Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1133–1141

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Weight loss associated with reduced intake of carbohydrate reduces the atherogenicity of LDL in premenopausal women☆

Ingrid Lofgren[a], Tosca Zern[a], Kristin Herron[a], Kristy West[a], Matthew J. Sharman[b], Jeff S. Volek[b], Neil S. Shachter[c], Sung I. Koo[a], Maria Luz Fernandez[a,*]

[a]*Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA*
[b]*Department of Kinesiology, University of Connecticut, Storrs, CT 06269, USA*
[c]*Department of Medicine, Columbia University, New York, NY 10032, USA*

Received 27 October 2004; accepted 30 March 2005

Abstract

The effect of a 3-tier intervention including dietary modifications (ie, moderate energy restriction, decreased carbohydrate, increased protein), increased physical activity, and the use of carnitine as a dietary supplement was evaluated on plasma lipids and the atherogenicity of low-density lipoprotein (LDL) particles in a population of overweight and obese premenopausal (aged 20-45 years) women. Carnitine or a placebo (cellulose) was randomly assigned to the participants using a double-blind design. Carnitine supplementation was postulated to enhance fat oxidation resulting in lower concentrations of plasma triglycerides. Seventy women completed the 10-week protocol, which followed a reduction in their energy intake by 15% and a macronutrient energy distribution of 30% protein, 30% fat, and 40% carbohydrate. In addition, subjects increased the number of steps taken per day by 4500. As no differences were observed between the carnitine and placebo groups in all the measured parameters, all subjects were pooled together for statistical analysis. Participants decreased ($P < .01$) their caloric intake (between 4132.8 and 7770 kJ) and followed prescribed dietary modifications as assessed by dietary records. The average number of steps increased from 8950 ± 3432 to 12 764 ± 4642 ($P < .001$). Body weight, plasma total cholesterol, LDL cholesterol, and triglyceride were decreased by 4.5%, 8.0%, 12.3%, and 19.2% ($P < .0001$), respectively, after the intervention. Likewise, apolipoproteins B and E decreased by 4.5% and 15% ($P < .05$) after 10 weeks. The LDL mean particle size was increased from 26.74 to 26.86 nm ($P < .01$), and the percent of the smaller LDL subfraction ($P < .05$) was decreased by 26.5% ($P < .05$) after 10 weeks. In addition, LDL lag time increased by 9.3% ($P < .01$), and LDL conjugated diene formation decreased by 23% ($P < .01$), indicating that the susceptibility of LDL to oxidation was decreased after the intervention. This study suggests that moderate weight loss (<5% of body weight) associated with reduced caloric intake, lower dietary carbohydrate, and increased physical activity impacts the atherogenicity of LDL.

1. Introduction

Coronary heart disease (CHD) is the leading cause of death in the United States. In 1999, CHD claimed over 950 000 lives and resulted in $112 billion expense in direct costs [1]. Elevated concentrations of plasma total cholesterol (TC) (≥240 mg/dL) and LDL cholesterol (LDL-C) (≥160 mg/dL) classify individuals at risk for CHD [2,3]. Since the first recommendations for treatment were made, the number of known CHD risk factors has increased, and treatment has become more aggressive [2-6]. Recently, evidence has demonstrated that measures of LDL atherogenicity, LDL particle size, and oxidation potential, in addition to LDL-C, are associated with CHD incidence and progression [6-11].

Very low density lipoproteins (VLDL) are the initial substrates in the delipidation cascade, which produces primarily 2 LDL phenotypes, pattern A and pattern B. The LDL phenotype is dependent on the amount of hepatic VLDL secreted and which apolipoproteins (apos) are present [10]. Apolipoproteins transport hydrophobic lipids, activate and inhibit lipases and other lipid modifying enzymes, and act as ligands for the receptors responsible for lipoprotein removal from circulation by the liver and extrahepatic tissue [12]. These factors interact to produce

☆ Supported in part by NIH HL70006 to NSS.
* Corresponding author. Tel.: +1 860 486 5547; fax: +1 860 486 3674.
E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.03.019

heterogeneous LDL particles in relation to size, density [9], and apo content [13]. Generally, larger, more buoyant LDL particles characterize pattern A [9]. In contrast, pattern B is typified by smaller, denser, more triglyceride (TG)-rich particles, which are more atherogenic [9] and is associated with a 3-fold increase in CHD risk [14]. In addition, pattern B particles have decreased affinity for hepatic LDL receptors, extended residence time in circulation, increased migration into endothelial cells, increased propensity for oxidation [8,9], and enhanced coagulant activity [15]. Studies have shown that converting from pattern B to pattern A decreases CHD risk [8].

We previously reported that this population of premenopausal overweight/obese women under study were at higher risk for both diabetes mellitus type 2 and CHD [16]. For example, insulin resistance, determined by the homeostasis model assessment [17,18], was present in 37.5% of participants, and 12.5% of these women had the metabolic syndrome [16].

Because lifestyle modifications have been shown to influence expression of pattern A and pattern B LDL [8,10], we decided to explore the influence of dietary modifications, increased exercise, and the use of carnitine as a supplement on plasma lipids and LDL atherogenicity. Carnitine, a necessary component of the system responsible for transporting long-chain fatty acids across the mitochondrial membrane for β-oxidation [19,20], could also increase the use of lipids when provided in supplemental form [21-24]. Our hypothesis was that carnitine supplementation would improve the plasma lipid profile when given in combination with dietary modifications and increased physical activity.

Our second hypothesis was that lifestyle modifications leading to moderate weight loss would alter LDL metabolism resulting in a less atherogenic LDL particle. Consequently, the purpose of this study was to evaluate the effect of a dietary modification (energy restriction and modified macronutrient composition), increased physical activity (measured as increased number of steps per day), and carnitine supplementation on lipoprotein metabolism and LDL atherogenicity.

2. Materials and methods

2.1. Materials

Enzymatic cholesterol and TG kits were obtained from Roche-Diagnostics (Indianapolis, Ind). Acetyl coenzyme A, carnitine acyltransferase, EDTA, aprotinin, sodium azide, 5,5′ -dithiobis(2-nitrobenzoic acid), and phenyl methyl sulfonyl fluoride were obtained from Sigma (St Louis, Mo). Malonaldehyde bis(diethyl acetal) was obtained from Aldrich (Arlington Heights, Ill). Apolipoprotein E and CIII immunoturbidometric kits were obtained from Wako (Osaka, Japan). Carnitine and placebo supplements were provided by Lonza (Lonza Inc, Fair Lawn, NJ).

2.2. Study population

Eighty-five overweight and obese, premenopausal women were recruited from the University of Connecticut and surrounding communities to participate, and 70 completed the 10-week protocol. Reasons for attrition were personal reasons unrelated to the study (n = 5), time constraints because of the study (n = 5), and difficulty with adhering to dietary modifications (n = 5). The participants who dropped out of the study were not significantly different from those who remained in the study in regard to baseline diet and plasma profile. The 70 participants (74% white) who completed the protocol were between the age of 20 and 45 years, and body mass indexes (BMIs) ranged from 25 to 37 kg/m^2. Exclusionary criteria were pregnancy, lactation, and history of CHD, kidney or liver disease, and diabetes. Eight of the women were identified as current smokers, and 52 reported some alcohol consumption. Eight participants were taking a multivitamin, 5 were taking calcium, and 1 participant was taking calcium, vitamins A and E, and folic acid. Twenty-five participants were taking oral contraceptives, 8 were on thyroid medication (stable for at least 2 years), and 1 participant had been taking a cholesterol-lowering medication for over 5 years. Three participants reported anemia, but did not report taking any prescription or nonprescription drugs for the condition. Using the International Physical Activity Questionnaire [25], the majority of participants considered themselves to be sedentary to moderately active.

2.3. Experimental design

The 10-week study protocol was approved by the University of Connecticut Institutional Review Board, and informed consents were obtained from all subjects. All participants were placed on a energy-restricted diet (85% of energy needs based on the Harris-Benedict formula and a small activity factor). The distribution of energy in the diets was 30% protein, 30% fat, and 40% carbohydrate. The carbohydrate content of the diet (ie, 40% of total energy) was lower than current governmental recommendations and was chosen to enhance the likelihood of carnitine supplementation having a positive effect. Carnitine palmitoyltransferase I, the first of the carnitine-dependent long-chain fatty acid transport proteins, is inhibited when carbohydrate consumption is high [19,20]. A moderate (40% of total energy) rather than a more severe carbohydrate restriction was also chosen to improve dietary compliance. Participants received the food and the menus to follow based on their energy level and macronutrient composition. Based on their calculated energy expenditure, we assigned our participants into 5 different groups based on kilojoule intake: 5880, 6300, 6720, 7140, and 7560 kJ/day. An example of a week menu for the 5880-kJ group is presented in Table 1. Each participant also received an Omron HJ-104 pedometer (Omron Healthcare, Inc, Vernon Hills, Ill), which was set