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Original Article

EFFECT OF HIBISCUS SABDARIFFA LINN METHANOLIC EXTRACT ON HEART HYPERTROPHY INDEX AND PGC-1α IN OVERTRAINED RAT

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ABSTRACT

Objective: Studies have shown that prolonged physical exercise increases ventricular wall mass. Physiologically, this increase is followed by an increase of mitochondrial biogenesis. However, increases of ventricular mass in some cardiovascular diseases are not followed by an increase of PPARγ coactivator-1α (PGC-1α), a marker of mitochondrial biogenesis. No data regarding cardiac PGC-1α during an excessive physical exercise program that causes pathological conditions (overtraining) are available. Thus, we aimed to determine the effect of overtraining on cardiac hypertrophy index and PGC-1α level. Furthermore, we aimed to elucidate the cardio protective effect of *Hibiscus sabdariffa Linn*. (HSL) administration on these cardiac parameters.

Methods: Twenty-five male adult Wistar rats aged 8–10 w were randomly divided into five groups: control (C), control-HSL (C-HSL), aerobic training (A), overtraining (OT), and overtraining-HSL (OT-HSL). Treatments were conducted five times a week, for 11 w. Differences in heart mass were determined by measuring ratios of ventricular weight to body weight (hypertrophy index). PGC-1α levels were measured using an ELISA method.

Results: We found that overtraining increased ventricular wall mass; however, it did not increase cardiac PGC-1α levels, whereas mild-aerobic exercise robustly increased cardiac levels of PGC-1α. Furthermore, administration of a methanol extract of HSL did not show any significant effect on cardiac mass or PGC-1α level.

Conclusion: Thus, our study showed that ventricular hypertrophy elicited by overtraining conditions was not followed by an increase in cardiac PGC-1α, and administration of *H. sabdariffa* extract did not ameliorate this condition.

Keywords: Overtraining, Hypertrophy, *Hibiscus sabdariffa Linn.*, PGC-1α

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INTRODUCTION

The purpose of physical training, by and large, is to enhance physical performance. At whatever point the power, length, and repetitiveness of work begin to burden proper body function, physiological adjustments occur. Successful sports athletes are often faced with long-term training, especially when preparing for a match, that frequently causes a condition called overtraining (OT). OT can be characterized as an increase in exercise intensity that results in a significant loss of diminished achievement or described performance of the sport, and can be associated with mood disturbance. Endless resistance exercise can result in various reactions to OT contingent upon either training duration or excessive power exertion [1].

Physiologically, an increase in the workload of the heart during long-term physical exercise can lead to cardiac adaptation that causes structural and functional changes in heart tissue. Such physiological adaptations to environmental conditions can cause the heart to grow or shrink. In OT, physiological adaptation is expected to become delayed or even produce a pathological adaptation of the cardiovascular system. The heart can increase in size depending on the type, power, and duration of stimulation, resulting in physiological or pathological hypertrophy [2]. The heart can also incrementally decrease in size contingent upon the type, quality, and length of incitement that brings about physiological or neurotic hypertrophy [2].

Hypertension is related to unfavorable morphological and functional changes in the cardiovascular framework, including left ventricular hypertrophy (LVH). Epidemiological examinations demonstrate that prevalence of LVH in Chinese patients with basic hypertension was about 25% to 35%. LVH is viewed as a free hazard factor for cardiovascular disorders and heart failure [3]. Physiological hypertrophy is characterized by normal or enhanced contractile function coupled with normal architecture and organization of cardiac structure. Pathological hypertrophy is associated with increased cardiomyocyte death and fibrotic remodeling, and is characterized clinically by reduced systolic and diastolic function that often progresses towards heart failure [2]. As a macroscopic result, morphometric methods are needed whenever there is the necessity to describe and compare shapes of organisms or particular structures within living beings, including to assess changes in heart size [4].

Heart workload requires more ATP to maintain cardiac contractility, there is evidence that contractility disorders can be at risk for heart failure. The past decade has seen a resurgence of enthusiasm for heart mediators, as well as enormous advances in our comprehension of transcriptional systems that direct cardiovascular energetics. The dynamic range of cardiac activity is large, either exercise (acutely), and development, especially postnatal (chronically). As such, bioenergetic programs in the heart should be firmly controlled along these lines. PPARγ coactivator-1α (PGC-1α), whose expression and action are perfectly attuned to extracellular and physiologic signals, assumes a focal job in these pathways. For example, at the gene expression level, PGC-1α is stimulated in liver and heart by fasting, brown fat by cold-induced sympathetic stimulation, and skeletal muscle and heart by exercise [6]. The results of research conducted by Finck (2007), indicate that PGC-1α plays an important role in controlling cardiac energy metabolism and suggest that perturbations in the PGC-1α system could influence to cardiomyopathic remodeling. PGC-1α overexpression prevented cardiac hypertrophy or improved contractility in cultured cardiac myocytes [7]. Moreover, a study by Pereira et al. (2014) showed that PGC-1α is repressed in concert with reduced mitochondrial oxidative capacity and fatty acid oxidation (FAO) during pathological hypertrophy [8].

Hibiscus sabdariffa Linn. (HSL), or roselle, is an herbal plant used for cancer prevention, controlling blood pressure, and improving bowel movements. The most important ingredients in roselle flower are gassypetin, glucoside hibiscin, and anthocyanin–an antioxidant and

natural pigment that gives roselle infusion its red color. High levels of antioxidants in roselle petals can inhibit free radicals. These substances are believed to function as diuretics, reduce blood viscosity and blood pressure, and stimulate intestinal movements [9]. In the literature, only one previous study focused on the direct effects of anthocyanins to prevent cardiovascular disease [10].

Understanding the effects of OT on various organs is useful for determining amounts of exercise for athletes and approaches needed to reduce the risk of OT, for example by using the herbal supplement HSL. There is currently no research on the effects of overtraining on PGC-1α levels and cardiac mass changes in the heart, nor the potential related effects of HSL. Therefore, this study examined the effects of OT on PGC-1α levels and hypertrophy index changes in the heart, as well as the effects of HSL.

MATERIALS AND METHODS

Animals

Male Wistar rats (*Rattus norvegicus*), aged 8–11 w and with body weights ranging from 200–250 g were purchased from Faculty of Animal Science at the Institute Pertanian Bogor (Bogor, Indonesia). Animals were randomly divided into five groups: control (C, sedentary rat administered placebo), control-HSL (C-HSL, sedentary rat administered *H. sabdariffa* extract), aerobic training (A, subjected to aerobic program and administered placebo), OT (subjected to OT program and administered placebo), and OT-HSL (subjected to OT program and administered *H. sabdariffa* extract). Exercise was conducted 5 times a week, for 11 w. Rats were maintained on a 12-h light/dark cycle, with free access to food and water. All efforts were made to maintain animal welfare. Approval for this study was obtained from the Ethics Committee of Medical Research-Faculty of Medicine Universitas Indonesia/Cipto Mangunkusumo Hospital (FMUI/RSCM) No: 0955/UN2. F1/ETIK/2018.

Extract

Methanolic extract of *Hibiscus sabdariffa L.* was obtained from Pusat Studi Biofarmaka (PSB)-Institut Pertanian Bogor. Extract of *Hibiscus sabdariffa L.* was extracted from its calyxes. Administration of this extract was performed orally with a dose of 500 mg/kg, five times a week for 11 w, three hours before physical exercise training.

Physical exercise protocol: overtraining and aerobic programs

Rats underwent acclimatization for two weeks before commencing physical exercise programs. Rats that met inclusion criteria were acclimated to running on the treadmill during an adaptation procedure.

Overtraining program

The OT program corresponded to a protocol developed by Hohl [5]. The duration of the program was 11 w. First, rats underwent an adaptive phase of running five times a week for 4 w, with 24-h recovery time. This phase started with low-speed and short-duration running (10 m/min for 10 min) which was progressively increased every week until the velocity and duration reached 20 m/min for 60 min. Then, OT rats we submitted to a second adaptive phase for another 4 w. During this phase, the velocity and duration was fixed at 20 m/min and 60 min to adapt mice to a stable training load. For the last 3 w, the frequency of daily exercise sessions was increased to two-, three-, and four-fold followed by a reduction of recovery time between training sessions (4 h, 3 h, and 2 h, respectively) to create an imbalance between overload and recovery. The duration of running was measured manually using a stopwatch.

Aerobic exercise program

Rats that underwent the aerobic program were exercised on the treadmill with a constant velocity and duration twice a week for 11 w. The dose of running corresponded to aerobic exercise (i.e. 12 m/min for 10 min for each session).

Measurement of body weight, cardiac hypertrophy, and PGC-1α level

Twenty-four hours after the last training session, rats were sacrificed. Prior to sacrificing, body weights of rats were obtained. Hearts were removed from the mediastinum and aorta of each rat and all epicardial fats were carefully removed. Hearts were rinsed in phosphate-buffered saline (0.01M, pH 7.2–7.4) to remove excess blood. The heart was wiped with a towel and weighed as a whole heart measurement. Next, the atrium was removed and the remaining portion was measured as the ventricular weight. Subsequently, the left and right ventricles were separated by tracing the anterior and posterior longitudinal sulcus. The left ventricle, including the interventricular septum, was measured to determine left ventricular weight. A Ventricular Hypertrophy Index (VHI) was determined by the proportion of ventricular weight/body weight, while a Left Ventricular Hypertrophy Index (LVHI) was determined by the proportion of left ventricular weight/body weight. Both ratios are presented as percentages.

Heart tissues were then homogenized and analyzed for PGC1-α levels according to the manufacturer's instructions for the PGC1-α ELISA kit (CUSABIO, Wuhan, China).

Statistics

Data processing was performed using Prism 6.0 software (GraphPad, San Diego, CA). Body weight, VHI, and LVHI were evaluated with one-way ANOVA test followed by Tukey *post hoc* analyses to account for multiple comparisons between groups. Data were previously analyzed for normality with a Kolmogorov Smirnov test. All data are presented as means±standard error of the mean (SEM). For all comparisons, values of $p<0.05$ were considered significant.

RESULTS

Characteristic rat body weights among groups

Rat body weights were obtained as one parameter for determining VHI and LVHI. Before starting the physical exercise program, rats were randomly divided such that there was no difference in rat body weights among groups (fig. S1).

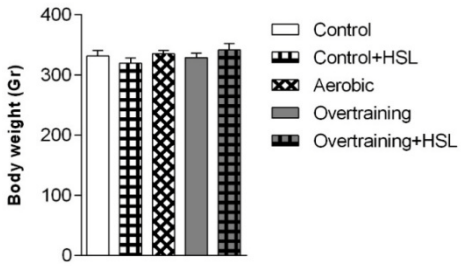

Fig. S1: Comparison of rat body weight among groups before intervention. There were no significant differences in rat body weights among groups. (Data represent mean±SEM; n = 5 rats/group. One-way ANOVA)

At the last training session, as expected, we found that OT rats (327±12.4 g) had lower body weights than rats in control (376.2±12.4 g, $p<0.01$) or aerobic (415.4±12.4 g, $P<0.001$) groups (fig. 1).

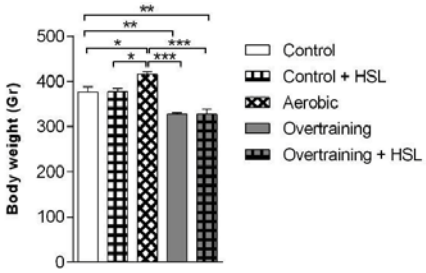

Fig. 1: Overtraining program caused a reduction of body weight, while administration of *H. sabdariffa* did not affect body weight, Overtraining condition rats had lower body weights compared with control and aerobic groups. Rats with aerobic exercise programs had the highest body weight among the groups. Administration of *H. Sabdariffa* had no effect on body weight in control or overtraining conditions. (Data represent mean±SEM; *n* = 5 rats/group. *p<0.05, **p<0.01, ***p<0.005 one-way ANOVA followed by Tukey post hoc analysis)

Rats undergoing aerobic training had slightly higher body weights. Administration of HSL did not affect the body weight of rats in control (376.2±12.4 g vs 376.8±12.4 g with HSL) or OT (327±12.4 g vs 326.8±12.4 g with HSL) groups. Thus, our OT program caused a reduction of body weight, one symptom of OT syndromes, and administration of HSL did not affect body weight.

Overtraining rats had increased cardiac mass compared with control and aerobic rats

Physiologically, an increase in cardiac workload during long-term physical exercise can lead to structural and functional adaptations of the heart. To observe cardiac mass changes in the heart, we calculated ratios of ventricular weight/body weight and left ventricular weight/body weight (as percentages). We found that OT rats (0.268±0.01049) had the highest ratio of ventricle weight to body weight compared with control (0.232±0.0058, p<0.05) and aerobic (0.2252±0.0104, p<0.019) groups (fig. 2A). We also found ratios of left ventricular weight/body weight in OT rats (0.2172±0.01073) were higher than aerobic rats (0.1774±0.01073, p<0.05; fig. 2B). Altogether, our data indicate that cardiac morphometric changes occurred in the OT condition, specifically an increase of ventricular mass (particularly left ventricle) representative of hypertrophic ventricular conditions.

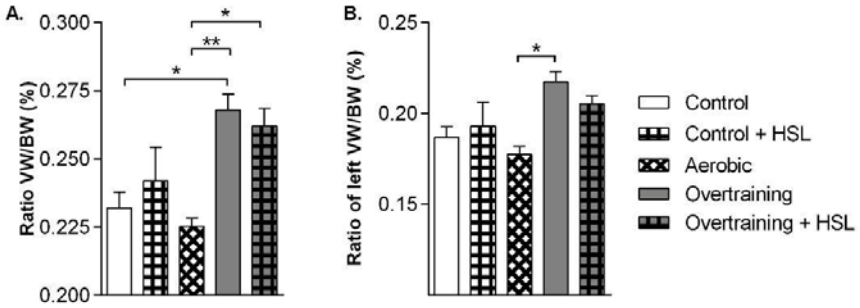

Fig. 2: Overtrained rats had the highest ratios of ventricle weight to body weight (%) and left ventricle weight to body weight (%). *Hibiscus sabdariffa L.* administration did not affect ratios in control or overtraining groups. (A) Comparison of ratios of ventricle weight/body weight percentages among groups. (B) Comparison of ratios of left ventricle weight/body weight percentages among groups. (Data represent mean±SEM; *n* = 5 rats/group. *p<0.05, **p<0.01, one-way ANOVA and Tukey post hoc)

Overtrained rats have lower levels of cardiac PGC-1α compared with aerobic rats

One factor that mediates cardiac responses to training conditions is proliferator-activated gamma coactivator 1-alpha receptor (PGC-1α), which is involved in mitochondrial biogenesis and metabolism in heart and skeletal muscle. In response to exercise training, PGC-1α production increases with increasing mitochondrial function. Our results showed lower PGC-1α levels in OT rats (5.87±3.621) compared with aerobic-trained rats (23.79±3.621, p<0.005). Thus, with overtraining, the pathological hypertrophy that occurs suppresses PGC-1α levels in the heart.

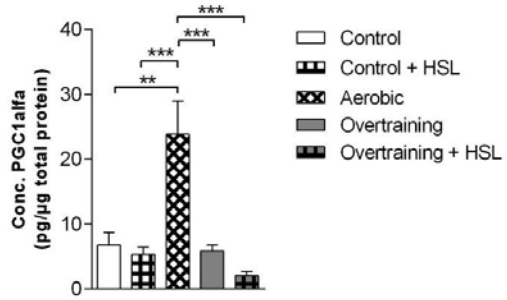

Fig. 3: Overtraining program did not increase cardiac levels of PGC-1α in contrast to an aerobic exercise program, nor did *H. sabdariffa* administration. Overtrained rats had lower concentrations of PGC-1α compared with others groups. Instead, the aerobic group had the highest concentration of PGC-1α. *H. sabdariffa* administration did not affect the concentration of PGC-1α in control or overtraining groups. (Data represent mean±SEM; *n* = 5 rats/group. **p<0.01, ***p<0.005, one-way ANOVA and Tukey post hoc)

DISCUSSION

Physical training is performed to increment and enhance physical performance. Physiological adjustments occur based on the force, duration, and daily work burden. Physiological adaptation of the heart to environmental conditions can cause the heart to grow or shrink. However, there is a genuinely unobtrusive delimitation between an outstanding performance and a decline resulting from overtraining (OT). Symptoms of OT may include muscular weakness and lesion, cytosine initiation, hormonal and hematological adjustments, mood swings, psychological depression, and nutritional disorders. In view of the premise that OT results from an imbalance between training and recuperation, neuroendocrine disruption has been proposed as a fundamental mechanism underlying OT [1].

Rats were weighed every day throughout the course of treatment, from the first week to the eleventh. We attempted to compare differences between average body weights among the five study groups. Differences in body weight were assessed based on changes in initial body weight until the eleventh week. From the results shown in fig. 1, body weights in the aerobic group were higher than in control and OT groups. This is consistent with findings described in Sengupta et al. (2003) suggesting that in the age range until maturity, training results in musculoskeletal maturity and increased muscle mass [11].

Fig. 1 also shows that weights of rats in the OT group were lower than in control and aerobic groups. Notably, this decrease was in line with Hohl's (2009) study indicating a natural decline in body weight in OT rats is a positive adaptation to physical exercise, although after reaching the eleventh week, weight continued to decline. Several studies have concluded that weight loss or loss of body mass can be used as a likely symptom or marker of OT in humans. According to Hohl (2009), this body weight decrease was related to hyper metabolism and proteolysis as a result of persistent physical workload [12].

Hypertrophy can be assessed functionally or anatomically, with an increase in heart mass being the most commonly assessed anatomical indicator. The simplest benchmark for assessing cardiac hypertrophy macroscopically is the weight of the heart and the left ventricle [13, 14]. The heart can increase in size depending on the type, strength, and duration of stimulation, resulting in physiological or pathological cardiac hypertrophy [2]. Increasing cardiac work can cause high blood pressure that can increase heart burden and in the end can cause decreasing if cardiovascular system function, with the occurrence of pathological ventricular hypertrophy [3]. Ventricles are a part of the heart that is primarily hypertrophic, such that measurements of the

left ventricle are important benchmarks of an increase in cardiac mass [15]. Therefore, to determined cardiac hypertrophy, heart weight and left ventricle weight is also need to studied together with body weight and calculate the ratio and then the number of increased heart weight is obtained. The results of this study indicate that ratios of ventricular weight/body weight and left ventricular weight/body weight (fig. 2) are low in the OT rat group compared with other groups, indicating that OT leads to cardiac hypertrophy.

PGC-1 is the key player in the mitochondrial biogenesis and metabolism in heart and skeletal muscle [12]. is important for mediating the cardioprotective effects of exercise and can be induced by integrators of transcriptional circuits that regulate mitochondrial function and biogenesis [16, 17]. PGC-1α expression is induced by physiological stimuli that increase ATP production and stimulate mitochondrial FAO, such as during meals, cold temperatures, and exercise. The results of this study showed increased PGC-1α levels in the aerobic group (fig. 3), consistent with the theory that PGC-1α expression is stimulated during exercise, whereby it supports mitochondrial biogenesis to increase ATP production and provide energy.

The results of research conducted by Finck (2007), indicate that PGC-1α plays an important role in controlling cardiac energy metabolism and suggest that perturbations in the PGC-1α system could influence to cardiomyopathic remodeling. PGC-1α overexpression prevented cardiac hypertrophy or improved contractility in cultured cardiac myocytes [7].

The results shown in fig. 3 indicated decreased PGC-1α expression in the OT rat group as a result of pathological cardiac hypertrophy, consistent with the results of Pereira *et al.* (2014) showing that during pathological hypertrophy, PGC-1α is repressed in concert with reduced mitochondrial oxidative capacity and FAO [8].

Notably, *H. sabdariffa* had no significant effects in the present study. This finding contrasts with an *in vivo* study conducted by Odigie *et al.* (2003) showing that water extract from HSL is potentially antihypertensive and cardioprotective with long-term administration at 250 mg/kg daily [18]. Thus, the cardioprotective effect of HSL is probably dependent on dose and duration of treatment, and should be examined in future studies.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest

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