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Effects of acute hyperinsulinemia on skeletal muscle mitochondrial function, reactive oxygen species production, and metabolism in premenopausal women

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ABSTRACT

Background. Acute metabolic demands that promote excessive and/or prolonged reactive oxygen species production may stimulate changes in mitochondrial oxidative capacity.

Purpose. To assess changes in skeletal muscle H_2O_2 production, mitochondrial function, and expression of genes at the mRNA and protein levels regulating energy metabolism and mitochondrial dynamics following a hyperinsulinemic-euglycemic clamp in a cohort of 11 healthy premenopausal women.

Methods. Skeletal muscle biopsies of the vastus lateralis were taken at baseline and immediately following the conclusion of a hyperinsulinemic-euglycemic clamp. Mitochondrial production of H_2O_2 was quantified fluorometrically and mitochondrial oxidation supported by pyruvate, malate, and succinate (PMS) or palmitoyl carnitine and malate (PCM) was measured by high-resolution respirometry in permeabilized muscle fiber bundles. mRNA and protein levels were assessed by real time PCR and Western blotting.

Results. H_2O_2 emission increased following the clamp ($P < 0.05$). Coupled respiration (State 3) supported by PMS and the respiratory control ratio (index of mitochondrial coupling) for both PMS and PCM were lower following the clamp ($P < 0.05$). IRS1 mRNA decreased, whereas PGC1α and GLUT4 mRNA increased following the clamp ($P \leq 0.05$). PGC1α, IRS1, and phosphorylated AKT protein levels were higher after the clamp compared to baseline ($P < 0.05$).

Abbreviations: ACC, acetyl-CoA carboxylase; AKT, protein kinase B; AS160, AKT substrate of 160 kDa; BMI, body mass index; CD36, fatty acid translocase; CPT1B, carnitine palmitoyltransferase 1B; DNP, 2,4-dinitrophenol; DRP1, Dynamin-related protein 1; DXA, dual-energy X-ray absorptiometry; ETS, electron transport system; GDR, glucose disposal rate; GLUT4, glucose transporter type 4; GS1, glycogen synthase 1; HOMA-IR, homeostatic model assessment of insulin resistance; IAAT, intra-abdominal adipose tissue; IR, insulin resistance; IRS1, insulin receptor substrate 1; ISI, insulin sensitivity index; LPL, lipoprotein lipase; MFN1, mitofusin 1; MFN2, mitofusin 2; PCM, palmitoyl carnitine and malate substrate condition; PDK4, pyruvate dehydrogenase kinase 4; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PMS, pyruvate, malate, and succinate substrate condition; RCR, respiratory control ratio; ROS, reactive oxygen species; SLN, sarcolipin; SOD1, Cu-Zn superoxide dismutase; SOD2, Mn superoxide dismutase; T2D, type 2 diabetes; UCP3, uncoupling protein 3.

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Conclusions. This study demonstrated that acute hyperinsulinemia induced H_2O_2 production and a concurrent decrease in coupling of mitochondrial respiration with ATP production in a cohort of healthy premenopausal women. Future studies should determine if this uncoupling ameliorates peripheral oxidative damage, and if this mechanism is impaired in diseases associated with chronic oxidative stress.

1. Introduction

The incidence of obesity and associated cardiometabolic diseases, such as insulin resistance (IR) and type 2 diabetes (T2D), continues to rise throughout the westernized world [1]. Skeletal muscle mitochondrial dysfunction and chronic overproduction of reactive oxygen species (ROS) have been considered as primary mediators in their development [2,3]. In contrast, it has been shown in a rodent model that acute increases in superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) from the electron transport system (ETS) may actually have beneficial effects on skeletal muscle insulin sensitivity [4]. Thus, assessing mitochondrial responses to acute metabolic challenges (such as hyperinsulinemia) may be a valuable way to differentiate between normal physiological responses following nutrient load and chronic disturbances (i.e. excessive caloric intake, inactivity, etc.) that have been linked to cardiometabolic diseases.

Mitochondria are known to exhibit a great deal of plasticity in order to respond adequately to chronic stimuli that may alter metabolic demands. For example, exercise training can increase mitochondria content [5] and less than two weeks of exercise training can increase mitochondrial content, enzyme activity, and capacity [6]. Additionally, chronic inactivity has been shown to decrease mitochondria content and oxidative phosphorylation capacity [7], whereas caloric restriction increases substrate oxidation that is linked to ATP production (coupled respiration) and cold stress increases substrate oxidation that is uncoupled from ATP production (uncoupled respiration) [8]. Mitochondrial responses to acute stimuli are less well characterized, but it is thought that mitochondria exhibit a number of adaptations to acute metabolic signals that reflect the present environment. For example, uncoupling mitochondrial respiration from ATP synthesis, by allowing protons to leak across the inner mitochondrial membrane independent of ATP synthase, may be a mechanism to create inefficiency in the presence of abundant substrate and low energetic demand [9,10]. Uncoupled respiration ensures continued utilization of substrate, promotes the continued movement of electrons through the ETS, and alleviates an elevated proton motive force despite decreased ATP demand [11]. This process is known to be stimulated by ROS production [12] and is thought to be a mechanism to prevent excessive ROS production that may lead to oxidative damage. However, whether this uncoupling action occurs following an acute metabolic insult known to promote ROS production in humans is unclear. Additionally, the morphology of the dynamic mitochondrial reticular network is altered by acute stimuli, which may also be a function of ROS production [13]. However, morphological properties of mitochondria relative to their function are not well characterized as ROS production has been associated with both fusion [14] and fission [15] processes. A better understanding of the regulation of acute mitochondrial function and dynamics in response to ROS production may yield therapeutic approaches to prevent or mitigate oxidative damage.

The hyperinsulinemic-euglycemic clamp technique has been used to assess acute mitochondrial responses to elevated substrate load [2,8,16]. Earlier investigations have shown lower insulin-stimulated ATP production in isolated mitochondria from individuals with T2D compared to controls [16], and lower ATP synthetic rates *in vivo* in patients with T2D [17] and insulin-resistant offspring [2] compared to controls. Thus, insulin-stimulated ATP production appears to be impaired in individuals with impaired insulin sensitivity, however no measure of oxidative phosphorylation efficiency was assessed in these studies. The ability to uncouple mitochondrial respiration from ATP production may be a key response to ROS production that protects against prolonged increases in ROS.

The purpose of the present study was to test the hypothesis that the mitochondria of healthy (premenopausal, non-diabetic) women would display an increase in uncoupled respiration following acute hyperinsulinemia, in conjunction with an increase in ROS production. To test this hypothesis, we examined changes in skeletal muscle H_2O_2 production, mitochondrial bioenergetics using high-resolution respirometry, and expression of genes at the mRNA and protein levels regulating energy metabolism and mitochondrial dynamics in permeabilized fiber bundles from skeletal muscle biopsies obtained before and following a hyperinsulinemic-euglycemic clamp.

2. Materials and Methods

2.1. *Participants*

Participants were 11 generally healthy premenopausal women. Inclusion criteria were a body mass index (BMI) of 18.5–35 kg/m^2, sedentary (<30 min of structured activity per week), and fasting serum glucose <100 mg/dL. Participants were excluded if they reported use of oral contraceptives, use of any medication known to affect metabolism or glucose tolerance, use of anti-hypertensive agents, history of eating disorder, use of tobacco, change in weight greater than 5 lbs in the previous 6 months, active engagement in unusual dietary practices (e.g. "low-carb" diets), or participation in extreme exercise. All testing was conducted in the first 10 days of the follicular phase of the menstrual cycle. All women provided written informed consent before participating in the study. This study was approved by the Institutional Review Board at the University of Alabama at Birmingham (UAB).