Running Performance at High Running Velocities Is Impaired but V’O₂max and Peripheral Endothelial Function Are Preserved in IL-6−/− Mice

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Abstract

It has been reported that IL-6 knockout mice (IL-6−/−) possess lower endurance capacity than wild type mice (WT), however the underlying mechanism is poorly understood. The aim of the present work was to examine whether reduced endurance running capacity in IL-6−/− mice is linked to impaired maximal oxygen uptake (V’O₂max), decreased glucose tolerance, endothelial dysfunction or other mechanisms. Maximal running velocity during incremental running to exhaustion was significantly lower in IL-6−/− mice than in WT mice (13.00 ± 0.97 m min⁻¹ vs. 16.89 ± 1.15 m min⁻¹, P < 0.02, respectively). Moreover, the time to exhaustion during running at 12 m min⁻¹ in IL-6−/− mice was significantly shorter (P < 0.05) than in WT mice. V’O₂max in IL-6−/− (n = 20) amounting to 108.3 ± 2.8 ml kg⁻¹ min⁻¹ was similar as in WT mice (n = 22) amounting to 113.0 ± 1.8 ml kg⁻¹ min⁻¹ (P = 0.16). No difference in maximal COX activity between the IL-6−/− and WT mice in m. soleus and m. gastrocnemius was found. Moreover, no impairment of peripheral endothelial function or glucose tolerance was found in IL-6−/− mice. Surprisingly, plasma lactate concentration during running at 8 m min⁻¹ as well as maximal running velocity in IL-6−/− mice was significantly lower (P < 0.01) than in WT mice. Interestingly, IL-6−/− mice displayed important adaptive mechanisms including significantly lower oxygen cost of running at a given speed accompanied by lower expression of sarcoplasmic reticulum Ca²⁺-ATPase and lower plasma lactate concentrations during running at submaximal and maximal running velocities. In conclusion, impaired endurance running capacity in IL-6−/− mice could not be explained by reduced V’O₂max, endothelial dysfunction or impaired muscle oxidative capacity. Therefore, our results indicate that IL-6 cannot be regarded as a major regulator of exercise capacity but rather as a modulator of endurance performance. Furthermore, we identified important compensatory mechanism limiting reduced exercise performance in IL-6−/− mice.

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Introduction

It is widely recognized that interleukins are secreted by macrophages and lymphocytes to coordinate the response of the immune system to injury and infection [1]. Although interleukin-6 (IL-6) is known as a key modulator of this response [2], it is also considered as an “exercise factor” released by contracting skeletal muscles into the circulation which adjusts substrate (lipid and carbohydrate) metabolism to increased energy demand during exercise [3,4]. Depending on the exercise intensity and its duration, plasma level of IL-6 in humans can be elevated even up to 100-fold after long lasting exercise (e.g. marathon run) and drops to its basal soon after the exercise is terminated [5]. IL-6 exerts its effects both on the whole body and skeletal muscles via endocrine and paracrine/autocrine manners, respectively, without activating classical pro-inflammatory pathways [6]. Namely, it has been postulated that IL-6 stimulates hepatic glucose release and lipolysis in adipose tissue as well as glucose uptake and fatty acid oxidation in skeletal muscles thus providing contracting skeletal muscle fibres with energetic substrates [4]. Since IL-6 is considered to be vital for regulation of glucose and lipid metabolism during exercise, its deficiency should lead to the impairment of exercise performance. Indeed, it was consistently reported that IL-6 knockout mice displayed compromised treadmill and swimming exercise capacity as evidenced by their reduced endurance time [7–9]. However, the data concerning regulation of the whole-body glucose and lipid metabolism in IL-6−/− animals are not so evident since one group referred to age-related insulin resistance and weight gain of IL-6−/− mice [10,11] while the other did not confirm this observation [12,13]. Therefore, these data cannot
explain the reduced exercise performance of IL-6−/− mice. It seems that, despite previous works, the mechanisms underlying impairment of exercise tolerance in IL-6−/− animals are still not known.

It has been also reported that, in spite of lower exercise tolerance of the IL-6−/− mice, their oxygen cost of running during exercise at submaximal intensities is significantly lower than in WT mice for unknown reason [7]. Faletz et al. [7] postulated that it could be due to progressive oxygen depletion in these animals. If indeed it would be the case, the maximal oxygen uptake (V'O2max) in IL-6−/− mice should be significantly lower than in the WT mice. In the present study we have hypothesized that lower exercise tolerance in the IL-6−/− mice will be accompanied by lower V'O2max. We also examined whether lower exercise tolerance in IL-6−/− could be linked to alterations in glucose tolerance, endothelial function or changes in locomotor muscle profile including activities of mitochondrial enzymes or expression of sarcoplasmic reticulum Ca2+-ATPase (SERCA) and uncoupling protein-3 (UCP-3).

Materials and Methods

Animals

The experiment protocol was approved by the Bioethics Committee of Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland (Permit Number: 914/2012). All experiments including treadmill exercise were preceded with acclimatization time to minimize stress of animals whereas surgery procedures were carried out under ketamine/xylazine anesthesia.

Eighty nine male IL-6−/− mice and their age-matched wild type IL+/+ (WT) littersmates used as controls in all experiments were bred in Medical University of Białystok, Poland. The animals were between 7 and 10-month-old once they were enrolled into the study and since then they were housed in single cages. They were maintained on a 12:12 h light-dark cycle and were given unlimited access to food and water for the duration of the study. At the end of the experiment, lack of functional IL-6 gene was confirmed by genotyping of liver samples. Namely, genomic DNA was isolated with Genomic Mini kit (A&A Biotechnology, Gdańsk, Poland) and PCR was performed using DreamTaq Green PCR Master Mix (ThermoScientific, Rockford, IL) and specific primers (M_II-6_Fwd 5’-CCATCCAGTGGCCATTCGTG-3’ and M_II-6_Rev 5’-AAGTGCACTCATGTTGTTCTCATA-3’). Subsequently, DNA was separated by electrophoresis on the agarose gel with ethidium bromide and liver samples of IL-6 knockouts were distinguished from WT control animals on the basis of their size (2400 bp for IL-6−/− and 1476 bp for WT mice).

Exercise capacity and endurance training protocols

WT and IL-6−/− mice were acclimatized to the motorized treadmills (closed one-lane treadmill for whole body gas exchange measurements and six-lane treadmill for the assessment of running performance capacities) (Columbus Instruments, Columbus, OH, USA) for three weeks. Subsequently, we assessed exercise capacity of both WT control as well as IL-6−/− knockout animals by measuring their (1) maximal oxygen consumption (V’O2max) during maximal incremental test, (2) oxygen consumption (V’O2) at maximal running velocity (vmax), (3) VO2 at vmax and (3) endurance time at 10 m·min−1 and 12 m·min−1. The experiment protocol was approved by the Bioethics Committee of Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland (Permit Number: 914/2012). All experiments including treadmill exercise were preceded with acclimatization time to minimize stress of animals whereas surgery procedures were carried out under ketamine/xylazine anesthesia.

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Monitoring of the oxygen consumption

For estimation of basal oxygen consumption (basal V’O2) and V’O2 during sub-maximal exercise, mice were acclimatized to the closed metabolic cage or closed one-lane treadmill equipped with oxygen and carbon dioxide sensors (Columbus Instruments, Columbus, OH, USA) for a week. The basal oxygen consumption (basal V’O2) was measured at a set time of the day (7–9 a.m.) for 1 h following 30 min of an animal acclimatization. To determine their V’O2 during sub-maximal exercise, mice were run at 6 m·min−1 for 1 hour.

Temperature measurements

Body temperature at rest was assessed rectally around 9 a.m. after measurement of basal V’O2 (see above) whereas body temperature after sub-maximal exercise was measured before and immediately after animals completed 1-hour run at 8 m·min−1 around 9 a.m.

Glucose tolerance test

Glucose tolerance was assessed in mice at the age of 10 and 12 months. Mice were starved for 18 hrs and, then their basal glucose concentration was measured in blood samples obtained from the tail vein and diluted with saline (ABX Pentra 400 biochemical analyser, Horiba ABX, France). Subsequently, mice were intra-peritoneally injected with glucose (2 g kg−1) and glucose concentration was measured at 15, 30, 45, 60 and 120 min time points.

Tissue samples collection

Mice were anesthetized with ketamine and xylazine (100 and 10 mg kg−1, respectively). Subsequently, aorta, liver and skeletal muscles (soleus and gastrocnemius) were harvested and snap-frozen in liquid nitrogen for further analysis except from aorta that was placed on Krebs buffer (118.06 mM NaCl, 4.69 mM KCl, 1.19 mM KH2PO4, 1.16 mM MgSO4, 2.52 mM CaCl2, 25 mM
NaHCO₃, 10 mM glucose, 2 mM pyruvic acid sodium, 0.030 mM EDTA), cleaned from the connective and adiponectin tissues and cut into 2-mm rings. Aortic rings were subsequently mounted in Multi Wire Myograph System (820M, DMT, Denmark) and maintained in KH buffer at 37°C equilibrated with 95%O₂-5%CO₂ to measure acetylcholine (ACh)- and sodium nitroprusside (SNP)-dependent endothelial function. For determination of post-exercise plasma lactate concentration, separate groups of IL-6⁻/⁻ (n = 22) and WT animals (n = 22) were subjected to either a single bout of submaximal exercise (8 m.min⁻¹ for 1 hour) or incremental test (as described above) and just after the run were sacrificed and their blood samples were collected. After centrifugation, plasma lactate concentration was determined with Stat Profile pHox (Nova Biomedical, Waltham, MA).

The blood variables and plasma lipid profile

Blood samples were collected in tubes containing EDTA and were used either to perform the blood count (animal blood counter Vet abc, Horiba Medical, France) or centrifuged to obtain plasma which was further aliquoted and stored at -80°C for determination of lipid profile at ABX Pentra 400 biochemical analyzer (Horiba ABX, France).

Cytochrome C oxidase (COX) and citrate synthase (CS) activities

Skeletal muscle samples (soleus and mixed gastrocnemius) were homogenized in the buffer containing 5 mM HEPES, 1 mM EGTA, 0.1% Triton X-100, 1 mM DTT and 10 N KOH. COX activity was measured as a time-course increase in absorbance at 550 nm due to oxidation of its substrate 1 mM reduced cytochrome C from horse (Sigma Aldrich, St. Louis, MO) according to the equation: reduced Cyt+O₂→oxidized Cyt+H₂O₂. CS activity was determined as the increase of absorbance at 412 nm according to reaction: acetylCoA(0.3 mM)+oxalacetic acid(0.5 mM)+H₂O→ citrate acid+CoASH+DTNB (0.1 M)→CoAS+H+ +C₂O₄₂⁻ [all reagents were from Sigma Aldrich, St. Louis, MO]. Subsequently, enzyme activities were normalized to protein concentration in the samples (measured with BCA assay) and the values were used to calculate COX and CS activities.

Western blot

Skeletal muscle samples of non-exercising mice were homogenized in the buffer containing protease and phosphatase inhibitors (Invitrogen Corp., Camarillo, CA). The protein concentration was determined by BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Subsequently, equal amounts of protein were separated by SDS-PAGE, transferred to nitrocellulose membrane and blocked with 5% non-fat dry milk/TBS-0.05% Tween solution. The washed blots were incubated with primary antibodies at following concentrations: anti-UCP3 (1 ug.ml⁻¹, ab3477), anti-SERCA ATPase (1:5000, ab6789), anti-GAPDH (ab3477), anti-SERCA ATPase (1:1000, ab2819), anti-GAPDH (ab3477), anti-SERCA ATPase (1:1000, ab2819), and anti-GAPDH (ab3477). When running at sub-maximal velocity of 6 m.min⁻¹ for 1 hour) or incremental test (as described above) and just after the run were sacrificed and their blood samples were collected. After centrifugation, plasma lactate concentration was determined with Stat Profile pHox (Nova Biomedical, Waltham, MA).

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Statistical analysis

Statistical analysis was performed in GraphPad Prism5, Statistica 10 and MS Excel 2003. P values lower than 0.05 were considered significant. The details regarding the specific statistical tests used are presented below relevant figures.

Results

Running performance during maximal incremental exercise test in IL-6⁻/⁻ (WT) control and IL-6⁻/⁻ mice

IL-6⁻/⁻ and WT mice were subjected to maximal incremental exercise treadmill test to assess their maximal running velocity in this protocol (vmax) which was by about 23% lower for IL-6 knockout (13.00±0.97 m min⁻¹, n = 12) when compared with WT controls (16.9±1.2 m min⁻¹, n = 12; P<0.02) (Figure 1A). Therefore, running exercise performed at absolute intensities determined by velocities of 10 and 12 m min⁻¹ was more intense for IL-6⁻/⁻ than WT mice (Figures 1B and 1C). Simultaneously, we also monitored oxygen consumption (V O2) (Figure 1E) and found that, in spite of no difference in maximal oxygen uptake (V O2max) between IL-6 knockout (100.3±2.8 ml kg⁻¹ min⁻¹, n = 22) and WT mice (113.0±1.8 ml kg⁻¹ min⁻¹, n = 22; P = 0.16) (Figure 1D), V O2 of IL-6⁻/⁻ animals at vmax was lower (104.5±2.4 ml kg⁻¹ min⁻¹, n = 21) when compared with WT mice 111.0±1.7 ml kg⁻¹ min⁻¹, n = 22; P<0.04) (Figure 1F). When running at sub-maximal velocity of 6 m min⁻¹ for 1 h, IL-6⁻/⁻ mice also displayed lower V O2 when compared with WT control mice (P<0.0001, F = 52.29, n = 6) (Figure 1G).

Running performance during prolonged exercise in IL-6⁻/⁻ (WT) control and IL-6⁻/⁻ mice

It was previously reported that IL-6⁻/⁻ mice displayed reduced endurance time when compared with control animals [7–9]. Our IL-6⁻/⁻ mice forced to run for 2 hours at 10 m min⁻¹ performed as well as their WT littermates (Figure 2A) though this exercise was more intense for them due to their lower maximal velocity (Figure 1A). However, increasing the speed up to 12 m min⁻¹ revealed compromised endurance time of IL-6 knockout animals (Figure 2B). The discrepancy between these two speeds originated from the fact that IL-6⁻/⁻ mice during running at 12 m min⁻¹ were actually exercising close to their maximal velocity (13±1 m min⁻¹; Figure 1A) whereas the run at 10 m min⁻¹ constituted only about 77% of their maximal running velocity (vmax) established for each mouse during the incremental running test (see Figure 1B).

Basal V O2, blood count/plasma lipid profile, body weight and temperature as well as glucose tolerance and endothelial function of IL-6⁻/⁻ (WT) control and IL-6⁻/⁻ knockout (IL-6⁻/⁻) mice

To answer why IL-6⁻/⁻ mice could also result from increased energy dissipation, we measured their body temperature as well as glucose tolerance and endothelial function of IL-6⁻/⁻ mice.
exercise (Figure 3D) between IL-6\(^{-/-}\) and WT mice. Moreover, our IL-6\(^{-/-}\) mice also did not display an impaired glucose tolerance either at 10 (Figure 3E) or 12 months of age (Figure 3F). Subsequently, we investigated vasodilation response to acetylcho-

**Figure 1. Running performance during maximal incremental exercise test in WT and IL-6\(^{-/-}\) mice.** Maximal velocity (v\(_{\max}\)) (A), running intensity at 10 m min\(^{-1}\) (B) and at 12 m min\(^{-1}\) (C), maximal oxygen consumption (V\(_{\text{O2max}}\)) (D), oxygen consumption during an incremental test with increasing speeds (E), oxygen consumption at maximal velocity (V\(_{\text{O2}}\) at v\(_{\max}\)) (F) and oxygen consumption during 1-hour run at submaximal velocity of 6 m min\(^{-1}\) (G). For determination of v\(_{\max}\) (A), V\(_{\text{O2max}}\) (D) and V\(_{\text{O2}}\) at v\(_{\max}\) (F), WT control and IL-6\(^{-/-}\) mice were run at an inclination of 0° with the increasing speed and their oxygen consumption (V\(_{\text{O2}}\)) was registered (E) whereas for measurement of V\(_{\text{O2}}\) during sub-maximal exercise, mice were run at velocity of 6 m min\(^{-1}\) for 1 h (G). Data are presented as the mean ± SEM. The symbols * denote values significantly different: *(P<0.05), ***(P<0.01), ****(P<0.001). Statistical analysis was performed in Statistica 10 (G; ANCOVA, n = 6, P<0.0001) or GraphPad Prism5 (two-sided T-test; n = 22-11). doi:10.1371/journal.pone.0088333.g001
and WT mice subjected to a single bout of submaximal exercise and found it lower by about 50% in IL-6/−/− (5.22±0.26 mmolL−1) when compared with WT control mice (10.13±0.25 mmolL−1), (n = 7–9, P<0.001) (Figure 4E). Moreover, maximal plasma lactate concentration determined after the maximal incremental running test in the IL-6/−/− mice amounting to 9.03±1.79 mmol L−1 was significantly (P<0.01) lower in the WT mice (amounting to 12.03±2.27 mmol L−1) (Figure 4F).

**Differences in skeletal muscle protein profile between IL-6/−/− and IL-6+/− mice**

As described above, IL-6/−/− mice displayed increased CS but not COX activities in oxidative soleus muscle in comparison with WT control animals (Figures 4A and 4C). Moreover, IL-6/−/− mice accumulated much less lactate in plasma during single-bout exercise which might be due to lower muscle lactate production (as described in Discussion section). These observations may indicate energy deficiency in skeletal muscles of IL-6/−/− mice which would trigger compensatory mechanisms leading to reduction of energy expenditure. Indeed, in soleus muscle of IL-6/−/− mice we observed decreased level of sarcoplasmic reticulum Ca2+-ATPase 1 (SERCA1) in IL-6/−/− (115.1±8.4 a.u.) as compared to WT mice (145.3±9.3 a.u., n = 7; P = 0.04) (Fig. 5A), that could indicate reduction of energy expenditure whereas the level of UCP3 (participating in energy dissipation) was not altered (Fig. 5B). In predominantly glycolytic gastrocnemius we did not detect any changes in SERCA1 and UCP3 levels (Figures 5C and 5D).

**Discussion**

We designed this study to elucidate the mechanisms of reduced endurance exercise capacity of IL-6/−/− mice reported earlier [7–9]. The main and original finding of this study is that our 10-month old IL-6/−/− mice displayed reduced exercise performance as evidenced by their lower maximal running speed (by about 23%) obtained during maximal incremental test (Figure 1A) and compromised endurance time while running on the treadmill at running velocity of 12 m min−1 (Figure 2B), despite no difference in VO2max when compared to WT mice. Therefore, poorer running capacity observed in these mice cannot be explained by lower VO2max in IL-6/−/− mice we observed as similar as in WT mice (Figure 1D). It should be noted that in our study the impairment of running capacity was evident only at high running velocities, whereas during 2 h run at lower running velocities (v = 10 m min−1), no difference in running performance between IL-6/−/− and WT mice was observed. These results are in agreement with the finding by Benrick et al. [11] showing no difference in endurance capacity between male IL-6/−/− and WT mice assessed during 50 minutes run at 75% of their VO2max. Taking into consideration the key role of VO2max in determining endurance exercise capacity, our results indicate that IL-6 cannot be regarded as a major regulator of exercise capacity but rather as a modulator of endurance performance.

It has been postulated that IL-6 might affect exercise tolerance by its effect on AMP-activated protein kinase (AMPK) which is considered as an energy sensor of the cell by monitoring the AMP/ATP ratio. It was demonstrated that elevated IL-6 levels during exercise [4] positively correlated with an increase in AMPK protein kinase activity [18] and, moreover, that AMPK could be activated by increased levels of IL-6 [18,19]. On the contrary, lack of IL-6 resulted in diminished AMPK activity both in sedentary and exercised IL-6/−/− mice [20]. Since it was shown that during a
single bout of exercise AMPK activation correlates positively with eNOS phosphorylation in the mouse aorta [21] and that AMPK co-immunoprecipitates with eNOS [22], we hypothesized that impaired exercise capacity of IL-6^{−/−} mice could be related to
Moreover, Fa¨ldt animals, which impairs their ability to continue running [7].

However, these authors postulated that reduced oxygen consumption during exercise causes a progressive oxygen depletion in these animals, which impairs their ability to continue running [7]. Moreover, Falldt et al. (2004) speculated that impaired heart function and reduced capilalisation in the IL-6−/− mice could be responsible for their poorer running capacity. In turn, our results suggest that the poorer running capacity reported in the IL-6−/− mice was not caused by limited oxygen delivery. We have found no differences in the V O2max between the IL-6−/− and the WT mice (see Figure 1D), indicating that the oxygen delivery to the working muscles as well as its utilization during exercise was well preserved in the IL-6−/− mice. Furthermore, lower V O2 observed during subsequent steps of the incremental test (see Figure 1E) and during running at 12 m min−1 (Figure 1F) was not due to limitation in V O2max in the IL-6−/− mice. Therefore, we strongly suggest that lower oxygen uptake observed during submaximal running in IL-6−/− is not a sign of a limited oxygen delivery as suggested previously [7] but is related to the adaptive mechanisms aimed to achieve enhanced mechanical efficiency in IL-6−/− mice.

In order to understand the physiological background of this mechanism, we measured some muscle proteins expression/activities involved in oxygen cost of work including sarcoplasmic reticulum Ca2+-ATPase1 (SERCA1), uncoupling protein-3 (UCP-3) as well as COX and CS activities (for overview see [24–26]). We have found lower expression of SERCA1 in soleus muscle in IL-6−/− than in WT mice (Figure 5A). This could at least partly explain the lower oxygen cost of running at sub-maximal running velocities in the IL-6−/− mice (for overview see [24–26]). Lower oxygen cost of the run in the IL-6−/− mice could be also due to lower expression of UCP-3 in their locomotor muscles and lower energy dissipation during exercise (see e.g. [27]). However, in the present study we found no difference between UCP-3 expression in the gastrocnemius muscle of the IL-6−/− mice when compared to the WT mice (see Figure 5D). Accordingly, no change in basal and post-running temperature was observed in IL-6−/− mice when compared to the WT (see Figures 3C and 3D). On the other hand, we found higher citrate synthase (CS) activity (considered as a marker of mitochondria volume density - see e. g. [27,28]) in the soleus muscle of the IL-6−/− mice when compared to the WT mice (Figure 4C). However, the maximal COX activity measured in the soleus muscle of IL-6−/− mice was not different when compared to WT mice (see Figure 4A). Moreover, the maximal COX activities measured in the gastrocnemius muscle of IL-6−/− mice and WT mice were not significantly different (see Figure 4B). This suggest that the oxidative phosphorylation activity of the soleus and the gastrocnemius muscles in IL-6−/− mice and WT mice are similar. Therefore, the lower oxygen cost of running observed in IL-6−/− mice cannot be explained by higher oxidative phosphorylation activity in their locomotor muscles.

In contrast to humans, mice can successfully perform prolonged endurance running close to their V O2max (as observed during the measurements of V O2max in this study). This suggests that mouse locomotor muscles possess greater abilities to tolerate metabolic acidosis during endurance running. Little, however, is known about the role of IL-6 in developing of metabolic acidosis during endurance running close to their V O2max. This is an original new finding showing lower lactatemia in IL-6−/− mice. This is an original new finding showing lower lactatemia in IL-6−/− mice. This is an original new finding showing lower lactatemia in IL-6−/− mice.

### Table 1. The blood count and plasma lipid profile of WT control and IL-6−/− mice.

|             | WT                  | IL-6−/−               |
|-------------|---------------------|-----------------------|
| WBC (K ul−1) | 3.03±1.33           | 3.23±1.36             |
| LYM [%]     | 72.5±10.7           | 77.7±7.61             |
| MON [%]     | 5.1±0.91            | 4.59±1.03             |
| GRA [%]     | 22.4±10.14          | 17.67±6.86            |
| LYM (K ul−1)| 2.14±0.84           | 2.5±1.1               |
| MON (K ul−1)| 0.12±0.08           | 0.21±0.02             |
| GRA (K ul−1)| 0.85±0.65           | 0.63±0.31             |
| RBC (M ul−1)| 9.35±0.76           | 9.78±0.56             |
| HGB (g dl−1)| 13.17±0.96          | 13.62±0.53            |
| HCT [%]     | 47.21±4.22          | 48.46±2.1             |
| MCV (fl)    | 50.4±1.58           | 49.6±1.1              |
| MCH (pg)    | 14.12±0.51          | 13.94±0.66            |
| MCHC (g dl−1)| 27.96±1.41        | 28.13±1.1             |
| RDW [%]     | 13.76±0.79          | 13.5±0.37             |
| PLT (K ul−1)| 1561.33±223.44      | 1363.8±198.31         |
| MPV (fl)    | 5.34±0.21           | 5.21±0.1              |
| LDL [mmol l−1]| 0.0975±0.05        | 0.092±0.027           |
| HDL [mmol l−1]| 0.83±0.19          | 1.02±0.27             |
| TC [mmol l−1]| 1.49±0.29          | 1.775±0.46            |
| TG [mmol l−1]| 0.41±0.18          | 0.49±0.26             |

WBC (white blood cells: LYM% (% of lymphocytes), MON% (% of monocytes), GRA% (% of granulocytes), RBC (red blood cells), HGB (hemoglobin), HCT (hematocrit), MCV (red cell) mean corpuscular volume), MCH (red cell) mean corpuscular hemoglobin, MCHC (red cell) mean corpuscular hemoglobin concentration, RDW (red cell distribution width), PLT (platelets), MPV (mean platelets volume), LDL (low-density lipoprotein), HDL (high-density lipoprotein), TC (total cholesterol), TG (triglycerides). Data are presented as means ± SEM. doi:10.1371/journal.pone.0088333.t001
Figure 4. Post-exercise plasma lactate and COX, CS activities in skeletal muscles in WT and IL-6−/− mice. COX and CS activities in soleus (A, C) and gastrocnemius (B, D) skeletal muscles were measured in lysates from non-exercising WT control mice and IL-6−/− mice. Post-exercise plasma lactate concentration (E) was assessed both in non-exercising mice (WT at rest and IL-6−/− at rest) as well as in animals subjected to single bout of exercise (1-hour run at 8 m.min−1). (F) Plasma lactate concentration at V̇O₂max during maximal incremental running test until exhaustion in WT mice and IL-6−/− mice. Data are presented as the mean ± SEM and symbols * denote values significantly different: **(P<0.01), ***P<0.001). Statistical analysis was performed in GraphPad Prism5 (two-sided T-test, n = 7–11).

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Figure 5. Skeletal muscle protein profile of WT and IL-6−/− mice. Whole-muscle soleus (A,B) and gastrocnemius (C,D) lysates were used to assess the levels of sarcoplasmic reticulum Ca²⁺-ATPase 1 (SERCA1) and uncoupling protein 3 (UCP3). Values on the graphs represent means ± SEM. Representative Western Blot images acquired for the same membrane at the same exposure time are shown to compare protein levels between WT and IL-6−/− mice with GAPDH used as the loading control for gastrocnemius. For soleus, we used Coomassie blue staining of protein gels as a loading control (not shown). The symbol * denotes values significantly different from WT controls: *(P<0.05). Statistical analysis was performed in GraphPad Prism5 (two-sided T-test, n = 7–8).

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could be related to lesser disturbances in muscle metabolic stability in their muscles during running at submaximal velocities when compared to the WT mice. It should be noticed that during this run (running at the same absolute running velocity amounting to 0 m min\(^{-1}\)) the relative exercise intensity in case of IL-6\(^{-/-}\) mice was much higher than in case of WT mice due to above-mentioned lower maximal running capacity (by about 25\%) found in IL-6\(^{-/-}\) mice (see Figure 1A) but still plasma lactate concentration in IL-6\(^{-/-}\) mice after this run was much lower than in WT mice. This is, indeed, an interesting new finding showing that IL6\(^{-/-}\) mice, despite of running at relatively higher running velocity, paradoxically accumulate much less lactate in the blood than WT mice. Interestingly, in the present study we have also demonstrated to our knowledge for the first time that the maximal plasma lactate concentration after fatiguing run to exhaustion in IL-6\(^{-/-}\) mice was significantly lower than in WT mice (see Figure 4F). Results collected in this study enable us to explain the reason for lesser accumulation of plasma lactate in IL-6\(^{-/-}\) mice as compared to WT mice since lower plasma lactate concentration in this case could be due to lower muscle lactate production and/or faster lactate uptake by various cells (see e.g. [30,31]). Nevertheless, our results clearly indicate that IL-6 is strongly involved in the mechanism responsible for plasma lactate accumulation during exercise both during sub-maximal and maximal running velocities. In physiological conditions higher plasma lactate concentration at a given power output is associated with poorer performance of sustained exercise (see e.g. [32–34]) and, therefore, lower plasma lactate concentration in IL-6\(^{-/-}\) mice should be associated with better tolerance of sustained exercise. This could explain the preserved good endurance capacity of the IL-6\(^{-/-}\) mice during low intensity running when compared to WT mice (see Figure 2A). On the other hand, low plasma lactate concentration observed in IL-6\(^{-/-}\) mice during run at 8 m min\(^{-1}\) when compared to WT mice could result from impaired anaerobic glycolysis as well as from faster glycogen depletion during exercise. Both these factors could be responsible for poorer running performance at high velocities found in this study in IL-6\(^{-/-}\) mice when compared to WT mice (see Figures 1C and 2B). Regarding the maximal incremental exercise, the lower plasma lactate concentrations found after this fatiguing run in IL-6\(^{-/-}\) mice when compared to WT mice (see Figure 4F) suggest that IL6\(^{-/-}\) mice possess poorer anaerobic glycolytic energy capacity than WT mice. This could at least partly explain their poorer maximal running capacity (near V\(\text{O}_2\text{max}\)) Interestingly, it was recently reported that poorer endurance swimming capacity of IL-6\(^{-/-}\) mice was accompanied by greater intramuscular glycogen depletion after fatigue exercise [8]. This is in accordance with the findings by Kelly et al. (2009) [33] that IL-6 increases substrate availability within the muscle cell by increasing glycogenolysis and lipolysis.

In the present study, we found no difference in glucose tolerance between IL-6\(^{-/-}\) mice and WT mice, which indicates that, at least at rest, lack of IL-6 has no effect on glucose homeostasis. Our results are in accordance with the findings by [36] that IL-6 is not necessary for glucose production during non-exhaustive exercise. It was also recently reported, in contrast to previous observations, that IL-6 release in humans during exercise was not directly correlated with the release or uptake of exogenous substrate, nor to muscle glycogen utilization [37]. This suggests that more studies are needed to establish the quantitative significance of IL-6 in the carbohydrate metabolism during exercise in humans.

Conclusions

The present study confirmed the previous reports showing that IL-6\(^{-/-}\) mice displayed reduced exercise performance as evidenced by lower maximal running velocity during maximal incremental test and shorter time to exhaustion at running velocity of 12 m min\(^{-1}\). We provided novel evidence suggesting that poorer running capacity in IL-6\(^{-/-}\) mice is not due to lower V\(\text{O}_2\text{max}\), impairment of peripheral endothelial function, glucose intolerance or impaired muscle oxidative capacity. Therefore, our results indicate that IL-6 cannot be regarded as a major regulator of exercise capacity but rather as a modulator of high intensity endurance performance. We also identified that IL-6\(^{-/-}\) mice displayed lower cost of running at a given sub-maximal running velocity linked to the lower expression of SREC1 in soleus muscle and lesser plasma lactate accumulation during running at sub-maximal and at maximal velocities. This response seems to constitute an important compensatory mechanism limiting reduced exercise performance in IL-6\(^{-/-}\) mice.

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Author Contributions

Conceived and designed the experiments: MW K. Kmiecik RVC SC JAZ. Performed the experiments: DF MO JJ BS AF MW K. Kmiecik. Analyzed the data: MW K. Kmiecik JM. Contributed reagents/materials/analysis tools: SC JAZ K. Kaminski. Wrote the paper: MW SC JAZ. Obtained permission to use the animals: MW.

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