Original Article

MOLECULAR METABOLISM

Skeletal muscle overexpression of nicotinamide phosphoribosyl transferase in mice coupled with voluntary exercise augments exercise endurance

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ABSTRACT

Objective: Nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme in the salvage pathway that produces nicotinamide adenine dinucleotide (NAD^+), an essential co-substrate regulating a myriad of signaling pathways. We produced a mouse that overexpressed NAMPT in skeletal muscle (NamptTg) and hypothesized that NamptTg mice would have increased oxidative capacity, endurance performance, and mitochondrial gene expression, and would be rescued from metabolic abnormalities that developed with high fat diet (HFD) feeding.

Methods: Insulin sensitivity (hyperinsulinemic-euglycemic clamp) was assessed in NamptTg and WT mice fed very high fat diet (VHFD, 60% by kcal) or chow diet (CD). The aerobic capacity (VO_2max) and endurance performance of NamptTg and WT mice before and after 7 weeks of voluntary exercise training (running wheel in home cage) or sedentary conditions (no running wheel) were measured. Skeletal muscle mitochondrial gene expression was also measured in exercised and sedentary mice and in mice fed HFD (45% by kcal) or low fat diet (LFD, 10% by kcal).

Results: NAMPT enzyme activity in skeletal muscle was 7-fold higher in NamptTg mice versus WT mice. There was a concomitant 1.6-fold elevation of skeletal muscle NAD^+. NamptTg mice fed VHFD were partially protected against body weight gain, but not against insulin resistance. Notably, voluntary exercise training elicited a 3-fold higher exercise endurance in NamptTg versus WT mice. Mitochondrial gene expression was higher in NamptTg mice compared to WT mice, especially when fed HFD. Mitochondrial gene expression was higher in exercised NamptTg mice than in sedentary WT mice.

Conclusions: Our studies have unveiled a fascinating interaction between elevated NAMPT activity in skeletal muscle and voluntary exercise that was manifest as a striking improvement in exercise endurance.

Keywords Nicotinamide adenine dinucleotide; Nicotinamide phosphoribosyl transferase; High fat feeding; Mitochondrial gene expression; Insulin sensitivity; Exercise

1. INTRODUCTION

Nicotinamide adenine dinucleotide (NAD^+) is an essential co-substrate for several enzyme classes such as sirtuins and poly ADP-ribose polymerases (PARPs) that regulate a myriad of signaling pathways governing metabolism, healthy aging, and lifespan extension [1]. NAD^+ can be generated *de novo* from dietary tryptophan or NAD^+ precursors. However, the NAD^+ salvage pathway is the dominant pathway for NAD^+ biosynthesis in mammals [2–4]. In this pathway, nicotinamide phosphoribosyl transferase (NAMPT), a homo-dimeric type II phosphoribosyl transferase [5,6], catalyzes the reversible condensation of nicotinamide (NAM) and 5′-phosphoribosyl-1-

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Abbreviations: CD, chow diet; HFD, 45% high fat diet; LFD, 10% low fat diet; Mck, muscle creatine kinase; NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyl transferase; NMN, nicotinamide mononucleotide; NMNAT, NMN adenylyl transferase; PARPs, poly ADP-ribose polymerases; PGC-1α, PPAR-γ coactivator-1 alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; PPi, pyrophosphate; R_d, insulin stimulated glucose disposal; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RER, respiratory exchange ratio; SIRT1, sirtuin-1; VCO2, carbon dioxide production; VHFD, 60% very high fat diet; VO_2, oxygen consumption

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pyrophosphate (PRPP) to yield nicotinamide mononucleotide (NMN) and pyrophosphate (PPi) [7,8]. NMN is subsequently converted to NAD^+ in the presence of ATP by the enzyme NMN adenylyl transferase (NMNAT) [7].

We previously showed that NAMPT protein content in skeletal muscle was higher in athletes compared to sedentary individuals, and positively correlated with mitochondrial function, insulin sensitivity, and oxidative capacity in humans [9]. Interestingly, the level of NAMPT protein in skeletal muscle was increased in sedentary individuals who were exercise trained for 3 weeks [9]. Exercise defends against environmental insults, such as high fat diet (HFD) feeding, that cause metabolic complications. While the molecular underpinnings of the beneficial effects of exercise are far from fully understood, emerging data has pointed to NAD^+ elevation as a key driver. For example, exercise increases the activity of the NAD^+-dependent deacetylase sirtuin-1 (SIRT1) [10,11] through elevation of NAD^+ [12,13]. In turn, SIRT1 controls the activity of peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator-1 alpha (PGC-1α) [14], a master regulator of mitochondrial biogenesis and function [10,15].

To further probe the putative salutary effects of increased NAMPT activity, we generated a mouse transgenic line that overexpressed NAMPT in skeletal muscle (NamptTg). We hypothesized that NAMPT overexpression in skeletal muscle would rescue mice from metabolic abnormalities that developed with HFD feeding and would increase oxidative capacity, endurance performance, and mitochondrial gene expression.

2. MATERIALS AND METHODS

2.1. Mice

Mice overexpressing the NAMPT transgene (C57BL/6J-Tg(Mck-NAMPT)Pbef2Srs) under the control of the muscle creatine kinase (Mck) promotor were generated at the Pennington Biomedical Research Center (PBRC) Transgenic Core by pronuclear injection using C57BL/6J embryos following standard techniques [16]. 1256 bp of the Mck promoter was amplified from p1256MCKCAT (a gift from Dr. M. W. Hulver) and inserted into pCMV-sport to make pCS-Mck1256. 2.6 kb of NAMPT cDNA was excised from pCMV-SPORT6-NAMPT and then inserted into pCS-Mck1256 between Mck and pA to yield pMck-NAMPT (Figure S1). To identify transgenic founder mice, DNA was isolated from tail biopsies at 21 days of age for PCR genotyping. Wild type (WT) C57BL/6J littermates were used as controls.

2.2. Animal studies

We performed three separate animal studies. Study 1 was conducted at the PBRC in Baton Rouge, LA, USA. Male C57BL/6J-Tg(Mck-NAMPT) Pbef2Srs (shortened to NamptTg for the remainder of the text) and wild type (WT) C57BL/6J mice were fed low fat diet (LFD) (10% by kcal) (D12450B, Research Diets, New Brunswick, NJ) or high fat diet (HFD) (45% by kcal) (D12451, Research Diets, New Brunswick, NJ) for 30 weeks from weaning. Body weight and body composition was assessed every 2 weeks at the same time of day. Studies 2 and 3 were conducted at the Sanford Burnham Prebys Medical Discovery Institute in Orlando, FL, USA. In study 2, male NamptTg and WT C57BL/6J mice were fed standard chow diet (CD) (2016, Harlan Teklad, Indianapolis, IN) or very high fat diet (VHFD) (60% by kcal) (D12492, Research Diets, New Brunswick, NJ) for 16 weeks from weaning. Body weight and body composition were assessed every week at the same time of day. Indirect calorimetry was performed and food intake, water intake, and spontaneous physical activity were assessed after 14 weeks on the diet. Hyperinsulinemic-euglycemic clamps were performed after 16 weeks on the diet. In study 3, male NamptTg and WT C57BL/6J mice were fed CD (2016, Harlan Teklad, Indianapolis, IN) for 11 weeks from weaning. Body weight and body composition were assessed every week at the same time of day. Half of the mice were given access to running wheels (Mini Run Around $4\frac{1}{2}''$, Super Pet, Elk Grove Village, IL) equipped with odometers (F12 Bike Computer, Easton-Bell Sports, Rantoul, IL) on week 4 of the study. Time spent running and distance completed were recorded every 24 h for the first 4 weeks that the mice had access to the wheels. Aerobic capacity (VO_2max) and exercise endurance tests were performed prior to giving mice access to the wheels and following 6 (VO_2max) and 7 (exercise endurance) weeks of voluntary exercise training, respectively. Mice were given one week between tests to recover. Wheels were removed from the cages 24 h prior to VO_2max tests and endurance tests. On week 11, mice were fasted for 5 h and then euthanized via CO_2 followed by cervical dislocation, and tissues were collected and weighed. Wheels were removed from cages 24 h prior to euthanasia.

All mice were individually caged and maintained at 22–24 °C with light from 7:00am to 7:00pm. Lights were equipped with a dimmer such that a gradual increase/decrease in light occurred 30 min prior to lights being fully on/off. All animal studies and procedures were approved by the appropriate Institutional Animal Care and Use Committees.

2.3. Quantitative reverse transcriptase-PCR

In study 1, tissues were snap-frozen in liquid nitrogen immediately following dissection. RNA was extracted via column purification using the Qiagen RNeasy Fibrous Mini Kit (Qiagen, Valencia, CA). RNA quantity was determined using an ND-1000 Nanodrop Spectrophotometer (Thermo Fisher Scientific). The concentration of NAMPT mRNA was determined by qRT-PCR using Taqman primers and fluorescent probes as the detection system on an ABI 7900HT (Applied Biosystems, Foster City, CA) using the following parameters: one cycle of 48 °C for 30 min, then 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. NAMPT expression data was normalized to the housekeeping gene peptidylprolyl isomerase B (PPIB). Primers and probes were designed using Primer Express version 2.1 (Applied Biosystems). Sequences of primer/probe sets are shown in Table S1. For all other genes in study 1, cDNA was synthesized with the iSCRIP cDNA synthesis kit (Bio-Rad, Hercules, CA) using 200 ng of RNA. qRT-PCR reactions were performed using 1 μM of primers and LightCycler® 480 SYBR Green Master (Roche, Branford, CT) on a LightCycler® 480 detection system (Roche). Calculations were performed by a comparative method (2-ΔΔCT) using 18S RNA as an internal control. Primers were designed using the Integrated DNA Technologies (IDT) software, and the primer sequences can be found in Table S2. In study 3, total RNA was isolated as previously described [17]. Briefly, RNA was isolated from 50 to 100 mg of skeletal muscle tissues (red quadriceps, white quadriceps) with Qiazol reagent (Invitrogen, Carlsbad, CA). The quantity and purity of RNA was determined using a ND-1000 Nanodrop spectrophotometer (Thermo Fisher Scientific). Primer-probe sets were pre-designed Single Tube Taqman® Gene expression assays. qRT-PCR reactions were performed using Taqman Fast Virus 1-step reaction mix Standard protocol (Life Technologies, Grand Island, NY). Data were normalized by dividing the target gene by the geometric mean of the internal control genes (*RPLP0*, *GAPDH*).

2.4. Western blots

Tissues were collected immediately following euthanasia and flash frozen in liquid nitrogen. Homogenates were prepared by Polytron homogenization in RIPA buffer containing protease inhibitor and

