Purpose

The overall purpose of this study is to investigate the response to acute endurance exercise and physical performance in children and how it may change during puberty.

Specific aims

1. To follow children through puberty and investigate their physical activity and physical performance in relation to biological maturation
2. To describe the response of circulating cytokines and circulating immune cell populations to 45 min endurance exercise and the immediate recovery
3. To investigate the mitochondrial function and mitochondrial content in immune cells of children in different puberty stages
4. To relate exercise-induced changes in the immune cell populations, and their mitochondrial function, to age, pubertal maturation, and sex in children.
5. To investigate how the growth plate of children is affected by daily physical activity, physical performance and biological maturation.
6. How is physical self-esteem related to physical activity and -performance and how does it change during puberty?
7. Is there a difference in daily physical activity in different age groups in children, and how does it evolve in the same individuals during puberty?

Background

Physical activity in children has been associated with several positive outcomes, including, but not limited to, decreased cardiovascular risk factors, less adiposity, and enhanced motor skill development (1-3). Likewise, physical activity levels have also been linked to immune activity (4) with a subsequently decreased risk for infection (5). However, investigations including children and adolescents are far less prevalent compared to studies compared in adult populations (6). Acute exercise typically activates an immune cell response, and in general, children seem to have a lower immune response and a quicker recovery compared to adults, where shifts in natural killer cells (7), t-cells (8), neutrophils (9), interleukin-6 (10), and tumor necrosis factor α (9) is significantly lower. The blunted response and faster recovery in children have been hypothesized to in part be mediated by lower muscle damage following exercise (6, 11). A factor that complicates comparisons between studies on exercise-induced immune responses in adults and children in different ages is that slightly different exercise modalities are used as stimuli. Some studies also report factors such as age, pubertal maturation, and gender in children heavily influence the immune outcome (12). We, therefore, set to examine exercised-induced immune responses in the same children every year for five years, to follow the response through puberty. Circulating levels of immune cells and cytokines will be analyzed from blood collected during the immediate post-exercise window. As the immune cell response is influenced by physiological factors and maturity, participants will be characterized for body composition, fitness levels as well as pubertal maturation.

Moreover, children today are more and more sedentary, with increased screen time and decreased levels of physical activity (13). We therefor want to investigate how daily physical activity is changed from childhood to adolescence and how it is related to their strength and physical performance. Resistance exercise seems to rise in the younger population, previously being considered a controversioal topic due to risk of injury and beneficiary effects, something that in later years has been revised (14-15). It was previously believed that resistance exercise would prevent skeletal growth, not supported by more recent studies (16-17). We want to further investigate the relation between growth and physical activity and -performance by doing yearly measurements of the growth plate with both ultrasound and MRI-scans.

Overview study design

Seventy volunteering children age 9-12 will be recruited. They will conduct four sessions each year for five years, estimated to take 1-5 hours each.

The characterization tests include a maximal oxygen uptake (VO2max) tests on a treadmill, estimation of VO2max using cycle ergometer test, body composition measurements, echocardiography, muscle thickness, pennation angle and growth plate measurements with ultrasound and MRI-scans, and an estimation of puberty development using sex hormones and Tanner classification.

The acute aerobic exercise session will consist of 45 minutes of indoor track running. All participants will be instructed to run as far as possible during the 45 min. The possibility of stopping to recover and/or hydrating will be offered every 10^{th} min. A peripheral venous catheter (PVC) will be inserted into the antecubital vein and venous blood samples will be collected before, immediately after, and following 60 and 180 minutes of recovery.

After a familiarization session, strength will be measured in the legs using leg press and knee extension, as well as grip strength. During the familiarization sessions the child will get the chance to learn the proper technique to exercise in these machines safely and we will titrate the child's seven repetition maximum (7RM) for both exercises. There will on a separate occasion be a resistance exercise strength test where we will load the participant's 7RM in each machine

Seperately we will measure daily physical activity using smart watches (Fitbit) and wrist- and waist worn accelerometers for seven consecutive days in their home environment. We will also measure their physical self-esteem using the Swedish translation of the questionnaire "Children and Youth – Physical Self-Perception Profile" called "Sådan är Jag!", either during one of the physical visits or in a digital meeting.

Detailed description of each session:

The children and their caregivers will be informed both orally and in writing as to the risks and benefits of enrollment, after which they are free to ask any questions regarding study participation. Next, the children older than fifteen will give their oral and written consent for enrollment, as well as give their provision for their data to be collected. For the younger children their caregivers will give written consent.

All enrolled participants will conduct the following four sessions:

- **Session 1** is conducted at the Swedish School of Sport and Health Sciences. Participants will start of the session by measuring their weight and height. After this, participants perform an Ekblom-Bak submaximal cycle ergometer test to estimate their VO2max. Lastly, O2 and CO2 exchange is also measured for another estimation of VO2max by maximal treadmill running. Capillary lactate values will also be collected during the max treadmill running test. The time estimate for session 1 is 1.5h.

- **Session 2** is conducted at Nya Karolinska Sjukhuset (NKS) and estimated to take 2 h. During this meeting, an echocardiography is conducted. Following echocardiography, participants will be familiarized to the resistance exercise machines, leg press and knee extension, and their 7RM will be measured followed by grip strength measurement.
- **Session 3** will be conducted at the national athletic center Bosön and is estimated to take approximately 5h. This session will start between 7-10 am, and participants are instructed to consume a standardized breakfast prior to them reporting to Bosön. At arrival, participants will receive a PVC in their antecubital vein and a baseline blood sample will be collected. Participants will then be equipped with a heart rate monitor and asked to run for 45 min on an indoor running track. Distance covered, heart rate, as well as perceived exertion, will be collected during the running test. Venous blood samples will again be collected immediately post-exercise as well as at 60- and 180 min post cessation of exercise. A pediatrician will additionally estimate their Tanner puberty stage.
- **Session 4** will take place at Karolinska University Hospital Huddinge, estimated to take two hours. They will perform a MRI-scan of the muscle in the legs and the growth plate in the knee, followed by ultrasound also of the muscle and the growth plates.

Description of methods

VO2max assessment
Session #1
A submaximal cycle ergometer test will be conducted according to the standard praxis and the calculation estimate of VO2max will be conducted according to the updated Ekblom-Bak equation (19).

VO2max will also be measured during maximal treadmill running, employing a metabolic chart with continuous, breath-by-breath, gas exchange assessment. During the treadmill running test blood lactate will be measured using a portable system. Peripheral blood is sampled via a finger prick before and after the VO2max test. Perceived exertion will also be collected throughout the maximal running test to ensure the criteria for VO2max is met (20).

Echocardiography and echocardiogram, 7RM test and grip strength
Session #2
Cardiac function will be assessed using a non-invasive method called echocardiography (ECHO), which is an ultrasound examination of the heart measuring several parameters related to cardiac function including thickness of the walls, size of the four chambers and ejection fraction, i.e., the amount of blood the heart pumps out during each heartbeat. A small amount of gel is placed on the thorax to facilitate image rendering.

After the ECHO examination the children will be introduced to the strength training machines, leg press and knee extension. The machines will be adjusted to their bodies and they will be instructed how to use them properly. Participants will first warm up at lighter and comfortable loads. After this, more and more resistance per set is added to the weight stack of the exercise machine until the participant cannot perform seven repetitions. Participants will get 2-5 min rest between tries depending on their level of exhaustion. The participants will always be under supervision from a test leader to ensure that the exercises are conducted safely.

Grip strength will be measured using Jamar hand dynamometer, adjusted to the hand size of each participant. They will be seated in an upright position and be instructed to grip as hard as they can for three seconds. They will do the grip test for three times with each hand.

Monitoring during endurance exercise
Session #3
To assess strain during the endurance exercise bout all participants will wear a heart rate monitor (Polar), perceived exertion will be rated with the Borg scale, and number of laps will be counted by researchers.

Peripheral venous catheter, blood gas analysis and plasma collection
Session #3
The peripheral venous catheter (PVC) will be placed in the most accessible vein on the participants forearm. EMLA (local anesthetic cream) will be placed on the skin (1-3 hours before) to reduce discomfort and pain. Blood will be drawn a total of 4 times during session 3. The time points for collection are before the 45 min run, immediately after finishing and following 1 and 3h of recovery. A total of 36,5 ml will be collected from the children.

Cytokine and immune cell analysis
Session #3
PBMC are separated from the whole blood by CPT tubes. The mononuclear cell layer is removed following centrifugation with manual pipetting, washed one time in Hank's solution, and centrifuged for 10 min at room temperature and 450 g. Washed cells are resuspended in 1 ml of PBS. Analyses are performed on frozen cells, which are stored at -80°C. Plasma and serum will be prepared from whole blood using standard centrifugation protocols. Subtypes of PBMCs (monocytes, different types of leukocytes) will be quantified using fluorescence-activated cell sorting (FACS) based on combinations of cell surface markers. Gene expression of PBMCs will be assessed using qRT-PCR/RNA-seq and Western blot. Cytokine plasma levels will be assessed by ELISA and/or Luminex Multiplex assay (Invitrogen).

Measure muscle thickness, pennation angle and growth plate using ultrasound
Session #4
Architectural properties of the m. vastus lateralis, m. rectus femoris and m. gastrocnemius will be assessed non-invasively using 2D B-mode ultrasound (US). US images will be captured for each muscle to determine the muscle properties. In each image, muscle thickness will be measured as perpendicular distance between the deep and superficial aponeurosis as well as the fascicle pennation angles, i.e., the angles between the fascicle and the deep aponeurosis. Furthermore, the muscle-tendon unit and muscle belly of m. gastrocnemnius and the achilles tendon cross-sectional area, thickness and length will additionally be assessed. Their growth plates in their knee will also be measured.

Magnetic resonance imaging

Session #4
All participants will be imaged on a 3T whole-body scanner Skeletal maturation will be assessed using a merged fast field echo sequence with the following parameters: field of view 160 x 160 mm, resolution 3 x 3 x 3mm, time of echo/repetition time 20/7.61 ms, and a flip angle of 25°. Diffusion tensor imaging will be used to assess the microstructure of the growth plates, using the following technical parameters, 15 directions with b-value of 0 and 600s / mm^2, FOV 150 x 150 x 82 mm, resolution 2 x 2 x 2 mm, TE/RT 80/7100 ms (21).

Work plan and research team:
These experiments will be repeated yearly for five years, half of the group during spring and the other half during fall, startil fall -25. The work will be conducted by Ferdinand von Walden as PI. Co-investigators Petter Brodin, Jessica Norrbom, Ola Kvist and Sebastian Edman have documented previous experience conducting research on children, the immune system, and assessments of fatigue with exercise.

References

1. Saakslahti et al. Physical activity as a preventive measure for coronary heart disease risk factors in early childhood. Scand J Med Sci Sports 2004. 14 (3): 143–149.
2. Fisher et al. Fundamental movement skills and habitual physical activity in young children. Med Sci Sports Exerc 2005. 37 (4): 684–688.
3. Moore et al. Does early physical activity predict body fat change throughout childhood? Prev Med 2003. 37 (1): 10–17.
4. Carlsson et al. High physical activity in young children suggests positive effects by altering autoantigen-induced immune activity. 2016. Scand J Med Sci Sports. 26(4):441-50.
5. Ostrzyżek-Przeździecka et al. Association of low physical activity with higher respiratory tract infections frequency among pre-school children. Pediatr Res. 2023. 10-1038.
6. Timmons. Exercise and Immune Function in Children. Am J Lifest Med. 2007. 1:59.
7. Shore & Shephard. Immune responses to exercise and training: a comparison of children and young adults. Pediatr Exerc Sci 1998. 10:210-226.
8. Nieman et al. Effects of brief, heavy exertion on circulating lymphocyte subpopulations and proliferative response. Med Sci Sports Exerc. 1992. 24:1339-1345.
9. Timmons et al. Immune responses to strenuous exercise and carbohydrate intake in boys and men. Pediatr Res. 2004. 56:227-234.
10. Timmons. Immune responses to exercise in children: a brief review. Pediatr Exerc Sci. 2005. 18:290.
11. Marginson et al. Comparison of the symptoms of exercise-induced muscle damage after an initial and repeated bout of plyometric exercise in men and boys. J Appl Physiol. 2005. 99:1174-1181
12. Timmons et al. Immunological changes in response to exercise: influence of age, puberty, and gender. 2006. Med Sci Sports Exerc. 38(2):293-304.
13. Van Sluijs et al. Physical activity behaviours in adolescence: current evidence and opportunities for intervention. Lancet. 2021. 398(10298):429-442.
14. Beijar. Idrottsvanor bland ungdomar: En kartläggning över föreningsidrott och spontanidrott. 2014.
15. Behringer et al. Effects of strength training on motor performance skills in children and adolescents: a meta-analysis. Pediatr Exerc Sci. 2011;23(2):186-206.

16. Malina. Weight training in youth-growth, maturation, and safety: an evidence-based review. Clin J Sport Med. 2006. 16(6):478-87.
17. Faigenbaum & Myer. Resistance training among young athletes: safety, efficacy and injury prevention effects. Br J Sports Med. 2010. 44(1):56-63.
18. Specker et al. Does Exercise Influence Pediatric Bone? A Systematic Review. Clin Orthop Relat Res. 2015. 473(11):3658-72.
19. Bjorkman et al. Validity of the revised Ekblom Bak cycle ergometer test in adults. Eur J Appl Physiol. 2016. 116(9):1627-38.
20. Edvardsen et al. End criteria for reaching maximal oxygen uptake must be strict and adjusted to sex and age: a crosssectional study. PLoS One. 2014. 9(1):e85276.
21. Kvist et al. A cross-sectional magnetic resonance imaging study of factors influencing growth plate closure in adolescents and young adults. Acta Paediatr, 2021. 110(4):1249-1256.