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MOLECULAR METABOLISM

Exercise-induced molecular mechanisms promoting glycogen supercompensation in human skeletal muscle

Janne R. Hingst [1], Lea Bruhn [1], Mads B. Hansen [1], Marie F. Rosschou [1], Jesper B. Birk [1], Joachim Fentz [1], Marc Foretz [2,3,4], Benoit Viollet [2,3,4], Kei Sakamoto [5,10], Nils J. Færgeman [6], Jesper F. Havelund [6], Benjamin L. Parker [7], David E. James [7,8], Bente Kiens [1], Erik A. Richter [1], Jørgen Jensen [1,9], Jørgen F.P. Wojtaszewski [1,*]

ABSTRACT

Objective: A single bout of exercise followed by intake of carbohydrates leads to glycogen supercompensation in prior exercised muscle. Our objective was to illuminate molecular mechanisms underlying this phenomenon in skeletal muscle of man.

Methods: We studied the temporal regulation of glycogen supercompensation in human skeletal muscle during a 5 day recovery period following a single bout of exercise. Nine healthy men depleted (day 1), normalized (day 2) and supercompensated (day 5) muscle glycogen in one leg (exercised leg) while the contralateral leg served as a resting control. Euglycemic hyperinsulinemic clamps in combination with leg balance technique allowed for investigating insulin-stimulated leg glucose uptake under these 3 experimental conditions. Cellular signaling in muscle biopsies was investigated by global proteomic analyses and immunoblotting. We strengthened the validity of proposed molecular effectors by follow-up studies in muscle of transgenic mice.

Results: Sustained activation of glycogen synthase (GS) and AMPK in combination with elevated expression of proteins determining glucose uptake capacity were evident in the prior exercised muscle. We hypothesize that these alterations offset the otherwise tight feedback inhibition of glycogen synthesis and glucose uptake by glycogen. In line with key roles of AMPK and GS seen in the human experiments we observed abrogated ability for glycogen supercompensation in muscle with inducible AMPK deletion and in muscle carrying a G6P-insensitive form of GS in muscle.

Conclusion: Our study demonstrates that both AMPK and GS are key regulators of glycogen supercompensation following a single bout of glycogen-depleting exercise in skeletal muscle of both man and mouse.

1. INTRODUCTION

Glycogen is formed as a branched polymer of glucose serving as an essential energy depot in times of nutritional surplus that can be readily mobilized when energy is needed [1]. While liver glycogen directly contributes to glucose homeostasis by releasing glucose into the blood, skeletal muscle lacks this ability but accounts for ~80% of glucose disposal under insulin-stimulated conditions [2]; in this way, muscle glucose uptake and storage becomes crucial for controlling blood glucose levels. The majority of glycogen is stored in skeletal muscle (a total of ~400 g) and the "set-point" for glycogen concentration in human skeletal muscle appears to be tightly controlled at a level of ~1.5 g/100 g muscle. This is mediated by an efficient feedback-regulation of glycogen synthase (GS) by muscle glycogen concentration [3]. Intriguingly, when a single bout of exercise is followed by intake of carbohydrates, the muscle cell favors glycogen synthesis far beyond resting levels (i.e. glycogen supercompensation) and muscle glycogen concentration can increase by up to ~4 g/100 g muscle. In 1966, Bergström and Hultman demonstrated that the ability to supercompensate muscle glycogen is restricted to the prior exercised muscle [4]. The authors concluded that a single bout of exercise enhances factors within the prior exercised muscle that were

[1]Section of Molecular Physiology, Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, DK-2100, Copenhagen, Denmark [2]INSERM, U1016, Institut Cochin, 75014, Paris, France [3]CNRS, UMR8104, 75014, Paris, France [4]Université Paris Descartes, Sorbonne Paris Cité, 75014, Paris, France [5]MRC Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, United Kingdom [6]Department of Biochemistry and Molecular Biology and VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Campusvej 55, DK-5230, Odense, Denmark [7]Charles Perkins Centre, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, 2006, Australia [8]School of Medicine, University of Sydney, Sydney, NSW, 2006, Australia [9]Department of Physical Performance, Norwegian School of Sports Sciences, Oslo, Norway

[10] Present address: Nestlé Institute of Health Sciences SA, Lausanne, Switzerland.

*Corresponding author. Faculty of Science, Department of Nutrition, Exercise and Sports, Section of Molecular Physiology, University of Copenhagen, 13, Universitetsparken, DK-2100, Copenhagen, Denmark. E-mail: jwojtaszewski@nexs.ku.dk (J.F.P. Wojtaszewski).

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maintained for several days after exercise, elevating the "set-point" for muscle glycogen storage. It is noteworthy that the molecular nature of these exercise-induced "enhancing factors" that allow for glycogen synthesis far beyond normalized muscle glycogen concentration remains to be demonstrated.

The activity of GS, the key regulatory enzyme in glycogen synthesis, is controlled by the allosteric activator glucose-6-phosphate (G6P), and by covalent phosphorylation at inhibiting residues [5,6]. Insulin enhances glycogen synthesis through increased glucose uptake and by promoting de-phosphorylation and thus activation of GS [1,7]. Compared to insulin, exercise results in a more pronounced de-phosphorylation and hence higher activation of GS in skeletal muscle [8,9] than during insulin stimulation. A synergy exists between allosteric and covalent regulation of GS such that GS affinity for the allosteric activator G6P is enhanced in the de-phosphorylated state [10,11]. It remains an open question whether this molecular regulation of GS is involved in the phenomenon of glycogen supercompensation in skeletal muscle.

AMP activated protein kinase (AMPK) serves as a cellular energy regulator by monitoring cellular nucleotide status [12]. AMPK binds to the glycogen particle [13–15] and may also act as a cellular fuel sensor as muscle glycogen is a negative regulator of AMPK activation [16,17]. Supporting this concept, prolonged AMPK activation *in vivo* by different pharmacological means (e.g. AICAR, PF739 and MK-8722) is accompanied by elevated muscle glycogen concentration [18–20]. Moreover, missense mutation (gain-of-function) of the AMPK γ3 or γ1 regulatory subunits is associated with elevated muscle glycogen content [21–25]. Thus, a tight coupling exists between muscle glycogen and AMPK. AMPK in human skeletal muscle exists in three different complexes (α2β2γ1, α1β2γ1 and α2β2γ3) and kinase activity increases in a trimer-specific manner during exercise [27–29], and may stay elevated for several hours into exercise recovery [30]. Studies in rodents suggest that this AMPK activation is necessary for insulin sensitization of skeletal muscle in the period immediately following exercise [31,32]. However, whether AMPK is involved in the regulation of glycogen supercompensation in skeletal muscle remains to be investigated.

Here, we have used an invasive study in man and advanced muscle proteomics to revisit the phenomenon of glycogen supercompensation in skeletal muscle. In the course of 5 days both *in vivo* physiological measurements and classical biochemical as well as proteomic analyses in muscle biopsies revealed temporal regulation of insulin sensitivity for muscle glucose uptake, intracellular signaling, capacity for glucose uptake as well as glucose/fatty acid oxidation.

2. METHODS

2.1. One-legged glycogen supercompensation regime in man

The study was approved by the regional ethics committee in Denmark (Journal number: H-4-2013-071) and performed in accordance with the Declarations of Helsinki II. Each subject gave written informed consent before participating. Nine healthy male subjects (age, 26 ± 3 years; BMI, 23.5 ± 1.7 kg/m^2; VO_2 peak, 45 ± 4 ml O_2/min/kg; data presented as means ± SD) underwent a glycogen supercompensation regime. The study design is illustrated in Figure 1A and a detailed description of preclinical investigation of the subjects and the experimental design is provided in Supplemental I. The subjects were familiarized to the one-legged knee extensor model at light intensity prior to the testing. The working leg was chosen randomly in order to avoid possible confounding effects of leg dominance. On a separate day, the peak workload (PWL) of the knee extensors during one-legged

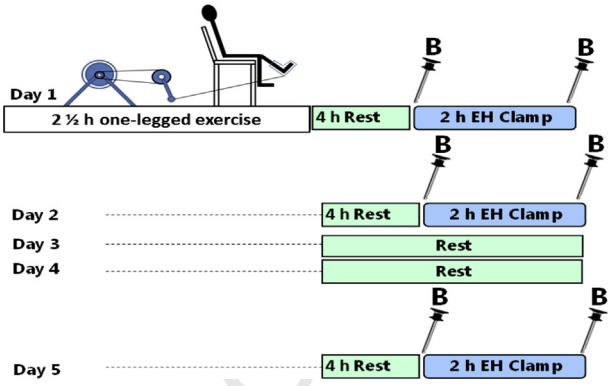

Figure 1: Study design for one-legged glycogen supercompensation in man. Nine lean and healthy male subjects underwent a one-legged glycogen supercompensation regime. Muscle glycogen content was reduced (day 1), normalized (day 2), and supercompensated (day 5) in one leg while remaining unchanged in the resting control leg. Muscle insulin action was evaluated under these three different situations by euglycemic hyperinsulinemic clamps (EH Clamps) and femoral catheterization in combination with measurement of femoral leg blood flow enabled to calculate insulin-stimulated leg glucose uptake. Muscle biopsies were obtained from both legs before and after insulin infusion. EH Clamp: Euglycemic hyperinsulinemic clamp. B: Biopsy from *vastus lateralis* muscle.

extensor exercise was determined as previously described [33]. On day 1, the subjects performed one-legged knee extensor exercise until exhaustion. This exercise protocol consisted of one-legged knee extensor exercise for 1 h at 80% of PWL interspersed by 5 min bouts at 90% of PWL every 10 min. This was followed by interval exercise until exhaustion containing 4 min bouts starting at 100% PWL followed by 1 min at 50% of PWL. When the subjects were unable to maintain kicking frequency during these intervals, the exercise intensity was lowered by 10% and finished when the subjects were unable to finish 4 min at 60% of PWL.

After 4 h of rest, a 120 min hyperinsulinemic- (∼100 μU/ml) euglycemic clamp was performed. This procedure was repeated in the same subjects in the rested state (without prior exercise) at day 2 and day 5 in order to investigate insulin-stimulated glucose uptake during the glycogen supercompensation regime. Catheters were inserted in the femoral veins of both legs and in a heated hand vein for sampling of arterialized venous blood in order to measure insulin-stimulated glucose uptake across the legs using A-V balance technique combined with ultrasound Doppler measurements of femoral arterial blood flow. Muscle biopsies from m. vastus lateralis were obtained before (basal state) and after 120 min of insulin infusion (insulin-stimulated state) from both legs at day 1, day 2, and day 5. Throughout the 5 day supercompensation regime, the subjects consumed a eucaloric diet composed of 80 E% carbohydrates, 10 E% fat and 10 E% protein.

2.2. Proteomic analyses of human muscle biopsies

Muscle biopsies for proteomic analyses were processed and analyzed as previously described [34]. A detailed description can be found in Supplemental I. For MS data repository and the complete data set, see Supplemental III.

2.3. Muscle processing and western blotting

Homogenates from human muscle biopsies were prepared from freeze-dried muscle, dissected free of visible fat, blood and connective tissue. Homogenates from mouse muscle were prepared from wet muscle. Muscle tissue was subsequently homogenized as described in detail in Supplemental I. Muscle homogenates and lysates were

