion and for the next several hours (i.e., 7 h), the plasma L-ARG concentration remains three- to seven-fold higher than that in the postabsorptive state [19,20]. An intravenous compared with an oral administration of L-ARG allows standardizing the availability of L-ARG in plasma, given the considerable wide range (i.e., 20% to 70%) in the bioavailability of orally administered L-ARG across individuals [21].

Materials and methods

Subjects

Eight, healthy, Caucasian, older men participated in this study after the purpose, procedures, and risks associated with the experiments had been explained and informed written consent was obtained from each subject. All subjects participating in the study were determined to be healthy based on medical history reports, physical examinations, resting electrocardiograms, and routine blood and urine tests. The exclusion criteria included smoking, a body mass index higher than 30 kg/m^2, hypertension, diabetes, heart disease, peripheral vascular disease, history of liver or kidney disease, and use of any prescribed or over-the-counter medications. The physical and clinical characteristics of the subjects are presented in Table 1. The percentage of body fat was determined using bioelectrical impedance analysis. The study protocol was approved by the institutional review board at Arizona State University.

Experimental protocol

All subjects underwent two lipid challenge studies with the ingestion of whipping cream. The studies were carried out on two different days separated by at least 1 wk and were performed in a randomized, crossover fashion. On one occasion subjects received an intravenous infusion of L-ARG (L-ARG trial) after the ingestion of whipping cream; on another occasion, they received a saline infusion instead of L-ARG as a control (CON trial). On both occasions, subjects were instructed to abstain from any form of exercise, maintain their regular diet, and avoid alcohol consumption for 3 d before the infusion study.

Subjects were admitted to the Clinical Research Unit at Arizona State University in the morning at ~06:30 and after at least a 9-h overnight fast. After compliance with the instructions had been verified, the subjects were laid in bed, and an intravenous catheter was inserted into an antecubital vein of each arm for blood sampling and infusions, respectively. An hour later, at ~08:00, a blood sample was collected for baseline measurements, after which the subjects ingested fat in the form of whipping cream (0.4 g of fat/kg of body weight) enriched with [1,1,1-^{13}C]-triolein (4 mg/kg of body weight; Cambridge Isotope Laboratories, Inc., Andover, MA, USA) over 15 min. On average, the participants ingested ~35 g of fat (range 31–41 g). The macronutrient composition of whipping cream (100 g) was 345 kcal, 2.1 g of protein, 2.8 g of carbohydrate, and 37.0 g of fat (23.0 g of saturated fat, 10.7 g of monounsaturated fat, 1.4 g of polyunsaturated fat). Immediately after the fat ingestion, subjects received an 1-h infusion of L-ARG (0.5 g/min; 10% arginine HCl injection; R-Gene 10, Pharmacia & Upjohn Co., New York, NY, USA) designed to mimic the pattern in plasma L-ARG response after L-ARG ingestion [21] or saline.

Blood samples were collected at hourly intervals for 8 h after the ingestion of the fat for the measurement of plasma concentrations of TGs, free fatty acids (FFAs), 3-hydroxybutyrate (3-HB), and insulin. Breath samples for the determination of the rate of oxidation of ingested fat and blood samples for the determination of labeled lipid in TG of TG-rich lipoprotein (TRL) fractions were collected at 2-h intervals during the postprandial period. Plasma was immediately separated by centrifugation (1500 × *g* for 15 min at 4°C). TRL subfractions were then isolated from plasma within 48 h for the determination of the ^{13}C enrichment in TG of plasma TRL with a Svedberg flotation index higher than 400 (i.e., large TRL fraction) that contains primarily chylomicrons, and TRL with a Svedberg flotation index between 20 and 400 (i.e., small TRL fraction) that contains predominately very low-density lipoproteins. The remaining plasma was stored at −80°C and used for the measurement of the blood chemistry parameters indicated earlier and the ^{13}C enrichment of oleate in plasma FFAs. For the determination of the rate of oxidation of ingested fat, rates of expired carbon dioxide (CO_2) were measured for 20 min using a metabolic cart (TrueMax 2400, Parvo Medics, Salt Lake City, UT, USA) immediately before the ingestion of fat and at the 2-h intervals during the postprandial period by having the subjects breathe under a ventilated hood. After each of these measurements, a breath sample was collected into an Exetainer tube purchased from Metabolic Solutions (Metabolic Solutions, Inc., Nashua, NH, USA). For the determination of $^{13}CO_2$ enrichment in the expired air.

Table 1
Physical and clinical characteristics of the subjects ($n = 8$)

Age (y)	67.8 ± 1.3
Weight (kg)	88.4 ± 2.7
Height (cm)	181.3 ± 1.6
Body fat (%)	26.7 ± 1.0
Plasma lipids (mg/dL)	
Triacylglycerols	81.9 ± 11.5
Total cholesterol	182.4 ± 16.1
HDL-C	52.9 ± 4.4
LDL-C	96.4 ± 15.5
Plasma glucose (mg/dL)	92.3 ± 2.8
Plasma insulin (μIU/mL)	6.6 ± 1.1
ALT (IU/L)	26.1 ± 4.9
AST (IU/L)	30.0 ± 6.7
SBP (mmHg)	123.8 ± 4.1
DBP (mmHg)	75.9 ± 1.9

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure
Values are means ± SEM.

Analyses of samples

Large and small TRL subfractions were isolated from plasma by density gradient ultracentrifugation, as described previously [9]. These plasma TRL subfractions were stored at −80°C until analysis. Blood glucose concentrations were determined using an automated glucose analyzer (YSI 2300, YSI Incorporated, Yellow Springs, OH). Commercially available kits were used for the measurement of the concentrations of plasma TG (Sigma-Aldrich, St. Louis, MO, USA), FFA, 3-HB (Wako Chemicals, Richmond, VA), and insulin (ALPCO Diagnostics, Windham, NH, USA). For consistency across variables, these chemistry parameters were measured in the 2-h plasma samples unless otherwise noted. For the measurement of ^{13}C-oleate enrichment in plasma lipids, TGs in large and small TRL subfractions and FFAs in plasma were isolated using thin-layer chromatography as described previously [9]. The ^{13}C-oleate enrichment in the plasma lipids (i.e., their fatty acid methyl esters) was determined using gas chromatography–mass spectrometry (Thermo Scientific Trace GC Ultra-DSQ GC/MS system; Thermo Scientific, West Palm Beach, FL, USA) by selected ion monitoring of