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Original Research

Conjugated linoleic acid prevents high glucose–induced hypertrophy and contractile dysfunction in adult rat cardiomyocytes

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ABSTRACT

Diabetes mellitus is associated with increased risk and incidence of cardiovascular morbidity and mortality, independently of other risk factors typically associated with diabetes such as coronary artery disease and hypertension. This promotes the development of a distinct condition of the heart muscle known as diabetic cardiomyopathy. We have previously shown that conjugated linoleic acid (CLA) prevents endothelin-1–induced cardiomyocyte hypertrophy. However, the effects of CLA in preventing alterations in cardiomyocyte structure and function due to high glucose are unknown. We therefore hypothesized that CLA will have protective effects in an in vitro model of diabetic cardiomyopathy using adult rat cardiomyocytes exposed to high glucose. Our results demonstrate that subjecting adult rat cardiomyocytes to high glucose (25 mmol/L) for 24 hours significantly impaired the contractile function as evidenced by decreases in maximal velocity of shortening, peak shortening, and maximal velocity of relengthening. High glucose–induced contractile dysfunction was inhibited by pretreatment with CLA (30 μmol/L; 1 hour). In addition to contractile aberrations, exposing adult rat cardiomyocytes to high glucose for 48 hours induced cardiomyocyte hypertrophy. High glucose–induced cardiomyocyte hypertrophy was likewise prevented by CLA. The antihypertrophic effects of CLA were abolished when cardiomyocytes were pretreated with the pharmacologic inhibitor of peroxisome proliferator–activated receptor γ, GW9662 (1 μmol/L). In conclusion, our findings show that exposing cardiomyocytes to high glucose results in cardiomyocyte functional and structural abnormalities, and these abnormalities are prevented by pretreatment with CLA and mediated, in part, by peroxisome proliferator–activated receptor γ activation.

Abbreviations: CLA, conjugated linoleic acid; DGKζ, diacylglycerol kinase ζ; +dL/dt, maximal velocities of shortening; –dL/dt, maximal velocities of relengthening; ET1, endothelin-1; PKCε, protein kinase Cε; PPAR, peroxisome proliferator–activated receptor; PBS, phosphate-buffered saline; PS, peak shortening; ROS, reactive oxygen species; TPS, time to peak shortening; TR90, time to 90% relengthening.

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1. Introduction

Diabetes mellitus is a common chronic condition that is a major global health issue. It is expected that the diabetic population will reach 552 million by 2030 compared with 366 million in 2011 [1]. Individuals with diabetes are predisposed to increased morbidity and mortality because they are at a higher risk for developing cardiovascular complications such as coronary heart disease and heart failure. However, clinical and animal data have also demonstrated that diabetes increases the risk of cardiovascular disease independently of traditional cardiovascular risk factors such as coronary artery disease and hypertension, thereby promoting a distinct disease of the heart muscle referred to as *diabetic cardiomyopathy* [2]. Diabetic cardiomyopathy is characterized by alterations in cardiac structure (eg, cardiac hypertrophy) and function (eg, contractile dysfunction) that present clinically as early-onset diastolic and late-onset systolic abnormalities [3,4]. Pathophysiological contributors to diabetic cardiomyopathy reportedly include hyperglycemia, myocardial lipotoxicity vis-a-vis lipid accumulation, enhanced generation of reactive oxygen species (ROS), impaired calcium homeostasis, myocardial fibrosis, apoptosis, and insulin resistance [5]. Here, we focused on hyperglycemia as a major trigger as several studies link chronic hyperglycemia or poor glycemic control with the development of diabetic cardiomyopathy and the increased risk of cardiovascular events, both in experimental models of diabetes and in diabetic patients [6,7]. Proposed mechanisms include formation of advanced glycation end-products [8], oxidative stress [9], metabolic abnormalities in the heart [10], cardiomyocyte hypertrophy [11], cardiomyocyte contractile dysfunction [12], and cardiomyocyte apoptosis [13].

Conjugated linoleic acid (CLA) belongs to a family of positional and geometric isoforms of linoleic acid that are characterized by the existence of conjugated double bonds [14]. In CLA, the double bonds are situated in the 9 and 11 or 10 and 12 positions and are in *cis* or *trans* configurations [14]. Most of the physiological actions of CLA have been attributed to two isoforms: *cis*-9, *trans*-11-, and *trans*-10, *cis*-12-CLA [15]. Conjugated linoleic acid is found in meat and dairy products from ruminant animals [16]. It has been shown to have numerous biological benefits, including anticarcinogenic [14], antiatherosclerotic [17], and body fat mass–lowering properties [18]. Currently, very little is known about the effects of CLA on the heart structure and function, although cardioprotective effects of CLA may be explained by its antiarrhythmic actions [19]. We have previously reported that CLA has cardioprotective effects both in vitro by preventing markers of hypertrophy stimulated via endothelin-1 (ET1) in neonatal rat cardiomyocytes and in vivo by inhibiting cardiac remodeling as observed by a decrease in cardiac hypertrophy in the spontaneously hypertensive heart failure rats [20]. These protective effects of CLA in cardiomyocytes were attributed to stimulation of peroxisome proliferator–activated receptors (PPARs) [20]. Peroxisome proliferator–activated receptors belong to the nuclear hormone receptor family of transcription factors that function mainly to control the expression of genes involved in fatty acid metabolism [21]. To our knowledge, the protective effects of CLA in the context of diabetic heart disease have not yet been examined.

We hypothesize that CLA prevents high glucose–induced cardiomyocyte aberrations in an in vitro model of diabetic cardiomyopathy. The objectives of this study were as follows: (i) to investigate the protective potential of CLA in preventing high glucose–induced hypertrophy and contractile abnormalities in adult rat cardiomyocytes, (ii) and to investigate the role of PPARγ in the protective effects of CLA against cardiomyocyte hypertrophy in response to high glucose. To test this hypothesis, adult rat cardiomyocytes were pretreated with CLA for 1 hour and then exposed to normal and high glucose concentrations for 24 hours to measure cardiomyocyte contractility, or 48 hours to measure cardiomyocyte hypertrophy. Hypertrophic growth of cardiomyocytes was evaluated by measuring cell size and de novo protein synthesis. Cardiomyocyte contractility was measured using the IonOptix HyperSwitch Myocyte System (IonOptix Inc, Milton, MA, USA).

2. Methods and materials

Conjugated linoleic acid was purchased from Nu-Chek Prep (Waterville, MN, USA). The CLA mixture used in this study consisted of a combination of different CLA isomers (40.7% t10,c12 and 39.1% c9,t11 CLA); as reported by the manufacturer, this preparation also contained other CLA isoforms including the following: 1.8% c9,c11 CLA; 1.3% c10,c12 CLA; 1.9% t9,t11 and t10,t12 CLA; and 1.1% c9,c12 linoleic acid. D-glucose, L-glucose, and chemicals used in the isolation of adult rat cardiomyocytes were obtained from Sigma-Aldrich (Ontario, Canada). [3H]-Leucine was obtained from PerkinElmer Life Sciences (Waltham, MA, USA). GW9662 was from Biomol (Ann Arbor, MI, USA). 5-(6)-Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was from Invitrogen Corp (Carlsbad, CA, USA).

2.1. Animal care

The experimental protocols used in this project were approved by the University of Manitoba Office of Research Ethics and Compliance and Animal Care Committee, and were conducted in accordance with guidelines by the Canadian Council for Animal Care.

2.2. Ventricular cardiomyocyte isolation from adult rat hearts

Adult rat ventricular cardiomyocytes were isolated following the procedure established by us previously [22]. Twelve-week-old male Sprague-Dawley rats weighing between 200 and 250 g were anesthetized with 3% isoflurane and injected with heparin into the saphenous vein (1000 U/mL at 1 mL/kg body weight). The hearts were washed with calcium-free buffer (mmol/L: NaCl 90, KCl 10, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 5.0, $NaHCO_3$ 15, taurine 30, glucose 20, pH 7.4) for approximately 5 minutes and then transferred to a Langendorff apparatus and perfused for 30 minutes with calcium-free buffer containing 179 U/MI collagenase II. After perfusion, hearts were transferred from the Langendorff apparatus and ventricles were excised, minced, and transferred to recirculated collagenase buffer (at 37°C) for further digestion. Individual cardiomyocytes were resuspended in