Supporting Information

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SI Materials and Methods

Incremental Exercise Test to Exhaustion. Participants' exercise capacity was measured through an incremental exercise test to exhaustion on a modified, electrically braked cycle ergometer (Mijnhardt KEM3; Cardiokinetics). At the start of exercise participants were given 30 s to achieve the required pedaling frequency (~65 revolutions per min) before the workload was increased by 20 W per min until exhaustion. Exhaustion was defined as the point at which the participants could no longer maintain the required pedaling frequency. Venous blood was sampled at the end of each work load (i.e., once per minute) to measure venous blood lactate concentration. Participants breathed through a mouthpiece, with their nose occluded. Respired gases were sampled through a catheter and analyzed continuously by mass spectrometry. Respiratory volumes were measured by means of a turbine volume-measuring device. Breath-by-breath data were averaged for each min of exercise. Room temperature during exercise was ~21 °C.

Small Muscle Mass Exercise for Investigation with Magnetic Resonance Spectroscopy. Participants reported to the laboratory early on the second day after an overnight fast. Skeletal muscle ^{31}P magnetic resonance spectroscopy (MRS) was performed in a Siemens Tim Trio 3.0-Tesla magnet with a 60-cm clear bore, interfaced with Syngo software. The participants were positioned supine and a dual-tuned ^{31}P and ^{1}H 6-cm-diameter surface coil was secured under the right calf muscle. The right foot was fastened to a custom-built nonmagnetic plantar flexion exercise apparatus and was immobilized with the leg straightened. The plantar flexion exercise apparatus was similar to the one previously described by Tran et al. (1). The exercise protocol consisted of 3 min of rest followed by three 5-min periods of exercise interspersed with 7-min recovery periods. The work rates for subsequent exercise periods were 3 W, 4 W, and 5 W. The calf was exercised at 1 Hz, in time to a digital metronome, which was played through the magnet room speakers. Participants were monitored continuously, and none had difficulty keeping time or completing the exercise.

Transverse gradient echo scout images of the lower leg were acquired to ensure correct positioning of the participant's leg and to select a region of interest within the calf muscle for localized shimming. Three baseline scans were acquired to allow corrections for partial saturation caused by the rapid magnetic pulse repetition time and nuclear Overhauser enhancement (NOE). The data acquisition consisted of a series of 444 ^{31}P spectra acquired throughout the exercise protocol described above with a temporal resolution of 5 s. The acquisition parameters were as follows: 500 ms repetition time, 0.35 ms short echo time, 4,000 Hz spectral width, 10 averages, 1,024 data points, nominal 25° flip angle, and 10 rectangular NOE pulses, with a pulse duration of 10 ms, an interpulse delay of 10 ms, and an excitation flip angle of 180°.

After phasing and baseline correction, spectra peaks were fitted using the automated AMARES algorithm, within the jMRUI software package (version 2.2) (2, 3). Absolute millimolar concentrations of phosphorus metabolites were calculated assuming that the concentration of cytosolic ATP at rest is 8.2 mM; this commonly used standard concentration is based on values extensively reported in the published literature (4).

Intramuscular pH was calculated on the basis of the chemical shift (σ) of phosphate relative to phosphocreatine in parts per million:

$$\mathrm{pH} = 6.75 + \log_{10}\left(\frac{\sigma - 3.27}{5.63 - \sigma}\right) \quad [1]$$

Standard Meal Tolerance Test. The standardized meal consisted of Rice Krispies (Kellogg Company) semiskimmed milk, dried skimmed milk powder, fresh orange juice, and a chocolate drink. The chocolate drink contained cocoa powder (2 g), palm oil (14 g), safflower oil (14 g), emulsifier (0.4 g), sweetener (1.2 g), and hot water (35.5 g). The caloric content of the fiber-free standard meal was scaled to each participant's body weight and height, so that it corresponded to about 40% of the daily energy requirement. The proportions of energy sources were constant for all participants. Protein provided 13% of the total energy intake, fat 38%, and carbohydrates 49%.

Participants rested on a bed throughout the study. Cannulae were inserted under local anesthetic into both the femoral artery and retrogradely into a vein draining the deep structures of the forearm. These cannulae were kept patent by a slow infusion of 0.9% (wt/vol) saline. Two sets of blood samples were taken 30 min apart in the fasting state. Following the standardized meal, paired arterial and venous blood samples were taken at varying time points over the subsequent 5 h. Two minutes before each sample was taken, a wrist cuff was inflated to 200 mm Hg to prevent contamination of the venous blood from the forearm vein by blood from the hand. Blood samples were used to quantify glucose, insulin, lactate, and pyruvate as described elsewhere (5, 6). Forearm muscle blood flow was assessed by strain-gauge plethysmography (7).

Muscle Biopsy Analyses. A portion of frozen muscle tissue was used to determine muscle fiber composition. The muscle was homogenized in a pyrophosphate extraction buffer (pH 9.5) supplemented with protease and phosphatase inhibitors (Sigma). Actomyosin was extracted from the homogenate by vibrating for 1 h in a cold room (0 °C) and then centrifuging at 10,000 × *g* for 10 min at 2–8 °C. The supernatant was mixed 1:1 with standard Laemmli sample buffer, boiled, and then run on a 5% SDS/PAGE gel for 18 h at 70 V to separate the myosin heavy-chain isoforms. The bands were identified with silver staining. The gel then was scanned using a Duo Scan T1200 Agfa scanner, and bands were quantified using the Quantity One program (Bio-Rad). Optical densities were adjusted by subtracting the local background. All values were normalized to the intensity of the actin band. Estimation of muscle fiber composition using this approach has been shown to be in good agreement with a standard histochemical approach for determining fiber composition on muscle sections (8).

A second portion of the frozen muscle was freeze dried, dissected free of visible connective tissue, and powdered. Muscle glycogen content of this powdered tissue was measured using a modified version of the spectrophotometric method of Harris et al. (9).

A further portion of the frozen muscle was used to determine pyruvate dehydrogenase complex total activity (PDCt), that being the total (dephosphorylated) active form of the complex. Briefly, PDCt activity was assayed in a buffer containing sucrose, Tris, EDTA, with Mg^{2+}, Ca^{2+}, and dichloroacetate added to activate the pyruvate dehydrogenase complex (PDC) phosphatase and inhibit PDC kinase, respectively. PDCt was expressed as rate of acetyl-CoA formation [nmol · (min · mg protein)$^{-1}$] at 37 °C using a radioactive substrate. Further details are as described elsewhere (10).

Another portion of muscle was homogenized on ice in a solution containing KH_2PO_4 50 mmol L^{-1}, EDTA 1 mmol L^{-1}, and Triton X-100 0.05% using a glass homogenizer. The muscle extract then was centrifuged at 20,000 × *g* for 3 min, and the supernatant was used to measure the activities of glutamate dehydrogenase, citrate synthase, 3-hydroxy-acyl-CoA dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase, as described by Opie and Newsholme (11) and Zammit and Newsholme (12). Protein concentration was determined using Peterson's method (13).

A final portion of muscle from the Chuvash polycythemia (CP) patients was used for quantitative RT-PCR. Because there was not enough material from the matched control samples, other biopsies taken from healthy adult participants were used for this analysis. The assays used Taqman chemistry and were undertaken on an ABI Prism 7000 sequence detector (Applied Biosystems) according to the manufacturer's protocol. Hydroxymethyl bilane syllane was used as the internal control.

Statistical Analysis. Differences between the CP patients group and the control participants group were assessed by means of two-tailed Student's paired *t* test; pairing was undertaken based on age, gender, height, weight, and weekly level of physical activity. For the quantitative RT-PCR results for which there were no paired control samples, an unpaired *t* test was used. Statistical significance was set at $P < 0.05$. Variables are presented as means ± SD, unless otherwise stated.

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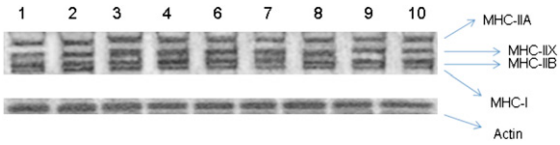

Fig. S1. SDS/PAGE analysis of myosin heavy-chain isoforms after silver staining. Myosin heavy- chain isoforms (MHC-I, MHC-IIA, MHC-IIX, MHC-IIB) in vastus lateralis muscle were obtained from CP patients (lanes 2, 4, 6, 9, 10) and control participants (lanes 1, 3, 7, 8). Fiber type did not differ significantly between the two groups. The proportion of fibers type I, IIA, IIX, and IIB for the control group were 19.8 ± 2.4%, 25.7 ± 2.1%, 35.4 ± 4.9%, and 19.1 ± 2.9%, respectively, and for the CP group were 17.2 ± 2.5%, 23.9 ± 1.4%, 31.9 ± 2.9%, and 27.0 ± 2.8%, respectively.

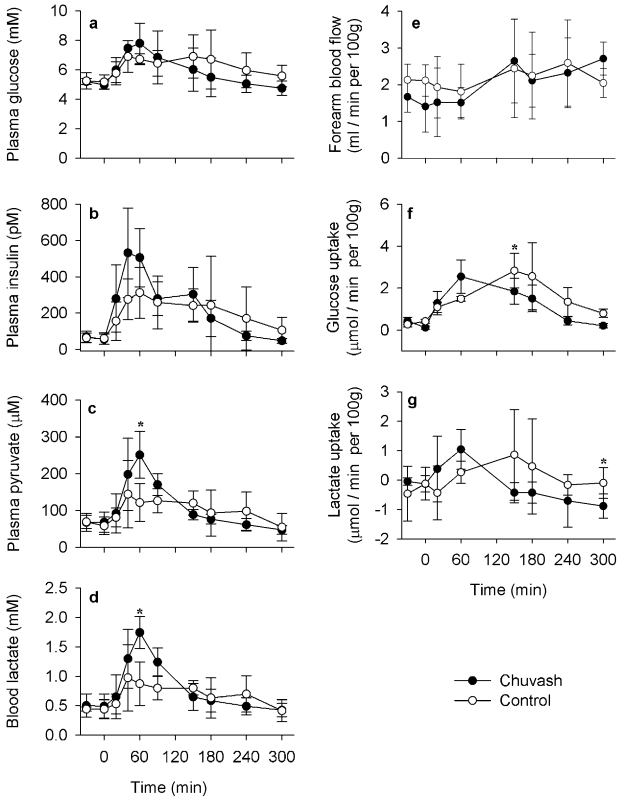

Fig. S2. Results from Chuvash polycythemia patients and control participants for arterial (*A*) glucose, (*B*) insulin, (*C*) pyruvate, and (*D*) lactate and forearm (*E*) blood flow, (*F*) glucose uptake, and (*G*) lactate uptake measured through the standard meal tolerance test. Empty circles show results from the control group; filled circles show results from the Chuvash polycythemia group. Data are mean ± SD. Values are for $n = 5$, apart from lactate for the control group where $n = 3$, at times −30, 20, 150, and 240 min. *$P < 0.05$.