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Original article

High fat, high sucrose diet causes cardiac mitochondrial dysfunction due in part to oxidative post-translational modification of mitochondrial complex II

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ABSTRACT

Background: Diet-induced obesity leads to metabolic heart disease (MHD) characterized by increased oxidative stress that may cause oxidative post-translational modifications (OPTM) of cardiac mitochondrial proteins. The functional consequences of OPTM of cardiac mitochondrial proteins in MHD are unknown. Our objective was to determine whether cardiac mitochondrial dysfunction in MHD due to diet-induced obesity is associated with cysteine OPTM. *Methods and results*: Male C57BL/6J mice were fed either a high-fat, high-sucrose (HFHS) or control diet for 8 months. Cardiac mitochondria from HFHS-fed mice (*vs.* control diet) had an increased rate of H_2O_2 production, a decreased GSH/GSSG ratio, a decreased rate of complex II substrate-driven ATP synthesis and decreased complex II activity. Complex II substrate-driven ATP synthesis and complex II activity were partially restored *ex-vivo* by reducing conditions. A biotin switch assay showed that HFHS feeding increased cysteine OPTM in complex II subunits A (SDHA) and B (SDHB). Using iodo-TMT multiplex tags we found that HFHS feeding is associated with reversible oxidation of cysteines 89 and 231 in SDHA, and 100, 103 and 115 in SDHB. *Conclusions*: MHD due to consumption of a HFHS "Western" diet causes increased H_2O_2 production and oxidative stress in cardiac mitochondria associated with decreased ATP synthesis and decreased complex II activity. Impaired complex II activity and ATP production are associated with reversible cysteine OPTM of complex II. Possible sites of reversible cysteine OPTM in SDHA and SDHB were identified by iodo-TMT tag labeling. Mitochondrial ROS may contribute to the pathophysiology of MHD by impairing the function of complex II. This article is part of a Special Issue entitled 'Mitochondria'.

1. Introduction

Obesity-related metabolic syndrome increases the risk for metabolic heart disease (MHD), a common cardiomyopathy characterized by impaired energetics [1,2] and myocardial dysfunction [3]. The mechanism responsible for impaired energetics in MHD is not understood. Another potentially important feature of MHD is increased ROS production within the mitochondria [1,4]. While the role of mitochondrial ROS in the pathophysiology of MHD is not known [5], it is increasingly evident that elevated levels of ROS can adversely affect cell function by causing oxidative post-translational modifications (OPTM) that regulate protein activity [6]. Relatively little is known about OPTM of mitochondrial proteins [7], and no prior study has assessed the functional consequences of cardiac mitochondrial protein OPTM in MHD.

We recently described the cardiac phenotype in a mouse model of metabolic syndrome induced by a "Western-style", high fat/high sucrose (HFHS) diet [3]. In these mice HFHS feeding causes MHD with diastolic dysfunction and elevated oxidative stress in the myocardium — both of which are ameliorated by antioxidant therapy [3]. In a preliminary assessment of cysteine OPTM using iodo-TMT multiplex tags we found that HFHS feeding is associated with reversible OPTM of cysteines in several cardiac mitochondrial proteins [8]. Together these observations raised the possibility that impaired energetic function in MHD is due to OPTM of mitochondrial proteins. Accordingly, the goal of this study was to test the hypothesis that impaired ATP synthesis in

Abbreviations: BIAM, biotin-iodoacetamide; DTT, dithiothreitol; GSH/GSSG, ratio of reduced to oxidized glutathione; HFHS, high fat/high sucrose; MHD, metabolic heart disease; OPTM, oxidative post-translational modifications; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SDHA, succinate dehydrogenase subunit A; SDHB, succinate dehydrogenase subunit B; SDHC, succinate dehydrogenase subunit C; SDHD, succinate dehydrogenase subunit D.

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HFHS-fed mice is due to reversible cysteine OPTM of one or more mitochondrial proteins. As a first approach we evaluated the effect of HFHS feeding on mitochondrial function by measuring mitochondrial ATP production, ROS generation, electron transport chain complex function, and the ability of a reducing environment to restore function. We then used the results of the functional analysis to direct the assessment of reversible OPTM using a biotin switch assay and targeted analysis of a dataset in which reversible cysteine OPTM were labeled with iodo-TMT multiplex tags [8].

2. Methods

2.1. Experimental animals

Male C57BL/6J mice 9 weeks of age were fed *ad libitum* either a control chow diet (Research Diets, product no. D09071703, 10% kcal lard, 0% sucrose) or a HFHS diet (Research Diets, product no. D09071702; 58% kcal lard, 13% kcal sucrose) for 8 months. The diets were matched for caloric value and full composition is provided in Table S1. By two months on the diet, HFHS-fed mice weighted more than CD-fed mice and the degree of obesity increased progressively over time through 8 months (Fig. 1S). The protocol was approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

2.2. Mitochondrial isolation

Heart mitochondria were essentially isolated as previously described by us with minor modifications [9]. All steps were performed at 4 °C. Briefly, tissues were rinsed in a buffer containing 100 mM KCl, 5 mM EGTA and 5 mM HEPES at pH 7.0, and thereafter homogenized in 2 ml of HES buffer (HEPES 5 mM, EDTA 1 mM, sucrose 0.25 M, pH 7.4 adjusted with KOH 1 M) using a Teflon-on-glass electric homogenizer. The homogenate was centrifuged at 500 ×*g* for 10 min at 4 °C. The supernatant was then centrifuged at 9000 ×*g* for 15 min at 4 °C and the mitochondrial pellet was resuspended in 100–200 μl of HES buffer with 0.2% of BSA fatty acid-free. Protein was quantified using BCA (Pierce) and the value of HES–BSA buffer alone was subtracted.

2.3. Mitochondrial H_2O_2 production

Mitochondrial H_2O_2 production was measured using the Amplex Ultra Red-Horseradish peroxidase method (Invitrogen) as described previously with minor modifications [10]. This assay is based on the Horseradish peroxidase (2 units/ml) H_2O_2-dependent oxidation of non-fluorescent Amplex Ultra Red (50 μM) to fluorescent resorufin red. In short, 10 μg mitochondria were diluted in 50 μl reaction buffer (125 mM KCl, 10 mM HEPES, 5 mM $MgCl_2$, 2 mM K_2HPO_4, pH 7.44) to determine complex I (pyruvate/malate, 5 mM) or complex II (succinate, 5 mM) driven H_2O_2 production with and without inhibitors (rotenone 2 μM, antimycin A 0.5 μM). Mitochondrial H_2O_2 production was measured after the addition of 50 μl of reaction buffer containing Horseradish peroxidase and Amplex Ultra Red. Fluorescence was followed at an excitation wavelength of 545 nm and an emission wavelength of 590 nm for 20 min. The assay is performed in a 96-well plate using a Tecan M1000 plate reader. The plate is set up so that all experimental conditions for each animal, as well as a standard curve for each run, are acquired and monitored over time simultaneously in duplicate. The slope of the increase in fluorescence is converted to the rate of H_2O_2 production with a standard curve. All of the assays were performed at 25 °C. The results are reported as pmol/min/mg protein.

2.4. Mitochondrial reduced (GSH) and oxidized (GSSG) glutathione measurements

Reduced and oxidized glutathione were simultaneously measured as previously described with minor modifications [11,12]. Briefly, the mitochondria were lysed in the presence of iodoacetic acid (10 mM) and derivatized with fluorescent dansyl chloride. Derivatized samples were separated and analyzed with hydrophilic interaction liquid chromatography on a Restek Ultra Amino 3 μm 100 × 3.2 mm HPLC column

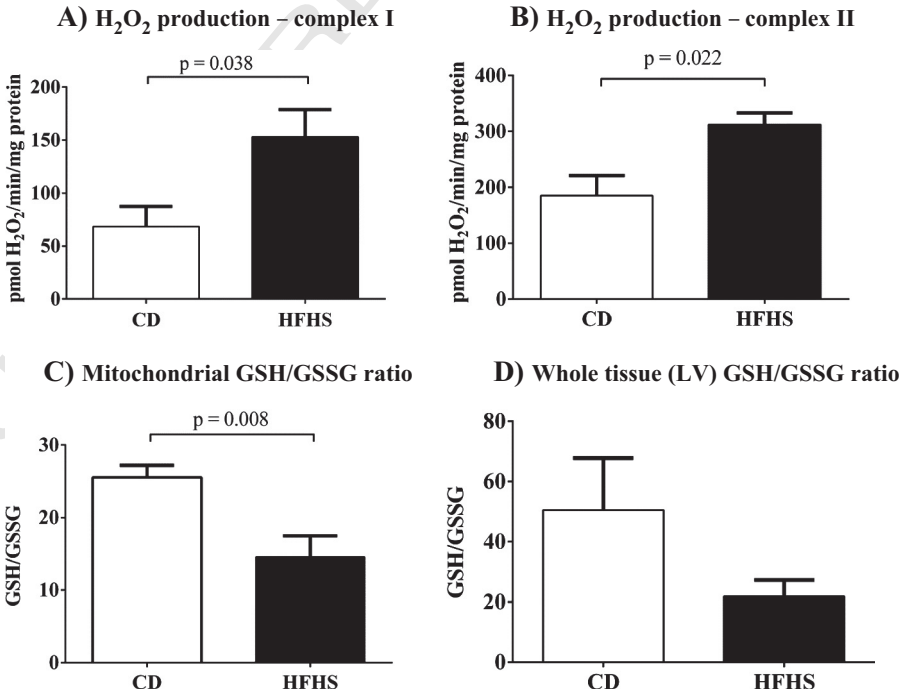

Fig. 1. Increased H_2O_2 production rate and decreased GSH/GSSG ratio in cardiac mitochondria from mice fed a HFHS *vs.* control diet. A) H_2O_2 production rate with a complex I substrate (5 mM pyruvate + 5 mM malate); B) H_2O_2 production rate with a complex II substrate (5 mM succinate) and inhibition of reverse electron transport (2 μM rotenone); C) Mitochondrial GSH/GSSG ratio; D) Whole tissue (LV) GSH/GSSG ratio (n = 4–5 per group).

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