Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 34 (2016) 8–16

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Conjugated linoleic acid and nitrite attenuate mitochondrial dysfunction during myocardial ischemia

Patrick M. Van Hoose, Natia Qipshidze Kelm, Kellianne M. Piell, Marsha P. Cole*

Department of Biochemistry and Molecular Biology, School of Medicine, University of Louisville, Louisville, KY, 40202, USA

Received 8 January 2016; received in revised form 1 April 2016; accepted 11 April 2016

Abstract

Cardiovascular health is influenced by dietary composition and the western diet is composed of varying types/amounts of fat. Conjugated linoleic acid (cLA) is an abundant dietary unsaturated fatty acid associated with health benefits but its biological signaling is not well understood. Nitrite is enriched in vegetables within the diet and can impact signaling of unsaturated fatty acids; however, its role on cLA signaling is not well understood. Elucidating how nitrite may impact the biological signaling of cLA is important due to the dietary consumption of both cLA and nitrite in the western diet. Since co-administration of cLA and nitrite results in cardioprotection during myocardial infarction (MI), it was hypothesized that cLA and nitrite may affect cardiac mitochondrial respiratory function and complex activity in MI. C57BL/6J mice were treated with cLA and nitrite for either 10 or 13 days, where MI was induced on day 3. Following treatment, respiration and complex activity were measured. Among the major findings of this study, cLA treatment (10 days) decreases state 3 respiration *in vivo*. Following MI, nitrite alone and in combination with cLA attenuates increased state 3 respiration and decreases hydrogen peroxide levels. Further, nitrite and cLA co-treatment attenuates increased complex III activity after MI. These results suggest that cLA, nitrite and the combination significantly alter cardiac mitochondrial respiratory and electron transport chain activity *in vivo* and following MI. Overall, the daily consumption of cLA and nitrite in the diet can have diverse cardiovascular implications, some of which occur at the mitochondrial level.

Keywords: Conjugated linoleic acid; Myocardial infarction; Nitrite; Mitochondria; Diet; Cardiovascular health

1. Introduction

Diet has diverse effects on overall well being and is important with regard to cardiovascular health. Diet remains one of the main risk factors for cardiovascular disease and according to the World Heart Federation, dietary changes will impact all other (nondietary) cardiovascular risk factors. While fat is regularly consumed, special attention must be paid to not only the amount but also the type of fats ingested, as they have varied effects on overall health. For instance, studies have demonstrated that dietary intervention using unsaturated fatty acids instead of saturated fatty acids reduces LDL cholesterol [1], indicating that the type of fat plays a strong role in the health outcome. The Mediterranean diet, which consists of green leafy vegetables containing nitrite (NO_2^-), is also associated with overall beneficial health outcomes. While a plethora of information exists about the end result of a high fat and the Mediterranean diet, it is important to further elucidate the mechanistic aspects that may contribute to overall health status following these diets.

Mitochondria play an essential role in cell fidelity and are a key contributor to cardiovascular function. Importantly, mitochondria are highly susceptible to changes in dietary consumption. For instance, studies have shown that diet fatty acid composition modifies mitochondrial membrane composition and can alter organelle function, leading to an imbalance in organelle oxidative status [2]. Studies such as these implicate mitochondria as a key player in oxidative stress and show that the status of such stress can be manipulated by dietary intake. Overwhelming evidence suggests that diet plays a vital role in cardiovascular health and that mitochondria are involved; therefore, it is important to investigate the effect of dietary fat and nitrite consumption on mitochondrial function in health and disease.

Conjugated linoleic acid (cLA) is considered a healthy fat and has become a more commonly consumed dietary component due to its predicted health benefits. cLA is an 18:2 unsaturated fatty acid and is abundant in ruminant meat and dairy products. The majority of dietary cLA exists as the cis-9, trans-11 isomer. The dietary intake of cLA within the United States is approximately 0.2 g/day [3], but this can increase as the amount of products containing cLA is consumed. cLA plays a role in lipid metabolism *via* activation of the peroxisome proliferator activated receptor (PPAR) [4] and can directly mediate effects on mitochondria by inhibiting beta oxidation [5], increasing carnitine palmitoyltransferase I activity [6] and mitochondrial citrate carrier protein expression [6]. Since cLA is consumed in the diet, it

* Corresponding author. University of Louisville, Department of Biochemistry and Molecular Biology School of Medicine, 580 South Preston Street, Delia Baxter II Building, Room 221B, Louisville, KY, 40202, USA. Tel.: +1-502-852-7157.

E-mail address: marcie.cole@louisville.edu (M.P. Cole).

http://dx.doi.org/10.1016/j.jnutbio.2016.04.001
0955-2863/

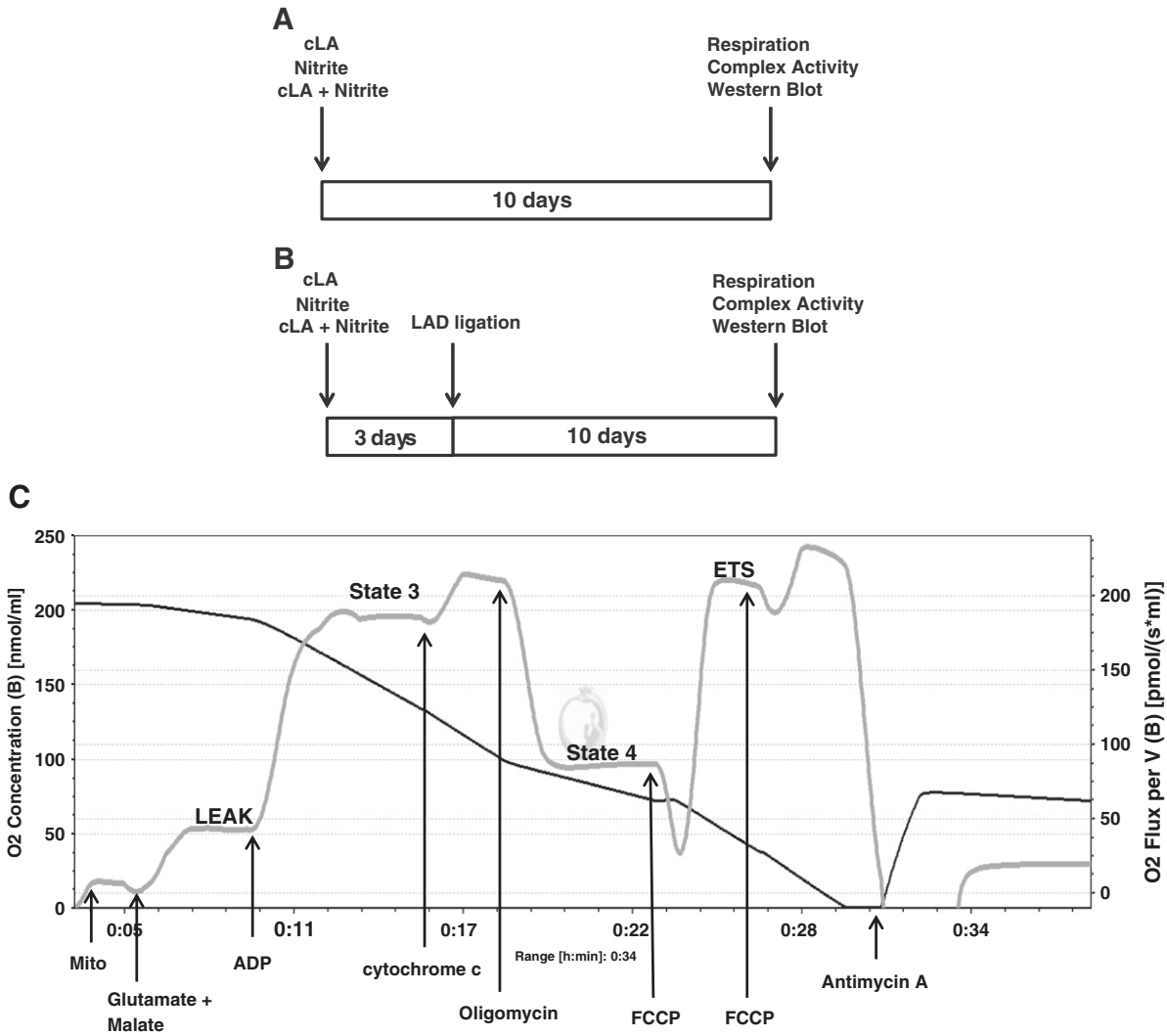

Fig. 1. Experimental design and mitochondrial respiration protocol. (A) Male C57BL/6J mice were treated with cLA, nitrite or cLA + nitrite for 10 days followed by assessment of cardiac mitochondrial respiration and complex activity. (B) Male C57BL/6 mice were pretreated with cLA, nitrite or cLA + nitrite for 3 days prior to ligation of left coronary artery (LAD) and treatment continued for 10 days after ligation followed by assessment of cardiac mitochondrial respiration and complex activity. (C) Mitochondrial respiration monitors simultaneous changes in oxygen concentration (black line, left *y*-axis) and oxygen flux per V (gray line, right *y*-axis) over time following substrate addition where indicated.

becomes important to investigate the biological effects of cLA on cardiac mitochondrial function.

Nitrite is a dietary constituent found within spinach and beet juice. Dietary nitrite represents an endogenous reservoir of nitric oxide (NO), as nitrite can be reduced to NO [7]. NO plays an important role in cardiovascular health due to its role in regulating vascular tone [7] *via* activation of soluble guanylyl cyclase. More recent evidence suggests that nitrite is cardioprotective *via* mitochondrial dependent mechanisms, including inhibition of mitochondrial pore transition opening [8], mitochondrial fusion [9] and posttranslational modifications on complex I [10]. Nitrite has also been shown to mediate changes upstream of mitochondria, including activation of protein kinase A that phosphorylates and inactivates dynamin related protein 1 leading to increased mitochondrial fusion [9]. Nitrite also serves to activate adenine monophosphate kinase [11], a metabolic sensor involved in modulating metabolism. Due to the dietary nature of nitrite and its reported effects on mitochondria *in vitro*, it is important to assess how nitrite influences cardiac mitochondrial function *in vivo*, as well as following myocardial infarction (MI).

Co-treatment of cLA and nitrite rescues cardiac dysfunction in aged mice, as well as in a murine model of MI [12,13]. Based upon these findings, it was hypothesized that cLA, nitrite and the co-treatment would impact cardiac mitochondrial function *in vivo*. This study investigates how cLA, nitrite and co-treatment affect cardiac mitochondrial respiration, electron transport chain (ETC) activity and mitochondrial protein subunit expression of complex I (NDUFB8), complex II (SDHB), complex III (UQCRC2), complex IV (MTCO1) and ATP synthase (ATP5A) *in vivo*, under physiological conditions and during MI. This study establishes a link between the dietary components cLA and nitrite and their impact on cardiac mitochondrial function.

2. Materials and methods

2.1. Animals and experimental design

Male C57BL/6J mice were obtained from Jackson Laboratory and all animal studies were approved by the University of Louisville Institutional Animal Care and Use Committee. Mice were fed standard chow and water *ad libitum* and divided into eight groups: control, cLA, nitrite, cLA/nitrite, MI-control, MI-cLA, MI-nitrite and MI-cLA/nitrite. cLA (cis-9, trans-11 isomer; Nu-Chek Prep Inc.) was given *via* osmotic minipump (20 mg/kg/day for non-MI and 10 mg/kg/day for MI) for 3 or 10 days and sodium nitrite (50 ppm) was supplemented in drinking water.