Original Article

MOLECULAR METABOLISM

Skeletal muscle autophagy and mitophagy in endurance-trained runners before and after a high-fat meal

Michael D. Tarpey [1], Kevin P. Davy [1,2,3], Ryan P. McMillan [1,2], Suzanne M. Bowser [1], Tanya M. Halliday [1], Nabil E. Boutagy [1], Brenda M. Davy [1,3], Madlyn I. Frisard [1,2,3], Matthew W. Hulver [1,2,3,*]

ABSTRACT

Objective: We tested the hypothesis that skeletal muscle of endurance-trained male runners would exhibit elevated autophagy and mitophagy markers, which would be associated with greater metabolic flexibility following a high-fat meal (HFM).

Methods: Muscle biopsies were collected to determine differences in autophagy and mitophagy protein markers and metabolic flexibility under fasting conditions and 4 h following a HFM between endurance-trained male runners ($n = 10$) and sedentary, non-obese controls ($n = 9$).

Results: Maximal oxygen consumption ($ml \cdot kg \cdot min^{-1}$) was approximately 50% higher ($p < 0.05$) in endurance-trained runners compared with sedentary controls (65.8 ± 2.3 and 43.1 ± 3.4, respectively). Autophagy markers were similar between groups. Mitophagy and mitochondrial dynamics protein markers were significantly higher in skeletal muscle of endurance-trained runners compared with sedentary controls in the fasted state, although unaffected by the HFM. Skeletal muscle metabolic flexibility was similar between groups when fasted ($p > 0.05$), but increased in response to the HFM in endurance-trained athletes only ($p < 0.005$). Key mitophagy markers, phospho-Pink1^{Thr257} and phospho-Parkin^{S65} ($r = 0.64$, $p < 0.005$), and phospo-Parkin^{Ser65} and phospho-Drp1^{Ser616} ($r = 0.70$, $p < 0.05$) were correlated only within the endurance-trained group. Autophagy and mitophagy markers were not correlated with metabolic flexibility.

Conclusion: In summary, mitophagy may be enhanced in endurance-trained runners based on elevated markers of mitophagy and mitochondrial dynamics. The HFM did not alter autophagy or mitophagy in either group. The absence of a relationship between mitophagy markers and metabolic flexibility suggests that mitophagy is not a key determinant of metabolic flexibility in a healthy population, but further investigation is warranted.

Keywords Metabolic flexibility; Autophagy; Mitophagy; Endurance training; Skeletal muscle

1. INTRODUCTION

The maintenance of a healthy, functional mitochondrial network requires turnover through regulated shifts in the balance between fission and fusion and mitochondrial biogenesis and mitophagy [1]. Concerted action between mitophagy and autophagy, the more general degradation pathway, selectively isolates and eliminates damaged or dysfunctional mitochondria, maintaining overall network quality and function. Impaired skeletal muscle mitochondrial function is a hallmark of obesity, insulin resistance, and type II diabetes. Skeletal muscle from obese, insulin resistance, and type II diabetic individuals is characterized by impaired mitochondrial function, which includes fewer and smaller mitochondria [2–5], reduced transport chain content [6–8] and gene expression [9,10], and lower oxidative capacity [3,11–14]. This implies that mitochondrial quality control is either insufficient or defective in these disorders. The latter may have important implications for disease progression.

Metabolic inflexibility, defined as diminished capacity to adjust substrate oxidation in response to changes in substrate availability [15,16], has been implicated in the pathogenesis of obesity and the development of insulin resistance. For example, the relative inability of skeletal muscle to coordinate compensatory increases in fat oxidation following lipid influx may lead to the accumulation of fat and lipid intermediates and, subsequently, a decline in insulin sensitivity [15,17]. Sedentary behaviors are associated with reduced metabolic flexibility [18] and are considered a prominent factor in the etiology of obesity, insulin resistance, and type II diabetes [19,20]. Conversely, exercise training is associated with improved metabolic flexibility and insulin sensitivity in obese [21,22] and type II diabetic patients [23,24].

The increases in metabolic flexibility observed with endurance training have been attributed to enhanced mitochondrial respiration in human skeletal muscle [25]. Similarly, restoration of skeletal muscle mitochondrial function in type II diabetic patients is accompanied by increased metabolic flexibility [24]. Besides highlighting potential

[1]Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, VA, USA [2]Metabolic Phenotyping Core, Virginia Tech, Blacksburg, VA, USA [3]Fralin Translational Obesity Research Center, Virginia Tech, Blacksburg, VA, USA

*Corresponding author. 295 West Campus Drive, 338 Wallace Hall, Virginia Tech, Blacksburg, VA 24060, USA. E-mail: hulvermw@vt.edu (M.W. Hulver).

Received August 25, 2017 • Revision received October 4, 2017 • Accepted October 16, 2017 • Available online xxx

https://doi.org/10.1016/j.molmet.2017.10.006

disparities in skeletal muscle mitochondrial quality between endurance-trained and sedentary individuals, as well as in those with metabolic disease, these findings also imply a causative link between mitochondrial function and metabolic flexibility. While the direction of causation remains controversial, restoration of metabolic flexibility in type II diabetic patients was associated with increased mitochondrial content [24]. Accordingly, metabolic inflexibility in obesity-associated insulin resistant individuals was correlated to reduced intermyofibrillar mitochondrial content, which could not be accounted for by differences in mitochondrion size, muscle fiber distribution, or maximal aerobic capacity [26]. Together, these findings indicate that while mitochondrial content is a contributor to metabolic flexibility, in of itself, it may not be the major determinant. Instead, factors governing the mitochondrial population and quality may provide an alternative explanation. While the relationship between endurance exercise and mitochondrial biogenesis in skeletal muscle has been widely studied [27–30], the role of autophagy and mitophagy is not well understood. Chronic endurance exercise training leads to increased markers of basal autophagy and mitophagy in murine skeletal muscle [29,31]. Meanwhile, single bouts of endurance exercise stimulate mitophagy in a fed state-dependent manner in endurance-trained human skeletal muscle in the absence of autophagy activation [32]. When the same endurance exercise bout was completed following a fast, the onset of mitophagy activation was delayed [32]. Ultra-endurance exercise, when conducted in a fed state, has been shown to increase markers of both autophagy and mitophagy activity in endurance-trained human skeletal muscle [33,34]. It remains unclear whether autophagy and mitophagy regulation differs in skeletal muscle of endurance-trained compared to sedentary individuals. Diet also modulates skeletal muscle autophagy. High-fat diets have been associated with lipid-induced insulin resistance and reduced basal autophagy activity in murine skeletal muscle [1]. If, and how high-fat feeding modulates autophagy and mitophagy in human skeletal muscle is unknown.

The focus of the current study was to investigate whether endurance-trained runners exhibit elevated markers of autophagy and mitophagy in skeletal muscle compared to non-obese, sedentary controls and if the groups adjust autophagy and mitophagy regulation similarly following a high-fat meal (HFM). Finally, we sought to establish whether markers of autophagy and mitophagy in the fasted state and following a high-fat meal were related to skeletal muscle metabolic flexibility and oxidative capacity.

2. MATERIALS AND METHODS

2.1. Participants

Nine healthy, non-obese, sedentary (<2 days, 20 min/day of low-intensity physical activity) males and 10 endurance-trained (≥5-h running per week, and 2 competitions in the past 12 months) male runners aged 18–45 years completed the study. Participants were weight stable (<±2.5 kg) for the past 6 months with a BMI > 18 or < 30 kg/m^2 and were not taking any medications or supplements known to affect study measures. All participants had blood pressure <140/90 mmHg, fasting glucose <126 mg/dL, total cholesterol <240 mg/dL or triglycerides <300 mg/dL, and percentage of habitual calorie intake composed of <40% fat and <15% saturated fat. Participants were non-smokers with no personal history of metabolic or cardiovascular disease. All study procedures were approved by the Virginia Tech Institutional Review Board. Prior to participation, all procedures, benefits, and any potential risks associated with the study were explained to the participants before written consent was provided.

2.2. Experimental design

Following successful completion of screening procedures, all participants underwent a maximal treadmill test to volitional exhaustion to determine maximal oxygen consumption (VO_{2max}). Participants refrained from exercise for 36-h prior to a HFM challenge and muscle biopsies. Muscle biopsies were taken from the vastus lateralis following a 12-h overnight fast and 4-h after a HFM for assessment of markers of skeletal muscle autophagy, mitophagy, and metabolic flexibility. A schematic of the study design is presented in Figure 1.

2.3. HFM challenge

The HFM consisted of two sausage, egg, and cheese biscuits containing of 58 g fat (24 g saturated fat), 52 g carbohydrate, 24 g protein and a total of 820 kcal. Participants were required to consume the HFM within 10 min and remain seated and awake for the duration of the meal challenge. Following the initial biopsy and prior to the HFM, an intravenous catheter was placed in an antecubital vein for baseline and hourly blood sampling. The biopsies taken before and following the meal were from right and left legs, respectively.

2.4. Measurements

2.4.1. Body mass and composition

Body weight was measured to the nearest ±0.1 kg on a digital scale (Model 5002, Scale-Tronix, White Plains, NY). Height was measured to the nearest ±0.1 cm using a stadiometer. Body composition (total fat and fat-free mass) was analyzed by dual-energy x-ray absorptiometry (General Electric, Lunar Digital Prodigy Advance, software version 8.10e Madison, WI).

2.4.2. Dietary assessment

Participants completed four-day food diaries for the assessment of dietary intake, as previously described [34].

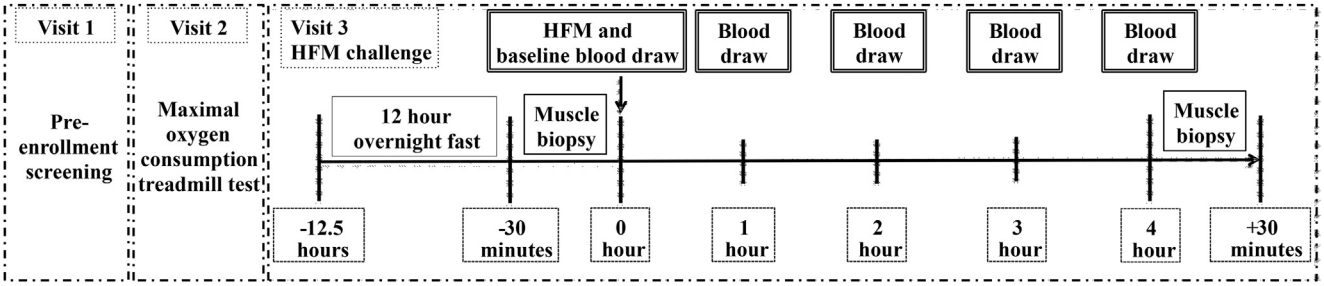

Figure 1: Schematic of study design. Participants completed a pre-enrollment screening prior to completing a maximal oxygen consumption test. Participants fasted for 12-h overnight prior to a baseline skeletal muscle biopsy taken at least 36-h after last exercise bout. Participants consumed a HFM and rested for 4-h before completing a second follow-up skeletal muscle biopsy. Blood was drawn every hour. HFM, high-fat meal.

