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Short Communication

Grape Juice, Red Wine, Resveratrol and Exercise, In the Expression of FNDC5 and UCP2 in Cardiac and Skeletal Muscles of Wistar Rats Submitted To High-Fat Diet - a

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ABSTRACT

Introduction: Diets high fat can lead to obesity and chronic non-communicable diseases. Genes related to thermogenesis regulation may be a possibilty to obesity and its comorbidities prevention.

Objective: Evaluate grape juice, red wine and resveratrol and exercise effects, in gene expression modulation of FNDC5 and UCP2 in cardiac (CM) and skeletal (SM) muscles of Wistar rats.

Method: Rats Wistar, 90 days, 230g, divided into 05 groups (n=10/group): a) Control Group (CG) b) High Fat Group (HG); C) Resveratrol Group (RG) (15mL resveratrol solution 4%/animal/day); D) Grape Juice Group (JG) (15mL/animal/day); E) Red Wine Group (WG) (10mL/animal/day); HG, RG, JG and WG received a high fat diet (20%). The animals performed a treadmill running protocol, speed 10m/min for 10 minutes, 5 days/week, for 60 days. After, they were anesthetized; CM and SM were removed for PCR analysis. Results were expressed as mean ± standard deviation. ANOVA one way and Tukey as post test were performed, and a $p < 0.05$ was considered significant.

Results: FNDC5 on CM: RG = 2,0 ± 0,8; JG = 2,1 ± 0,43 and WG = 1, 7 ± 0,5 displayed higher expression than CG = 0, 7 ± 0,4. FNDC5 on SM: JG = 1, 6 ± 0,6 displayed higher expression when compared to CG = 0, 63 ± 0, 2. UCP2 on CM: RG = 0,4 ± 0,1 and WG = 0, 4 ± 0,27 lower expression when compared to CG = 0, 8 ± 0, 26. UCP2 on SM: RG = 1, 9 ± 1, 4; JG = 2, 4 ± 1, 7 e WG = 2, 7 ± 0, 8 presented greater expression when compared to CG = 0, 5 ± 0, 4.

Conclusion: Polyphenol-rich beverages associated with exercise were able to modulate FNDC5 and UCP2 genes expression in CM and SM.

Keywords: Resveratrol; Grape juice; Red wine; Gene expression; Cardiac muscle; Skeletal muscle; Thermogenesis

ABBREVIATIONS

FNDC5: Fibronectin Type III Domain Containing protein 5; UCP2: Uncoupling Protein 2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GC: Control Group; HG: High-Fat Group; RG: Resveratrol Group;JG: Grape Juice Group; WG: Red Wine Group; CM: Cardiac Muscle; SM: Skeletal Muscle; RT-qPCR: Real Time quantitative Polymerase Chain Reaction

INTRODUCTION

Consumption of a diet rich in saturated fat and high energy density, along with a sedentary lifestyle are responsible for overweight and obesity in individuals, in addition of causing various chronic noncommunicable diseases (CNDs) such as cardiovascular diseases, representing a major public health problem [1-3].

It is widely known in the literature that the beneficial effects are attributed to polyphenols present in red wine and grape juice, specially the main component, resveratrol. This effect include the ability to inhibit the production of reactive oxygen species, cardioprotective antioxidant effects on myocardial ischemia, and protection from apoptosis caused by oxidative damage in cardiovascular disease and antioxidants and anti-inflammatory effects, act on atherosclerosis prevention. Accordingt these results, red wine, grape juice and resveratrol has cardioprotective actions in different situations caused by oxidative stress [4-8].

Induction of FNDC5 gene expression may lead to a higher irisine concentration and consequent thermogenesis increase, with higher activity of uncoupling proteins (UCPs) [9]. Increased energy expenditure through thermogenesis may induce weight loss for individuals [10]. Based on the above information, studying mechanisms inducing genes expression related to thermogenesis becomes relevant. Therefore, the aim of this study was to evaluate consumption of grape juice, red wine, resveratrol and physical exercise effects on gene expression modulation of FNDC5 and UCP2 in skeletal and cardiac muscles of Wistar rats submitted to a high fat diet.

METHOD

Adult female Norvergicus Wistar Albinus rats (90 days). All experimental procedures used during these experiments complied with the guidelines of the Ethics Committee for the use of laboratory animals, protocol number Comissao de Etica no Uso de Animais-CEUA473/2013.

Fifty rats were used and divided into 5 groups (n = 10/ group): 1) Control Group (CG) receiving balanced diet, based on casein (AIN93M); 2) High-Fat Group (HG) receiving high-fat diet (20%), casein-based; when the usal fat content is 4 - 8%. Resveratrol Group (RG) receiving high-fat diet (20%), casein-based and 15 mL of a resveratrol solution 4% (diluted water) per day; 4) Grape Juice Group (JG) receiving high-fat diet (20%), casein-based and 15 mL of whole grape juice per day and 5) Red Wine Group (WG), receiving high-fat diet (20%), casein-based and 10 mL of red wine per day. All animals (all groups) received diet and filtered water ad libitum and followed for 60 days. Both whole grape juice (Aurora Tinto Integral®) and red wine (Goes Tempos®) were purchased at the local market; the resveratrol solution was prepared every day and dispensed to the animals. Experimental control diet composition was made according to AIN93M recommendations. High fat diet was made of lard, which was purchased at the local market. Animals underwent physical training on a treadmill at a constant speed of 10m/s for 10 minutes, 5 times a week throughout the whole experiment.

After 60 days of protocol, after overnight fasting, animals were anesthetized and sacrificed. Cardiac and skeletal muscles were collected for analysis.

Tissue samples were collected, homogenized in TRIzol® and frozen at -80°C. cDNA synthesis was carried out using a two-step cDNA synthesis kit (Promega), using UCP2 and hFNDC5 primers [11,12] (Table 1). One microgram of RNA was reverse transcribed into cDNA using GoScript™ reverse transcriptase (Promega) according to the manufacturer's protocol using a total reaction of 20 mL. Real time quantitative PCR (RT-qPCR) was performed using 5 mL of Gotaq qPCR Master Mix (Promega). For determination of the initial relative

quantity of cDNA, samples were amplified with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (reference gene).

Variables were expressed as mean ± standard deviation. For comparison of means among groups, the one-way analysis of variance (ANOVA) test was performed, and Tukey as post-test. A $p < 0.05$ was considered significant. GraphPad software (version 3.00 for Windows XP, GraphPad Software) was used to perform statistical analysis.

RESULTS AND DISCUSSION

Figure 1A displays FNDC5 expression in the skeletal muscle. It's possible to observe higher FNDC5 expression in JG skeletal muscle in relation to the others groups.

Studies have shown polyphenol-rich beverages ability on FNDC5 and UCP2 expression modulation in Wistar rats skeletal muscle submitted to a high fat diet [8]. Rocha et al. [13] showed increased FNDC5 expression in SM along grape juice or resveratrol consumption, however, in the present study, when these beverages consumption was associated with physical exercise, increase FNDC5 expression was observed only in with grape juice consumption. Therefore, it's suggested that physical exercise may, somehow, potentiate the effect of grape juice on FNDC5 gene expression in SM, something not observed when receiving resveratrol associated with physical exercise. This association between resveratrol and physical exercise has not yet been fully elucidated in the literature, however further studies are needed on this association [14,15].

Figure 1B displays skeletal muscle UCP2 expression. This expression was higher in groups receiving drinks rich in polyphenols (RG, JG and WG).

Rocha et al. [13] observed higher expression of UCP2 in groups consuming high fat diet, red wine or resveratrol. It is known that high fat diets consumption may be responsible for regulating the increased UCP2 muscle expression, through increased lipid oxidation [16].

Table 1: Primer sequences for cDNA of hFNDC5 and UCP-2 [11,12].

Gene	Forward primer	Reverse primer
hFNDC5	Aagcacaaggactgactcaagc	catgtccttgatggctggat
UCP2	Gctcgtaatgccattgtca	acagtggccagcgctactgta

Jia et al. [17] observed during a diet with a high lipid content consumption, and a consequent increase in non-esterified fatty acids, may be related to muscle tissue response in increasing UCP2 activity.

However, a high fat diet associated with exercise in the present study did not show an UCP2 increased expression in HG group, although groups receiving wine or resveratrol (WG or RG) maintained a higher expression compared to control group. In addition, JG also had an increased skeletal muscle UCP2 expression, suggesting an ability of grape juice association with exercise gene expression modulation.

In figure 2A, cardiac muscle FNDC5 gene expression was higher in RG, JG and WG groups compared to CG.

FNDC5 gene expression could be regulated by others mechanisms besides physical exercise, and evidence suggests heart failure inflammation reducing this gene expression [18,19]. In addition to its importance as hormone irisine precursor, FNDC5 role in cardiomyocytes differentiation may be a new direction for an application in cardiac damage regeneration in heart failure situation [9,20-22]. In this context, this gene expression modulation in cardiac muscle by beverages associated with physical exercise may have therapeutic properties for damages in cardiac tissue.

The figure 2B displays cardiac muscle UCP2 gene expression. It was observed lower UCP2 gene expression in RG and WG compared to control group. These results suggest EROs and possible oxidative stress presence as a cause of myocardial dysfunction, since a higher expression of UCP2 in this muscle is related to protection against oxidative stress [23].

UCP2 gene can be expressed in several tissues and shows different functions [24,25], thus, mechanisms investigation on their expression modulation are essential for better elucidation on this gene functions and effects.

CONCLUSION

The association among polyphenol-rich beverages and physical exercise has been shown to be relevant in FNDC5 and UCP2 gene expression modulation having an important role in thermogenesis and cardiovascular system.

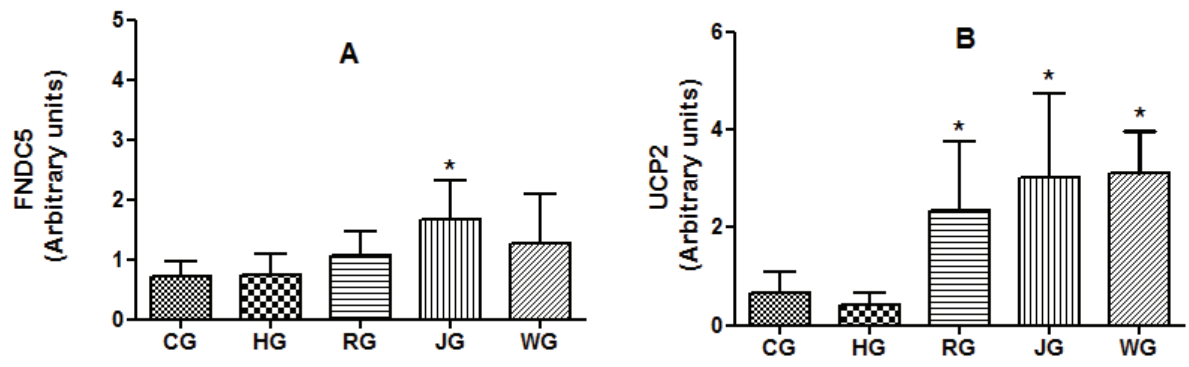

Figure 1: A) FNDC5 gene expression in skeletal muscle and B) UCP2 gene expression in skeletal muscle Control Group (CG); High-Fat Group (HG); Resveratrol Group (RG) Grape Juice Group (JG); Red Wine Group (WG) (n-10/group). Results are presented as mean standard deviation. Significant when p<0.05 (*). GAPDH was used as the reference gene.

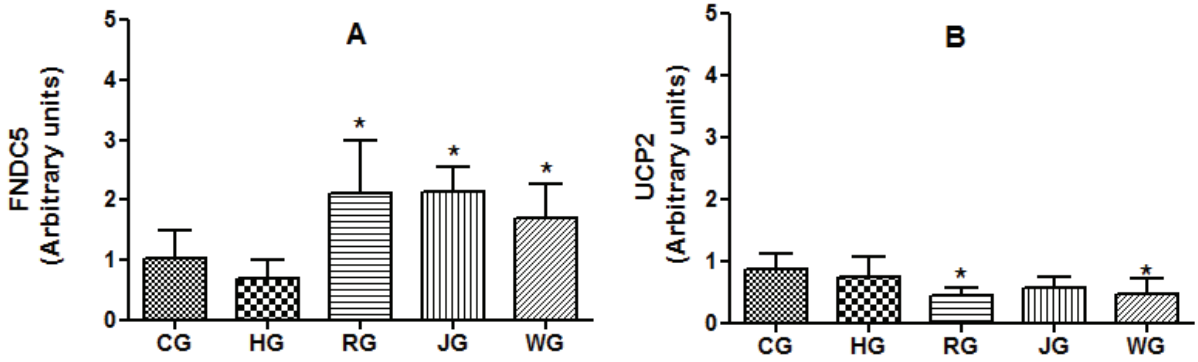

Figure 2: A) FNDC5 cardiac muscle gene expression and B) UCP2 cardiac muscle gene expression: Control Group (CG); High-Fat Group (HG); Resveratrol Group (RG) Grape juice Group (JG):; Red wine Group (WG)(n=10/group). Results are presented as mean standard deviation. Significant when p<0.05(*)/. GAPDH was used as the reference gene.

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