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High-fat diet induces metabolic changes and reduces oxidative stress in female mouse hearts☆

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Abstract

After an acute myocardial infarction, obese patients generally have a better prognosis than their leaner counterparts, known as the "obesity paradox". In addition, female sex is associated with a lower risk of cardiac ischemic events and smaller infarct size compared to males. The objective of the present work was to study the metabolic phenotype and mitochondrial function associated to female sex and short-term high-fat diet. ^{1}H NMR spectra of mice heart extracts were analysed by mRMR variable selection and linear discriminant analysis was used to evaluate metabolic changes. In separate experiments, O_2 consumption and H_2O_2 production were measured from isolated mitochondria as well as serum oxidation susceptibility. Fingerprinting showed that male hearts contained more myo-inositol, taurine and glutamate than female hearts. HFD reduced the levels of creatine, taurine citrate and acetate. Profiling showed increased alanine and fumarate in HFD suggesting altered glycolitic and Krebs cycle pathways. Female mice contained less glucose than males. Female sex nor HFD altered mitochondria oxygen consumption but both conditions reduced the amount of H_2O_2 produced in an additive manner. Serum of females had lower oxidation susceptibility than serum from males but there were no differences associated with HFD. In conclusion, female sex and short-term HFD have an effect on the myocardial metabolic pattern and reduce the amount of H_2O_2 produced by mitochondria in an additive manner suggesting different mechanisms of action. This could explain, at least in part, the protection afforded by female sex and the "obesity paradox".

Keywords: Metabolomics; Antioxidant capacity; NMR; Obesity; Myocardial metabolism; Mitochondrial function; Gender differences

1. Introduction

Cardiovascular risk is a leading cause of death and decreased quality of life despite improvements in treatment over the last decades [1]. Dyslipidemia is a modifiable risk factor associated to cardiovascular diseases through the accumulation of cholesterol esters in coronary atherosclerotic plaques that can lead to an acute cardiovascular event. In order to reduce the incidence of cardiovascular events, guidelines recommend the reduction of dietary lipids, in particular saturated fat, in order to reduce circulating levels of LDL cholesterol [2]. However, once an event has occurred, obese patients generally have a better short- and long-term prognosis than their leaner counterparts with equal cardiovascular burden, including myocardial infarction [3] and acute cardiovascular diseases [4]; this is the so-called "obesity paradox".

Female sex is associated with a reduced risk of developing cardiovascular disease when compared to males [5]. Moreover, studies in animal models of myocardial infarction show reduced damage in females than in males [6] and, in patients with ST-segment elevation myocardial infarction (STEMI), infarct size was smaller in women once corrected for confounding factors [7] highlighting an increased tolerance to ischemia in females as compared to males. This protective effect associated with female sex has been attributed to a hormone effect [8] and to several cellular signalling mechanisms [9]. In addition, there is evidence suggesting sex-related differences in cardiac metabolism [10].

Reactive oxygen species play an important role in the cardiovascular system patho-physiology including cardiac hypertrophy and myocardial infarction [11]. Also, serum oxidative susceptibility is associated with an increased risk of acute myocardial infarction [12]. Diet, and in particular western or high-fat diet (HFD), is associated with increased oxidative stress [13] and is able to modify serum oxidative stress factors [14]. Sex also plays a role in serum

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oxidation susceptibility as men show a higher level of oxidation than women [15].

Metabolomics consists in the unbiased measure of the metabolites in a biofluid or tissue. The metabolome is downstream of the genome and transcriptome [16]. Also, because the metabolome is affected by environmental factors including diet, metabolomics has become an important tool to study dietary interventions [17]. ^{1}H NMR is a robust analytical approach that is easy to automate so it suited for its use in the clinical setting. There are two main approaches to analyse NMR spectra. Fingerprinting treats NMR spectra as curves in order to obtain classifiers able to differentiate between various conditions (*e.g.* cases and controls) without prior knowledge of the metabolites that appear in the spectra. The second approach, known as metabolic profiling, requires the precise measure of metabolite concentration for each of the samples. The heart is a highly metabolic demanding tissue and has been extensively studied using metabolomics [18]; however, there is little information regarding the interaction of dietary fat and sex with metabolic profiles in myocardial tissue.

The objectives of the present work were to investigate whether metabolomics could be used to investigate the effects of short-term HFD on myocardial metabolism. The effects of HFD on mitochondrial function and its association with sex.

2. Methods

2.1. Ethical approval

This study was approved by the Research Commission on Ethics of the Hospital Vall d'Hebron and conform to the "Position of the American Heart Association on Research Animal Use" and the European Directive on the Welfare of Research Animals (2010/63/UE). (Study Reference 30.15).

2.2. Mice

Metabolomic experiments were performed on 23 mice C57BL/6 (Charles River Laboratories) of 16 to 28 weeks of age. Half of the animals (7 males, 5 females) were given a HFD consisting in 35% of fat (lard and soybean oil) accounting for 60% of the calories (Research Diets #D12492, New Brunswick, NJ) ad libitum for 2 weeks. The other half (6 males, 5 females) were given standard chow. A separate group of 24 animals (12 males, 12 females) were fed with either standard chow or HFD and used for mitochondria function experiments. All animals were housed under controlled conditions with a 12:12 h light dark cycle.

After 2 weeks of HFD or standard chow, mice were euthanized by exsanguination under deep anaesthesia (100 mg/kg ketamine, 10 mg/kg xylacine). Blood was obtained by puncture of the left ventricle of the heart; then the heart was rapidly excised, excess blood removed in a bath of ice-cold phosphate saline buffer and used immediately for mitochondria purification or frozen in liquid nitrogen for metabolite extraction.

To obtain serum, blood was allowed to clot over ice and then centrifuged for 5 min at 1500 g and 4C. Serum samples were kept at −80C until further processing.

2.3. Extraction

Metabolite extraction was performed according to the methanol: chloroform protocol as described previously [19]. Briefly, approximately 100 mg of tissue were pulverized with a mortar and pestle at liquid nitrogen temperature, transferred to a glass tube containing 3 ml of methanol:chloroform (2:1) mixture and left to stand on ice for 30 min with occasional mixing. The aqueous and organic phases were separated by the addition of 1.25 ml of chloroform and 1.86 ml of water. The remaining interface was re-extracted at room temperature and both aqueous phases pooled together.

The aqueous extract was lyophilized overnight and stored at -20C until NMR spectroscopy.

2.4. NMR

Prior to NMR, extracts were dissolved in 600 μl of deuterium oxide containing 1 mM of TSP as concentration and chemical shift standard. For serum spectra, 50 μl of serum was dissolved with 450 μl of deuterium oxide.

NMR spectroscopy was performed on a 9.7 T vertical bore magnet interfaced with a Bruker Avance 400 spectrometer. Spectra from extracts consisted in the accumulation of 64 scans with a 1D NOESY pulse sequence with the mixing time adjusted to 100 ms. In the case of serum, spectra were acquired with a CPMG pulse sequence with an effective T2 delay of 32 ms. All spectra were acquired at 30C.

2.5. Metabolic fingerprinting

The aliphatic part of the spectra, between 0.5 and 4.5 ppm was used for metabolic fingerprinting. Feature selection was done using a "minimal redundancy maximal relevance" (mRMR) method [20–23]. This approach provides good interpretability and the possibility of using a simple classifier as Fisher's discriminant analysis (LDA) from the selected variables.

For metabolic fingerprinting each sample spectrum is treated as a curve (that, in fact, is discretized to a high-dimensional vector). Then, the usefulness of the spectra to discriminate with respect to sex and diet is assessed via standard methods of supervised classification, combined with techniques of variable selection. In particular, this approach allows us to identify which variables in the spectra are more relevant for the factors under study.

2.6. Metabolic profiling

Metabolite identification and concentration measurements were done using Chenomx Profiler 8.0 software (Chenomx Inc., Canada). Concentration data is given as micromols per gram of wet tissue.

2.7. Mitochondrial function

Fresh mitochondria were isolated as described previously [24]. Oxygen consumption was measured using a Clark-type oxygen electrode (Hansatech, UK) at room temperature in the presence of malate 2 mM, glutamate 5 mM and ADP 1 mM; oxygen consumption rates were normalized by citrate synthase activity. Hydrogen peroxide (H_2O_2) was determined as fluorescent dimer production of homovallinic acid after oxidation by H_2O_2 catalysed by horseradish peroxidase at 440 nm using a fluorimeter (SpectraMax GeminiXS, Molecular Devices), using malate, glutamate or succinate substrates. Fluorescence values were normalized to values obtained at time zero (F_0).

2.8. Oxidation susceptibility

Oxidation susceptibility of the serum was measured according to the method described by Tynkkynen and cols [25]. Briefly, serum samples were treated with $CuS0_4$ 0.5 mM for 6 h at 37C. Lipid extracted using the chloroform:methanol approach. Lipid oxidation was measured with NMR spectroscopy and, each sample, compared to its own non-oxidized control. Results are given as % of oxidized lipids as compared to its control.