OXYGEN DELIVERY AND UTILIZATION DURING ACUTE DYNAMIC EXERCISE

EFFECTS OF POLYCYSTIC OVARY SYNDROME, TYPE 1 DIABETES, AND EXERCISE TRAINING

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in Auditorium PIII, Porthania, City Centre Campus, University of Helsinki, on May 20th 2017, at 10 o’clock in the morning.

Helsinki 2017
Man, he took his time in the sun
Had a dream to understand
A single grain of sand

- from the Nightwish masterpiece
  *The Greatest Show on Earth*
  (lyrics by Tuomas Holopainen)
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This thesis is based on the following original publications:


The publications are referred to in the text by their Roman numerals and have been reprinted with permission of their copyright holders. In addition to the original publications, some unpublished data are presented.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ANS-EXE</td>
<td>Autonomic Nervous System and EXercise in gestational diabetes</td>
</tr>
<tr>
<td>ARTEMIS</td>
<td>Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection of Discovery, Prevention and Knowledge Exchange</td>
</tr>
<tr>
<td>AT</td>
<td>anaerobic threshold</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>(a-v)O$_2$</td>
<td>arterial-venous oxygen difference</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BV</td>
<td>blood volume</td>
</tr>
<tr>
<td>CaO$_2$</td>
<td>arterial oxygen content</td>
</tr>
<tr>
<td>C(a-v)O$_2$</td>
<td>systemic arterial-venous oxygen difference</td>
</tr>
<tr>
<td>CO</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CPET</td>
<td>cardiopulmonary exercise test</td>
</tr>
<tr>
<td>CPO</td>
<td>cardiac power output</td>
</tr>
<tr>
<td>CPOi</td>
<td>cardiac power output index</td>
</tr>
<tr>
<td>CTI</td>
<td>contractility index (= dZ/d$t_{max}$)</td>
</tr>
<tr>
<td>CvO$_2$</td>
<td>mixed venous oxygen content</td>
</tr>
<tr>
<td>CVP</td>
<td>central venous pressure</td>
</tr>
<tr>
<td>DPF</td>
<td>differential pathlength factor</td>
</tr>
<tr>
<td>dZ/dt</td>
<td>rate of variation of thoracic impedance</td>
</tr>
<tr>
<td>dZ/d$t_{max}$</td>
<td>largest rate of variation of thoracic impedance during systole (= CTI)</td>
</tr>
<tr>
<td>EDGE</td>
<td>Exercise, Diet and GEnes in T1D</td>
</tr>
<tr>
<td>EDV</td>
<td>end-diastolic volume</td>
</tr>
<tr>
<td>EDVi</td>
<td>end-diastolic volume index</td>
</tr>
<tr>
<td>EF</td>
<td>ejection fraction</td>
</tr>
<tr>
<td>EIAH</td>
<td>exercise-induced arterial hypoxemia</td>
</tr>
<tr>
<td>ES</td>
<td>standardized effect size</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>forced expiratory volume in one second</td>
</tr>
<tr>
<td>FFM</td>
<td>fat-free mass</td>
</tr>
<tr>
<td>FinnDiane</td>
<td>Finnish Diabetic Nephropathy Study</td>
</tr>
<tr>
<td>FVC</td>
<td>forced vital capacity</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>[Hb]</td>
<td>hemoglobin concentration</td>
</tr>
<tr>
<td>HbA$_{1c}$</td>
<td>glycosylated hemoglobin A$_{1c}$</td>
</tr>
<tr>
<td>[HbCO]</td>
<td>carboxyhemoglobin concentration</td>
</tr>
<tr>
<td>HHb</td>
<td>deoxygenated hemoglobin</td>
</tr>
<tr>
<td>Δ[Hb]</td>
<td>relative concentration change in deoxygenated hemoglobin</td>
</tr>
<tr>
<td>%Δ[Hb]</td>
<td>normalized relative concentration change in deoxygenated hemoglobin</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>homeostasis model assessment of insulin resistance</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>IR</td>
<td>insulin resistance</td>
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LTPA leisure-time physical activity
MAP mean arterial blood pressure
MET metabolic equivalent (= resting pulmonary oxygen uptake)
NIP near-infrared spectroscopy inflection point
NIP_{TissueAT/RC} NIP observed in (leg muscle, arm muscle, or cerebral) tissue and being closest to anaerobic threshold or respiratory compensation point
NIRS near-infrared spectroscopy
NO nitric oxide
Oxygen
O_{2}Hb oxygenated hemoglobin
Δ[O_{2}Hb] relative concentration change in oxygenated hemoglobin
O_{2} pulse pulmonary oxygen uptake / heart rate
O_{2} pulse_{i} oxygen pulse index
PaCO_{2} partial pressure of arterial carbon dioxide
PaO_{2} partial pressure of arterial oxygen
PCOS polycystic ovary syndrome
PETCO_{2} end-tidal pressure of carbon dioxide of respired gases
PvO_{2} partial pressure of muscle venous oxygen
Q cardiac output
Q_{i} cardiac output index
Q_{VL} local microvascular blood flow in the vastus lateralis muscle
RC respiratory compensation point
RER respiratory exchange ratio
SD standard deviation
SHBG sex hormone-binding globulin
SpO_{2} arterial oxygen saturation
SV stroke volume
SV_{cal} stroke volume during a PhysioFlow calibration procedure
SV_{i} stroke volume index
SVR systemic vascular resistance
SVR_{i} systemic vascular resistance index
tHb total hemoglobin
Δ[tHb] relative concentration change in total hemoglobin
tHb-mass total hemoglobin mass
TSI tissue saturation index
V_{A} alveolar ventilation
V_{CO_{2}} pulmonary carbon dioxide output
V_{E} minute ventilation
VO_{2} pulmonary oxygen uptake
VO_{2max} maximal pulmonary oxygen uptake
VO_{2peak} peak pulmonary oxygen uptake
WEBWOMEX WELL-Being of WOMen during pregnancy: focus on individualized EXercise training
Z_{max} highest thoracic impedance during systole
Z_{min} lowest thoracic impedance during systole
ABSTRACT

Background and aims
Polycystic ovary syndrome (PCOS) and type 1 diabetes are relatively common endocrinopathies, often manifesting across most of the lifespan. Cardiovascular risk is increased in both PCOS and type 1 diabetes, affecting patients' quality of life and overall prognosis. Acute dynamic exercise is a widely used tool to examine an individual's cardiovascular health. Particularly, peak pulmonary O₂ uptake (VO₂peak), measured during maximal incremental dynamic exercise and reflecting the highest achievable level of whole-body oxidative metabolism, is a strong and independent predictor of cardiovascular morbidity and mortality. This is because VO₂ response to exercise consists of integrated serial steps of systemic O₂ delivery (i.e., pulmonary function, cardiac pump function, blood O₂ carrying capacity), peripheral O₂ delivery (i.e., skeletal muscle blood flow), and peripheral O₂ extraction and utilization (i.e., muscle O₂ diffusion and metabolism). Early identification of potential disease-related derangements in these integrated organ systems would provide important information on clinical manifestations of PCOS and type 1 diabetes; however, studies examining the issue from the integrated perspective have been sparse. Little evidence also exists on effects of long-term exercise training on the signs of the derangements related to type 1 diabetes.

The aim of this thesis was to study O₂ delivery and utilization during acute dynamic exercise in both healthy individuals and individuals with PCOS or type 1 diabetes. The adaptations induced by long-term exercise training were also examined in individuals with type 1 diabetes and compared with those in healthy individuals. Acute exercise was thus used as a physiological probe to identify early signs of cardiovascular dysfunction related to the disease states of PCOS and type 1 diabetes, whereas exercise training was employed to demonstrate whether it alleviates such early signs associated with type 1 diabetes.

Subjects and methods
Studies I-IV of this thesis belonged to a Canadian-Finnish research collaboration entitled “ARTEMIS – Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection of Discovery, Prevention and Knowledge Exchange”. Data on 22 healthy adult men (Study I), 15 adult overweight or obese women with and 15 without PCOS (Study II), seven adult men with and 10 without type 1 diabetes (Study III), and eight adult men with and eight without type 1 diabetes (Study IV) were included in the final analyses. The groups of Studies II-IV were matched for age, anthropometry, and leisure-time physical activity (II, III) or baseline VO₂peak (IV). Neither PCOS women (II) nor men with type 1 diabetes (III, IV) had clinically overt cardiovascular disease. The subjects performed either maximal incremental treadmill (I) or cycling (II-IV) exercise tests, and in Study IV the subjects did so both before and after a 1-year individualized exercise training intervention. Integrated data were collected during the exercise tests; alveolar gas exchange (volume turbine and mass spectrometry) (I-IV), arterial O₂ saturation (pulse oximetry) (I-IV), heart rate (electrocardiography) (I-IV),
cardiac pump function (PhysioFlow impedance cardiography) (II, III), and active leg muscle deoxygenation (I, III, IV) and blood flow (III) (near-infrared spectroscopy [NIRS]), were monitored. In Study I, less active arm muscle and cerebral deoxygenation were also monitored (NIRS). In Study IV, peak O2 pulse was calculated to indirectly reflect cardiac pump function. Total hemoglobin mass (I) and blood volume (III) were also determined (carbon monoxide rebreathing method), and blood samples were drawn particularly to measure hemoglobin concentration (I-IV) and to evaluate glycemic control (glycosylated hemoglobin A1c) (III, IV).

**Results**

I: Deoxygenation profiles, reflecting local imbalance between O2 delivery and utilization, in the tissues of interest were linked to alveolar gas exchange (i.e., whole-body) responses during maximal incremental treadmill exercise in healthy men. II: Reduced VO2peak, reduced peak systemic O2 extraction, and a pronounced cardiac response to increasing VO2 but otherwise intact systemic O2 delivery were observed in overweight and obese PCOS women. III: Reduced peak cardiac pump function, being associated with reduced blood volume, and independently deteriorated peak active leg muscle blood flow led to reduced VO2peak and were suggested to be associated with glycosylated hemoglobin A1c in men with type 1 diabetes. IV: The 1-year training intervention improved VO2peak and peak O2 pulse similarly in men with and without type 1 diabetes but had no effect on NIRS-derived local active muscle O2 extraction or glycosylated hemoglobin A1c in men with type 1 diabetes. In addition, consistent associations between training dose and responses were observed in healthy men but not in those with type 1 diabetes.

**Conclusions**

The findings of this thesis overall highlight the integrated nature of O2 delivery and utilization responses to acute dynamic exercise. The following conclusions on disease-related early signs of cardiovascular dysfunction can be drawn: In women with excess weight, PCOS per se is linked to alterations in peripheral adjustments to acute dynamic exercise rather than to limitations of systemic O2 delivery. In type 1 diabetes, both systemic and peripheral cardiovascular impairments, being affected by glycemic control, limit O2 delivery during acute dynamic exercise. Furthermore, regular exercise training improves VO2peak and probably cardiac pump function, and hence, improves the overall prognosis of cardiovascular morbidity in type 1 diabetes. However, exercise training, at least to the extent in Study IV, is not able to alleviate diabetes-related defects within active muscle microvasculature or to improve patients’ glycemic control. The lack of dose-response associations in men with type 1 diabetes may highlight the need for more individualized exercise regimens in individuals with type 1 diabetes.

These conclusions lead future multi-disciplinary research to identify more detailed mechanisms behind, the clinical significance of, and treatment for the observed disease-specific early signs of cardiovascular dysfunction.
TIIVISTELMÄ (ABSTRACT IN FINNISH)

Tausta ja tavoitteet

Munasarjojen monirakkulaoireyhtymä (PCOS) ja tyypin 1 diabetes ovat yleisiä tauteja ja vaikuttavat potilaiden elämään usein koko elinkaaren ajan. Molempien tautien liittyvä kohonnut sydän- ja verenkiertoelimistön sairastavuuden riski, mikä vaikuttaa sekä potilaiden elämänlaatuun että ennusteeen. Sydän- ja verenkiertoelimistön terveyden tutkimiseen yleisesti käytetty menetelmä on akuutti dynaaminen kuormitus; erityisesti maksimaalinen hapenotterkyky (VO₂peak), joka mitataan maksimaalisen portaittain nousevan dynaamisen kuormituksen aikana ja joka kuvaa koko kehon maksimialisti O₂-kulutuskyväyksi, on sydän- ja verenkiertoelimistöön liittyvää sairastavuutta ja kuolleisuutta vahvasti ja itsenäisesti ennustava muuttuja. Näin siksi, että VO₂peak muodostuu toisiinsa kokonaisvaltaisesti integroituneiden systeemisen O₂-jakelun (keuhkojen toiminta, sydämen pumppustoiminta, veren O₂-kuljetuskapasiteetti), perifeerisen O₂-jakelun (verenvirtaus luostolihaksissa) sekä perifeerisen O₂-ekstraktion - ja -käytön (O₂-diffusiio ja -käyttö luostolihaksissa) yhteistyön tuloksena. Näihin hengitys-, sydän- ja verenkiertoelimistön osatekijöihin liittyvien tautiksiotisen häiriöiden varhaisen tunnistamisen tarjotaan tärkeää tietoa PCOS:n ja tyypin 1 diabetes:ille liittyville sairastavuudesta ja henkilöiden varhaisille ennusille. Myös pitkäaikaisen liikuntaharjoittelun vaikutuksia tyypin 1 diabetes:ille liittyviin sydän- ja verenkiertoelimistön häiriöihin on tutkittu vähän.

Tämän tutkimuksen tavoitteena oli tutkia O₂-jakelua ja -käyttöä akuutin dynaamisen kuormituksen aikana sekä terveillä henkilöillä että PCOS- ja tyypin 1 diabetes -potilailla. Myös pitkäaikaisen liikuntaharjoittelun vaikutuksia tyyppin 1 diabetes -potilaisten k-liinisten ilmentymien osalta; tutkimustieto kokonaisvaltaisesta näkökulmasta on tältä tieteen alueelta vähäistä. Myös pitkäaikaisen liikuntaharjoittelun vaikutuksia tyypin 1 diabetes:ille liittyviin sydän-, verenkiertoelimistön sairastavuuden varhaisia ennusmerkkejä, kun taas liikuntakoulutuksen vaikutuksissa, pystytääanko sillä vaimentamaan kyseisiä tyypin 1 diabetekseen liittyviä varhaisia ennusmerkkejä.

Aineisto ja menetelmät


**Tulokset**

I: Tutkittujen kudosten O₂-tasapainoprofiilit, jotka kuvaavat paikallista O₂-jakelun ja -käytön välistä epätasapainoa, olivat yhteydessä alveolaarisen kaasujenvaihdon ja siten koko kehon tason vasteisiin maksimaalisen portaittain nousevan juoksumattokuormitukseen aikana terveillä miehillä. II: Ylipainoisilla ja lihavilla PCOS-naisilla havaittiin alentunut \(\dot{V}O_2\text{peak}\), alentunut systeeminen O₂-ekstraktio maksimikuormituksessa ja korostunut systeeminen O₂-ekstraktiokorosta suhteessa kuormituksen aikana kasvaneeseen O₂-kulutukseen. III: Tyypin 1 diabetesta sairastavilla miehillä havaittiin alentunut sydämen pumppaustoiminta, joka oli yhteydessä heidän alentuneeseen veritilavuuteensa, ja sydämen pumppaustoiminnasta riippumattomasti alentunut aktiivisen luostolihakseen verenvirtaus. Nämä tekijät johtivat tyypin 1 diabetes -miesten alentuneeseen \(VO_2\text{peak}\)yn ja olivat yhteydessä glykoituuteen hemoglobiini A1c:n tasoon. IV: Vuoden liikuntaharjoitteluinterventio paransi \(VO_2\text{peak}\):ää ja maksimaalista O₂-pulssia yhtä paljon terveillä ja tyypin 1 diabetes sairastavilla miehillä mutta sillä ei ollut vaikutusta NIRS-johdannaiseen aktiivisen luostolihakseen kuormituksenaikaiseen O₂-ekstraktiotoon eikä myöskään glykoituuteen hemoglobiini A1c:n tasoon tyypin 1 diabetes -miehillä. Lisäksi havaittiin, että liikuntaharjoittelun annoksen ja vasteen välillä oli johdonmukainen suhde terveillä miehillä mutta et tyypin 1 diabetesta sairastavilla.

**Johtopäätökset**

Tulokset korostavat kokonaisuudessaan, kuinka O₂-jakelu ja -käyttö reagoivat akuuttiin dynamiseen kuormituukseen systeemillisella ja perifeerisellä tasolla yhteen integroituneesti. Tautikohtaisten sydän- ja verenkiertosairastavuuden varhaisten ennusmerkkien osalta voidaan vetää seuraavat johtopäätökset: Ylipainoisilla naisilla PCOS itsessään on yhteydessä mieluummin perifeerisen O₂-jakelun ja tai -käyttön kuin systeemisen O₂-jakelun häiriöihin akuutin kuormituksen aikana. Tyypin 1 diabetesta sairastavilla miehillä O₂-jakelun on akuutin kuormituksen aikana rajoittunut sekä systeemisesti että perifeerisellä tasolla, ja nämä rajoitteet ovat yhteydessä pitkäaikaiseen veren glukoositason. Säännöllinen liikuntaharjoittelul mitattu
VO2peak:ä, mahdollisesti sydämen pumppaustoimintaa ja siten sydän- ja verenkiertoelimistön sairastavuuden ennustetta tyyppin 1 diabetesta sairastavilla miehillä. Pitkäaikainen liikuntaharjoittelu, ainakaan sellaisena kuin se toteutui osatutkimuksessa IV, ei kuilunkaan paranna aktiivisen luustolihaksen mikroverenkierron häiriöitä eikä pitkääikaista veren glukoositasapainoa tyyppin 1 diabeteksessa. Liikuntaharjoittelun annos-vastesuhteen puuttuminen tyyppin 1 diabetesta sairastavilla miehillä lisäksi korostaa, että liikuntaharjoittelun tässä potilasryhmässä tulee olla yksilöllisesti äärimmäisen räätälöityä suotuisien vastauksien aikaansamiseksi.

Nämä tulokset ja johtopäätökset ohjaavat tulevaisuuden poikkitieteellistä tutkimusta tunnistamaan havaittujen tautikohtaisten sydän- ja verenkiertoelimistön sairastavuuden varhaisten ennusmerkkien yksityiskohtaisempia mekanismeja, kliinistä merkittävyyttä ja hoitoa.
1 INTRODUCTION

PCOS is a complex endocrinopathy characterized by hyperandrogenism, chronic oligo- or anovulation, and polycystic ovaries (1-3). Depending on the population studied and the diagnostic criteria applied, PCOS affects approximately 6-20% or even more of women (4-7), making it one of the most common human disorders and the most common single endocrinopathy in reproductive-aged women (1). Diabetes mellitus, henceforth referred to as diabetes, is characterized by a chronic state of hyperglycemia. While 382 million people worldwide were estimated to have diabetes in 2013 and the prevalence is expected to reach 592 million people by 2035 (8), autoimmune-mediated type 1 diabetes accounts for 5-10% of all diabetes patients (9). Type 1 diabetes is typically diagnosed before the age of 15 years (10) and is one of the most common chronic diseases of childhood. Interestingly, type 1 diabetes has its highest incidence in Finland (~64/100,000/year below the age of 15) (11).

PCOS has important long-term health implications related to reproductive and hormonal, psychosocial, and metabolic and cardiovascular disturbances, with excess weight being a common factor contributing to all of these derangements (1-3,12). Insulin resistance (IR) has an essential role in the pathogenesis of PCOS, hence driving different phenotypic features of women with the syndrome (1-3), and also significantly increases patients’ risk of metabolic disturbances such as type 2 diabetes (13). Accordingly, vast evidence suggests that lifelong metabolic dysfunction in women with PCOS results in exaggerated risk of cardiovascular disease (1,2,14). In diabetes, clinical manifestations include well-known microvascular (i.e., retinopathy, nephropathy, and neuropathy) and macrovascular complications (i.e., coronary artery, cerebrovascular, and peripheral artery diseases). In addition, chronic hyperglycemia can also lead to diabetes-specific myocardial dysfunction known as diabetic cardiomyopathy (15-17), which has recently been shown to increase the risk of early development of heart failure already in relatively young adults with type 1 diabetes (18). Taken together, a common feature of PCOS and type 1 diabetes is that both are characterized by an increased risk of cardiovascular disease.

An individual’s cardiovascular risk and its magnitude can strongly and independently be predicted by VO2peak (19). VO2peak reflects the highest achievable level of whole-body oxidative metabolism and is typically measured during a maximal incremental cardiopulmonary exercise test (CPET) involving dynamic exercise, such as running or cycling, that features repetitive contractions of large muscle groups (20-22). VO2 response to such acute dynamic exercise consists of an integrated pathway for O2 from the atmosphere to the skeletal muscle mitochondria. The pathway contains serial steps of alveolar ventilation (VA), alveolar-to-capillary diffusion, circulation (including cardiac pump function, blood O2 carrying capacity, muscle blood flow), and muscle O2 diffusion and utilization. In consequence, as VO2peak reflects the highest achievable outcome of the integrated pathway and depends on all of the serial steps mentioned, acute dynamic exercise is an excellent physiological probe to identify possible disease-related defects in the serial organ systems responding for appropriate adjustments of O2 delivery and subsequent mitochondrial O2 utilization (23-30).
As overall cardiovascular risk is increased in PCOS and type 1 diabetes and performing acute dynamic exercise is able to reveal detailed signs of organ dysfunction, possibly limiting O₂ delivery and utilization, it is reasonable to examine responses to acute dynamic exercise in these patient groups. However, existing data on responses to acute dynamic exercise in women with PCOS are sparse. \( \text{VO}_{2\text{peak}} \) itself has been observed to be either reduced or intact in PCOS and is likely to depend on the magnitude of IR (31-35). By contrast, little is known of more specific adjustments of systemic O₂ delivery (i.e., pulmonary function, cardiac pump function, blood O₂ carrying capacity) or peripheral O₂ delivery (i.e., muscle blood flow) and utilization to exercise in women with the syndrome. Current literature on responses to acute dynamic exercise in type 1 diabetes is understandably more extensive than that in PCOS. While several studies have reported reduced \( \text{VO}_{2\text{peak}} \) in type 1 diabetes, it has also been well acknowledged that individuals with the disease are capable of attaining a similar \( \text{VO}_{2\text{peak}} \) to that of healthy individuals but this most likely depends on them maintaining good glycemic control (36). Meanwhile, numerous studies have observed separate systemic and peripheral determinants of \( \text{VO}_{2} \) response to exercise in patients with type 1 diabetes but no studies have simultaneously examined the integrated contribution of these systemic and peripheral derangements to \( \text{VO}_{2\text{peak}} \).

Exercise training is a planned form of physical activity. Ever since Greek physicians, with Herodicus and Hippocrates leading the way, around 400 B.C. began using exercise training as a therapy for various diseases (37), extensive scientific evidence has verified that exercise training is truly an important behavior for individual and population health (38). Hence, adherence to regular exercise training has also become a generally recommended part of overall treatment of patients with type 1 diabetes (39). Particularly endurance-type exercise training leads to various adaptations in the different components of the integrated O₂ pathway, thus increasing \( \text{VO}_{2\text{peak}} \) (40,41). However, such adaptations to long-term (i.e., >4-5 months) exercise training interventions have been sparsely studied in type 1 diabetes. Moreover, evidence of the effect of exercise training on glycemic control of type 1 diabetes patients is inconclusive, and long-term training intervention studies have not even been conducted to examine the issue.

The purposes of this thesis were 1) to study O₂ delivery and utilization during acute dynamic exercise in both healthy individuals and individuals with PCOS or type 1 diabetes, and 2) to examine the adaptations induced by long-term 1-year exercise training in individuals with type 1 diabetes. The large-scale aims were thus to identify early signs of cardiovascular dysfunction in the two patient groups and to examine whether long-term exercise training alleviates the early signs related to type 1 diabetes. The scientific infrastructure, including the integrated noninvasive methodology employed, was provided by a Canadian-Finnish research collaboration supplemented by Finnish research collaborations.

A reader of this thesis is first introduced to the topic through a narrative literature review. The literature review has been outlined so that it has its focuses on dynamic whole-body exercise, endurance-type exercise training, and data on human studies with some occasional complementary examples from animal models. The literature review is then followed by the integrative presentation of the methods, results, discussion, and conclusions of the four original studies.
2 REVIEW OF THE LITERATURE

2.1 OXYGEN DELIVERY AND UTILIZATION DURING ACUTE DYNAMIC EXERCISE

Oxidative phosphorylation within mitochondria of mammalian cells is the fundamental energy-producing biochemical process utilizing \( O_2 \) and making life possible. The basic equation describing mitochondrial regeneration of adenosine triphosphate (ATP), containing the high-energy bonds that are transduced into energy, is as follows (23):

\[
3 \text{ADP} + 3 \text{P}_i + \frac{1}{2} \text{O}_2 + \text{NADH} + \text{H}^+ \rightarrow 3 \text{ATP} + \text{NAD}^+ + \text{H}_2\text{O}
\]

where ADP is adenosine diphosphate, P\(_i\) is inorganic phosphate, H\(^+\) is a hydrogen ion, H\(_2\)O is water, and NADH and NAD\(^+\) are the reduced and oxidized forms of nicotinamide adenine dinucleotide, respectively.

At rest, whole-body \( \dot{V}O_2 \), traditionally measured as \( \dot{V}O_2 \), averages 3-4 mL/min/kg body weight, corresponding to resting \( \dot{V}O_2 \) of 0.15-0.4 L/min in young healthy humans weighing 50-100 kg. Most (~80%) of the \( O_2 \) utilized in a resting human is used by the brain, heart, liver and kidneys (22,41,42).

Dynamic exercise, such as walking, running, cycling, or different ball games, features skeletal muscle contractions performed over and over again (22). Acute dynamic exercise leads to elevated ATP turnover particularly within active skeletal muscles, thus increasing overall \( O_2 \) utilization according to Equation 1. In detail, during maximal exercise, henceforth referred to as peak exercise, \( \dot{V}O_2 \) can increase 10- to 15-fold, reaching 30-50 mL/min/kg in young untrained subjects and near 60 mL/min/kg in young trained subjects (22). Furthermore, a peak \( \dot{V}O_2 \) (\( \dot{V}O_2\text{peak} \)) of 70-85 mL/min/kg (or ~5.0-6.5 L/min) is typically reported in elite male endurance athletes (43-45). As muscle \( O_2 \) and high-energy phosphate stores are small, any sustained elevation of muscle ATP turnover requires that \( O_2 \) is delivered to the muscle mitochondria at a rate precisely matched to mitochondrial \( O_2 \) requirements (46). Consequently, acute dynamic exercise presents a great physiological challenge to the pulmonary, cardiovascular, and muscular systems, which strive to meet the increased requirements for \( O_2 \) delivery and subsequent mitochondrial \( O_2 \) utilization.

A pathway for \( O_2 \) from the atmosphere to the mitochondria has been detailed in several scholarly reviews (e.g., 23,26,28-30). The pathway contains an integrated series of steps: Pulmonary function comprises adequate \( V_A \) and gas exchange being responsible for diffusing \( O_2 \) from the environment to the blood, cardiac pump function delivers \( O_2 \) to the periphery within the limits of blood \( O_2 \) carrying capacity, and diffusion of \( O_2 \) then transfers \( O_2 \) from the microcirculation to the mitochondria within the peripheral tissues (i.e., particularly active skeletal muscles during acute dynamic exercise).

At the systemic whole-body level, the presented pathway for \( O_2 \) is mathematically distilled into the Fick principle (47):
\[
\dot{V}O_2 = \dot{Q} \times C(a-v)O_2
\]

where \( \dot{Q} \) is cardiac output and \( C(a-v)O_2 \) is systemic arterial-venous \( O_2 \) difference.

For \( \dot{Q} \) at rest, textbook values in young healthy males weighing around 70 kg are around 5 L/min, which is achieved as a product of heart rate (HR) of \( \sim 70 \) bpm and a stroke volume (SV) of \( \sim 70 \) mL (48-51). At the same time, while \( \sim 50 \) mL O\(_2\)/L (i.e., \( C(a-v)O_2 \)) is extracted from the peripheral circulation at rest, resting \( \dot{V}O_2 \) is \( \sim 250 \) mL/min or 3.5 mL/min/kg in a 70-kg human (22), as approximated above.

Regarding acute dynamic exercise and adaptations to exercise training, the different components of the Fick principle will be thoroughly covered in the following sections examining systemic and peripheral \( O_2 \) delivery as well as peripheral \( O_2 \) extraction and utilization during acute dynamic exercise along with \( \dot{V}O_2\)peak and effects of endurance-type exercise training on these integrated adjustments. Figure 1 simplifies how the text flows in Sections 2.1.1-2.1.2 towards the integrated concept of \( \dot{V}O_2\)peak, presented in Section 2.1.3.

**Figure 1** Simplified flow of Sections 2.1.1-2.1.2 towards the integrated concept of \( \dot{V}O_2\)peak, presented in Section 2.1.3.
2.1.1 SYSTEMIC OXYGEN DELIVERY DURING ACUTE DYNAMIC EXERCISE

The components determining systemic O₂ delivery (i.e., circulatory O₂ convection for the body as a whole) can be recognized by compartmentalizing the Fick principle (Equation 2) (23):

\[
\dot{V}O_2 = \dot{Q} \times C(a-v)O_2 = \dot{Q} \times (CaO_2 - CvO_2) = \dot{Q} \times CaO_2 - \dot{Q} \times CvO_2
\]

where \(CaO_2\) is arterial O₂ content and \(CvO_2\) is mixed venous O₂ content.

The minuend \((\dot{Q} \times CaO_2)\) of Equation 3 refers to systemic O₂ delivery. In detail, \(CaO_2\) is a product of arterial O₂ saturation \((SpO_2)\), hemoglobin concentration \([Hb]\), and 1.34, which is the physiological O₂ binding coefficient of hemoglobin \((Hb)\) (1.34 mL/g (52); range of reported values of nonsmokers: 1.30-1.39 mL/g (53)). Notably, ~98.5% of O₂ delivered from the lungs to the periphery is carried by \(Hb\), while the remaining ~1.5% is transported in the dissolved state in the water (51). Taken together, according to the chronological order of the O₂ pathway, systemic O₂ delivery is determined by pulmonary function (approximated by \(SpO_2\)), cardiac pump function (quantified as \(\dot{Q}\)), and blood O₂ carrying capacity (reflected by [Hb]) (23,26,28-30).

2.1.1.1 Pulmonary function

\(\dot{V}O_2\) increases linearly as a function of intensity during acute dynamic exercise (54,55). Eventually, \(\dot{V}O_2\) may or may not reach an observable plateau at peak exercise (56-58). \(\dot{V}O_2\) here refers to pulmonary O₂ uptake but closely reflects O₂ utilization of active leg muscles over the broad range of cycling intensities, from moderate to severe (54,55). In other words, \(\dot{V}O_2\) is tightly coupled with aerobic energetics according to Equation 1 and particularly reflects the increasing energetic demand of the exercising musculature in the limbs during acute dynamic exercise (46). It is notable that as a sign of their pronounced O₂ demand, respiratory muscles themselves may also receive a substantial amount of \(\dot{Q}\) at peak exercise (59). However, it is not fully elucidated whether this affects blood flow to and O₂ utilization within the exercising limb musculature (see Section 2.1.2.1).

In addition to the rise in \(\dot{V}O_2\), pulmonary CO₂ output (\(\dot{V}CO_2\)) also rises with exercise intensity due to the increased activation of metabolic pathways (particularly glycolysis). Furthermore, \(\dot{V}CO_2\) even accelerates if exercise intensity exceeds a certain threshold (60-62). This acceleration is due to an anaerobic component of muscle metabolism reflected by increased lactate and reduced bicarbonate concentrations in the blood (62,63). The threshold at which \(\dot{V}CO_2\) begins to accelerate in relation to \(\dot{V}O_2\) is termed an anaerobic threshold (AT) (60,61).

\(V_A\) and diffusive gas exchange across the alveolar membrane to the erythrocytes are the pulmonary components responsible for appropriate exercise-induced increases in \(\dot{V}O_2\) and \(\dot{V}CO_2\). Pulmonary ventilation is quantified as minute ventilation \((\dot{V}E)\), which reflects the volume of expired air exhaled from the lungs in one minute. \(\dot{V}E\) increases linearly when plotted against exercise intensity (or \(\dot{V}O_2\)) until reaching the respiratory compensation point (RC). Above RC, \(\dot{V}E\) rises more rapidly in order to compensate
acidosis caused by the anaerobic component of energy metabolism (60). Consequently, this hyperventilation results in decreases in partial pressure of arterial CO₂ (PaCO₂) and its surrogate (end-tidal pressure of CO₂ of respired gases [PETCO₂]) at exercise intensities above RC (60,64).

Regarding an interplay between $\dot{V}_A$ and partial pressures of alveolar O₂ and CO₂, modest hyperventilation is also needed to reduce alveolar CO₂ pressure so as to increase alveolar O₂ pressure enough to raise a driving pressure for alveolar-to-capillary diffusion of O₂ (41). In the average individual exercising at sea level, the driving pressure for O₂ diffusion (i.e., alveolar-arterial O₂ pressure difference) commonly increases from 5-10 mmHg at rest to about 25 mmHg at peak exercise (41). This is sufficient to maintain partial pressure of arterial O₂ (PaO₂) and SpO₂ near resting levels at light, moderate, severe, and even peak exercise intensities (65-68).

Thus, the lungs of healthy nonathletes perform their tasks of both $\dot{V}_A$ and alveolar-to-capillary diffusion extremely well under normoxic conditions.

Contrary to healthy nonathletes, fit athletes may undergo exercise-induced arterial hypoxemia (EIAH; SpO₂ decreasing [far] below 95% (69)) at near peak or peak exercise (70), but even during intensities of 40-50% of $\dot{V}O_{2peak}$ (70,71). Both an excessive alveolar-arterial O₂ pressure difference and inadequate compensatory hyperventilation contribute to EIAH, as do acid- and temperature-induced rightward shifts of the O₂ dissociation curve at any given PaO₂ (69). In more detail, inadequate $\dot{V}_A$ in relation to $\dot{Q}$ (i.e., ventilation-perfusion [$\dot{V}_A/\dot{Q}$] mismatch) and limited O₂ diffusion contribute equally to the excessive alveolar-arterial O₂ pressure difference, whereas exercise hyperventilation may be limited by expiratory flow limitation set by, for instance, mechanical constraints of airway diameter and/or respiratory muscle force production (69). Interestingly, EIAH may be more frequent in women than in men (72). In fact, even some untrained women may develop EIAH (73). This sex difference is particularly due to pronounced mechanical constraints in women (72-74).

Taken together, while pulmonary function does determine systemic O₂ delivery, and thus $\dot{V}O_{2}$, during acute dynamic exercise, it rarely sets any limitation on these in healthy nonathletes. However, such pulmonary limitations may be evident in trained athletes, but also in some untrained women; each 1% reduction in SpO₂ (or CaO₂) leads to 1-2% reduction in $\dot{V}O_{2peak}$ below the SpO₂ level of 95% (69).

### 2.1.1.2 Cardiac pump function

**Cardiac cycle**

Cardiac events occurring from the beginning of one heartbeat to the beginning of the next one constitute the cardiac cycle, which is presented in Figure 2. The cardiac cycle comprises a relaxation period called diastole, during which the heart fills with blood, followed by a contraction period called systole. Diastole begins with a period of isovolumic relaxation; the right and left intraventricular pressures decrease rapidly back to their low diastolic levels, while pulmonary, atrioventricular, and aortic valves are closed. The atrioventricular valves then open and the ventricles fill with blood during three phases (rapid inflow, diastasis, and atrial contraction) to reach their end-diastolic volumes (EDV). After the ventricular filling, systole is initiated; the ventricles
begin contracting and the ventricular pressures rise, causing the atrioventricular valves to close. During the following period of isovolumic contraction, the ventricles build up sufficient pressures to push the pulmonary and aortic valves open against the pressures in the pulmonary artery and aorta. After the pulmonary and aortic valves have opened, blood is ejected from the ventricles until the elevated pressures of filled and distended large arteries lead to the closing of the pulmonary and aortic valves. The volume pumped towards the periphery during systole is SV, the ratio of SV to EDV is termed ejection fraction (EF), and the difference between EDV and SV reflects end-systolic volume. After the systolic ejection, the next cardiac cycle begins (50).

Figure 2 Cardiac cycle for left ventricular function, demonstrating changes in left atrial pressure, left ventricular pressure, aortic pressure, ventricular volume, and electrocardiogram. Modified from Guyton and Hall (50).

Cardiac pump function during acute dynamic exercise

During acute dynamic exercise, $Q$, which is the product of HR and SV and has a key role in the Fick principle (Equation 2), rises in proportion to $\dot{V}O_2$. Reviews of extensive literature in the field have documented that $Q$ increases between 5 and 6 L for every 1 L increase of $\dot{V}O_2$ in healthy individuals (41,75). Based on the data collected before modern obesity and inactivity epidemics, typical peak values of $Q$ of 20-year-olds are 15-20 L/min in sedentary, 20-25 L/min in active, and 25-40 L/min in elite athlete
individuals (22). Since peak HR may increase up to ~200 bpm during acute dynamic exercise rather regardless of fitness level (22,48), these values indicate that peak SV in these groups is correspondingly around 70-100 mL, 100-125 mL, and 125-200 mL, respectively (22). Anecdotally, \( \dot{Q} \) of 42.3 L/min in a world-class orienteer is probably the highest published value of cardiac pumping capacity (45).

The exercise-induced rise in \( \dot{Q} \) is mainly achieved through elevated HR (i.e., tachycardia), and to a lesser extent through increased SV (41,49). The relationship between HR and \( \dot{V}O_2 \) is linear (41,49). In humans, the rapid onset of tachycardia up to ~100 bpm is mainly attributable to the withdrawal of parasympathetic vagal activation, whereas HR increases above the level of ~100 bpm are primarily due to sympathetic activation of cardiac \( \beta_1 \)-adrenoceptors (76,77). SV is raised partly through increased ventricular filling pressure, which increases EDV via the Frank-Starling mechanism, and partly through increased myocardial contractility and EF, which reduce end-systolic volume (78-80); the former mechanism contributes \( \sim 2/3 \) and the latter \( \sim 1/3 \) to the increase in SV from seated rest to upright exercise (80). The rise in filling pressure is brought about by sympathetically mediated venoconstriction in the splanchnic circulation and the skeletal muscle pump, which compresses veins in the exercising limbs, and thus, both provides optimal filling pressure for the heart at all levels of exercise and simultaneously prevents central venous pressure (CVP) from falling (41,49). The increase in myocardial contractility is due to increased stimulation of cardiac sympathetic nerves and also to a lesser degree to the increase in circulating adrenaline (41,49).

The response of SV to incremental upright exercise was observed to be nonlinear as early as 1960 (78). Further observations of the SV response to acute exercise from the 1960s (45,81,82) eventually led to the common acceptance that after increasing from rest, SV plateaus at 40-50% of \( \dot{V}O_2 \)peak during incremental upright exercise. The first study to simultaneously measure pulmonary and systemic pressures and left ventricular volume in humans during upright exercise explained this as follows: at low intensities, SV increases via increases in ventricular filling pressures and EDV, whereas at high intensities, EDV decreases, possibly as a result of tachycardia, and SV thus is just maintained through a decrease in end-systolic volume (i.e., increase in contractility) (83). However, current evidence shows that SV response to incremental upright exercise varies between individuals depending on age, fitness level, genetics, and sex (84). In particular, a progressive increase in SV during exercise to \( \dot{V}O_2 \)peak may be seen in endurance-trained individuals (85-88) due to their higher left ventricular diameter, wall thickness, and mass (89-93), larger ventricular compliance (89,94), more rapid diastolic filling (85-87), and possibly also due to greater contractility (95,96) and longer ventricular ejection time (86,87). However, since a decrease in SV and \( \dot{Q} \) at high exercise intensities has also been observed in fit individuals (97), no consensus on this issue has been reached. Figure 3 illustrates the interindividual variability of SV responses to increasing exercise intensity, detailed elsewhere by Vella et al. (84).
Darcy's law defines the systemic relationships between $\dot{Q}$, mean arterial blood pressure (MAP), CVP, and systemic vascular resistance (SVR) (49):

$$\dot{Q} = \frac{(MAP - CVP)}{SVR}$$

where MAP is calculated by the standard equation ($MAP = \frac{[systolic\ arterial\ blood\ pressure + 2 \times diastolic\ arterial\ blood\ pressure]}{3}$), and CVP is close to 0 mmHg from rest to peak exercise (97).

Equation 4 assumes that blood flow from the left ventricle back to the right atrium is determined by the pressure difference between the two chambers, discounting the fact that the circuit is affected by the skeletal muscle pump, which also delivers energy to drive blood flow (41). However, as incremental exercise from rest to peak intensity causes only a modest rise in MAP (from 90 to 115 mmHg when measured from the aorta), the rise in $\dot{Q}$ and vasodilatation in the exercising skeletal musculature, the latter of which reduces SVR, are well compensated by each other, in accordance with Equation 4 (81,98).

$\dot{Q}$ has a critical role in the Fick principle (Equation 2), thus being an important determinant of $\dot{VO}_2$. The following key observations support that at peak exercise, $\dot{Q}$ (particularly SV) and hence systemic $O_2$ delivery are main determinants of $\dot{VO}_{2\text{peak}}$: First, $\dot{VO}_{2\text{peak}}$ is achieved by intense activation of only ~50% of the total muscle mass, and addition of more active muscle causes no further increase in $\dot{Q}$ or $\dot{VO}_{2\text{peak}}$ (22,41). Exceptions to this include cross-country skiers and rowers, who have highly trained arms, thus achieving 5-10% higher $\dot{VO}_{2\text{peak}}$ when arm exercise is added to leg exercise (99,100). Even so, the heart cannot provide enough blood flow for all of the muscles.
In fact, with the aid of the development of the groundbreaking knee-extensor model to study an isolated exercising muscle in 1985 (101), Andersen and Saltin (102,103) and Richardson et al. (104) observed that the capacity of the skeletal muscles to receive blood flow is at least 2-3 times higher than the pumping capacity of the heart. Second, surgical opening of the pericardial sac of dogs has been shown to facilitate significant increases in their peak SV, peak Q̇, and VO2peak (105). This indicates that pericardium sets crucial mechanical constraints on ventricular filling and hence SV and the pumping capacity of the heart. Third, mitochondrial capacity to utilize O2 has been demonstrated to exceed VO2peak (106), although this may not always be the case (see Section 2.1.2.2). In summary, regarding the Fick principle (Equation 2), pronounced peak Q and particularly pronounced peak SV rather consistently contribute to higher V̇O2peak more than pronounced peak C(a-v)O2, especially in younger men and women (107). However, this sort of interpretation actually incorrectly dismisses the integrated nature of V̇O2peak (see Sections 2.1.3.2 and 2.1.4.3). Taken together, Q and systemic O2 delivery are main (or at least highly important) determinants of V̇O2peak, although peripheral determinants may be at least equally important in unfit subjects (26), as discussed later (see Section 2.1.2.2).

Methods to quantify cardiac pump function

The “gold standard” methods to determine cardiac pump function are considered to be the direct Fick method and indicator dilution methods (i.e., dye- or thermodilution methods) (51,108). The direct Fick method requires invasive measurements of CaO2 from any artery and CvO2 from mixed venous blood (a cardiac catheter with its tip in the pulmonary artery), along with a direct measure of V̇O2; hereby, Q can be calculated according to the Fick principle (Equation 3) (51,108,109). Dye-dilution method is similar to the Fick method with the exception that, instead of measuring O2, the concentration of injected dye (usually indocyanine green) in serial samples of arterial blood is measured. Eventually, Q is obtained by dividing the amount of injected dye by the average arterial dye concentration after a single circulation through the heart (51,108). Thermodilution method, for its part, is based on the same principle as dye-dilution except that, instead of dye, cold saline is injected into the right atrium through one channel of a double-lumen catheter. The temperature change in the blood is then recorded in the pulmonary artery by a thermistor in the other, longer side of the catheter. Consequently, the amount of cooling of the blood is inversely proportionate to Q (51,108).

The “gold standard” methods described above are invasive by nature and while they have been widely shown to give accurate and reliable determinations of Q at rest and during submaximal exercise their use during maximal exercise conditions is debatable due to the inherent risks and inaccuracy increasing along with exercise intensity (108). In consequence, several noninvasive methods, such as foreign gas (particularly acetylene (110,111)) rebreathing methods, Doppler echocardiography (112), and impedance cardiography, have been developed and are popular among clinicians and exercise scientists (108,113).

As listed above, impedance cardiography is one of the noninvasive techniques to quantify cardiac pump function. It is based on the measurement of transthoracic changes in electrical bioimpedance during the cardiac cycle (113,114). A small
alternating current (1.8-4 mA, 75-100 kHz) is passed through the chest using two sets of two skin electrodes (i.e., two transmitting+receiving pairs), and registered changes in electrical impedance are assumed to represent changes in SV. The principle has been applied to estimate beat-to-beat changes in SV and \( \dot{Q} \) since 1966 (115). The original Kubicek equation (115) to calculate SV was further modified by Sramek et al. (116) and Bernstein (117). However, all of these three equations rely on an evaluation of basal thoracic impedance, which depends on multiple factors such as thorax morphology, homogeneity of thorax perfusion, and fluid and gas content (115-119). The three equations could thus be confounded by perspiration, subcutaneous adiposity, and poor electrical contact (118). Consequently, Warburton et al. (113) concluded in their review in 1999 that impedance cardiography would not be able to provide accurate or reliable data during exercise.

A different approach to impedance cardiography was presented in 2000 and 2001, when a new impedance cardiograph device, PhysioFlow, provided valid and reliable estimates of SV and \( \dot{Q} \) at rest and from low to peak exercise (114,120). Charloux et al. (114) first observed in 40 normal-weight, overweight, or obese adults that mean differences and correlation coefficients between \( \dot{Q} \) estimated by PhysioFlow and \( \dot{Q} \) measured by the direct Fick method were 0.07 L/min (95% confidence interval -0.23 - +0.27 L/min) and 0.89, respectively, at rest, and 0.26 L/min (95% confidence interval -0.17 - +0.69 L/min) and 0.85, respectively, during dynamic submaximal supine leg exercise. Richard et al. (120) then reported in seven normal- or overweight adults that a mean difference and a correlation coefficient between \( \dot{Q} \) estimated by PhysioFlow and \( \dot{Q} \) measured by the direct Fick method were 0.58 L/min (95% confidence interval -4.04 - +2.89 L/min) and 0.94, respectively, during maximal incremental cycling exercise.

From a methodological point of view, the most important advancement of the PhysioFlow method is that evaluation of basal thoracic impedance is not needed for the calculation of SV (114). Using four impedance electrodes (i.e., two transmitting+receiving pairs) and two electrocardiography electrodes (Figure 4A), the PhysioFlow device first evaluates SV during a calibration procedure based on 24 consecutive heartbeats recorded at rest. This evaluation, called \( SV_{\text{cal}} \), retains the largest impedance variation during systole (\( Z_{\text{max}} - Z_{\text{min}} \)), and the largest rate of variation of the impedance signal (\( \frac{dZ}{dt_{\text{max}}} = \text{contractility index} [\text{CTI}] \)) (Figure 4B). Additionally, the SV evaluation depends on ventricular ejection time, which is reflected as thoracic flow inversion time. Thoracic flow inversion time is a time interval between the first zero value of \( \frac{dZ}{dt} \) following the beginning of systole and the first nadir after the peak ejection velocity (\( \frac{dZ}{dt_{\text{max}}} \)) (Figure 4B). As a result of the above concepts, \( SV_{\text{cal}} \) is calculated as follows (114):

\[
SV_{\text{cal}} = k \times \left[ \frac{(\frac{dZ}{dt_{\text{max}}})}{(Z_{\text{max}} - Z_{\text{min}})} \right] \times W(TFiT_{\text{cal}})
\]

where \( k \) is a constant and \( W(TFiT_{\text{cal}}) \) refers to a weighted algorithm of thoracic flow inversion time obtained during the calibration procedure. Furthermore, during the data acquisition phase (i.e., during ongoing exercise), SV values are derived by combining fluctuations of \( \frac{dZ}{dt_{\text{max}}} \) (CTI) and thoracic flow inversion time (114):
where “cal” indicates the parameters measured during the calibration phase.

Figure 4 combines the placement of PhysioFlow electrodes and waveforms obtained by PhysioFlow.

Concomitantly, $\dot{Q}$ is obtained by multiplying estimated SV by HR (114). The PhysioFlow device also provides estimates of EF and EDV: EF is calculated by an equation ($EF = 0.84 - 0.64 \times \text{pre-ejection period} / \text{ventricular ejection time}$), where pre-ejection period refers to time between ventricular depolarization and ejection (121). This equation has been shown to correlate strongly with EF obtained by gated pool scanning (121). EDV is calculated by the ratio of SV to EF. Overall, the PhysioFlow method, similarly to the acetylene rebreathing method (108,113), meets the criteria of being noninvasive, easy to use, reliable, and valid for use also at peak exercise (114,120).

In addition to all of the methods described above, SV can also be estimated by calculating O$_2$ pulse, which has its origins in the Fick principle (20,123):

\[
\dot{V}O_2 = \dot{Q} \times C(a-v)O_2 = SV \times HR \times C(a-v)O_2 \rightarrow \frac{\dot{V}O_2}{HR} = SV \times C(a-v)O_2 
\]

\[(7)\]

\[
SV = SV_{cal} \times 3\sqrt{\frac{CTI}{CTI_{cal} \times TFIT_{cal} / TFIT}}
\]
where the \( \dot{V}O_2/HR \) quotient is O\(_2\) pulse. The calculation of O\(_2\) pulse assumes that no anemia, lung disease, hypoxic conditions, or EIAH are present, and C(a-v)O\(_2\) is maximal (20,123). Thus, O\(_2\) pulse is mainly applicable to estimate SV at peak exercise.

### 2.1.1.3 Blood oxygen carrying capacity

Apart from pulmonary and cardiac pump functions, the third factor determining systemic O\(_2\) delivery is blood O\(_2\) carrying capacity, which is reflected by [Hb]. During acute dynamic exercise in thermally neutral circumstances, [Hb] may rise slightly above resting values since plasma water is lost to active myocytes and interstitial fluid as the concentration of osmotically active particles in the muscles rises (41,124). However, if no EIAH is present, CaO\(_2\) remains nearly constant (41) since SpO\(_2\) is maintained near resting levels at all exercise intensities (65-68).

[Hb] is formed as a ratio of total hemoglobin mass (tHb-mass) and blood volume (BV). Both tHb-mass and BV in endurance athletes with high \( \dot{V}O_2\)peak are up to 50% higher than in sedentary subjects (125). Accordingly, cross-sectional (126,127) as well as longitudinal (128) studies have shown that tHb-mass and BV are closely related to \( \dot{V}O_2\)peak; a change in tHb-mass by 1 g causes a change in \( \dot{V}O_2\)peak by \( \sim 4 \) mL/min (129). However, due to the “coinciding” training-induced increases of both tHb-mass and BV, no association between [Hb] and \( \dot{V}O_2\)peak is evident in very heterogeneously trained groups (127) or within groups characterized by similar endurance levels (125). Thus, rather than [Hb] alone, the balance between tHb-mass (affecting blood O\(_2\) carrying capacity and BV) and BV (affecting diastolic filling and hence SV and \( \dot{Q} \)) for its part determines \( \dot{V}O_2 \) response to acute dynamic exercise in nonanemic, normoxic conditions (129).

The methods to quantify tHb-mass and BV are all indirect and based on dilution of tracers injected into the circulation (130). A method using radioactive chromium as a tracer has previously been regarded as the criterion method on the basis of its reliability, reproducibility, and ease of routine clinical use (131). Another method uses inhaled carbon monoxide (CO) as a tracer and is thus known as a CO-rebreathing method; the subject first adopts a consistent posture for at least 20 min, after which basal capillary carboxyhemoglobin concentration ([HbCO]) is determined. Then CO is inhaled for two minutes via a spirometer. Seven minutes after inhaling the CO bolus, [HbCO] is again determined to derive tHb-mass (132,133):

\[
(8) \quad tHb\text{-mass} = \frac{\text{CO dose}}{1.34} / \Delta[HbCO]
\]

where CO dose is a volume of pure CO added to a rebreathing circuit, 1.34 (mL/g) is the physiological CO binding coefficient of Hb, and \( \Delta[HbCO] \) is a difference between [HbCO] seven minutes after inhaling the CO bolus and basal [HbCO]. BV is obtained by dividing tHb-mass by measured [Hb] (133).

The principle of this CO-rebreathing technique has originally been introduced more than 100 years ago (134), and its optimized, contemporary version has proved to be valid and reliable (132). In fact, the mean error of the method (a coefficient of variation of 2.2%) has been demonstrated to be lower than that of other methods (including the
radioactive chromium method) (133). Herewith, the CO-rebreathing method is currently recommended for determinations of THb-mass and BV (133).

2.1.2 PERIPHERAL OXYGEN DELIVERY, EXTRACTION, AND UTILIZATION DURING ACUTE DYNAMIC EXERCISE

2.1.2.1 Overview of blood flow distribution and regulation at the whole-body level

Section 2.1.1.2 describes how \( \dot{Q} \) reflects systemic blood flow and increases during incremental dynamic exercise. Here, Figure 5 illustrates how the distribution of \( \dot{Q} \) in a normal human changes from rest to higher exercise intensities. Blood flow increases with exercise intensity in skeletal, cardiac, and respiratory muscles, and to a lesser extent in the brain. In other words, “blood goes where it is needed”, as postulated by Scottish anatomist and surgeon John Hunter already in the 18th century (135). By far the largest increases in blood flow occur in the exercising skeletal musculature, the increasing energetic demand of which thus dominates that of the rest of the body (22,41,42,46). Coronary blood flow of cardiac muscle increases three- to fivefold, from \(~250\) mL/min up to \(~1.0\) L/min (41,136). As a result, skeletal muscle and cardiac muscle together receive 85-95% of \( \dot{Q} \) at maximal exercise intensities (22,41,42). Being involved in the portion of the exercising (skeletal) musculature, respiratory muscle work may require up to \(~15\)% of \( \dot{Q} \) at peak exercise (59). In fact, even evidence of competition for blood flow between the exercising limb muscles and the respiratory muscles has been presented (59,137). However, Romer et al. (138) have reported that at least in moderately fit subjects, neither inspiratory muscle work nor diaphragm fatigue limit performance during maximal incremental exercise. In addition, based on their simultaneous measurements of blood flow in both locomotor and intercostal respiratory muscles, Vogiatzis et al. (139) have suggested that at least intercostal muscles do not “steal” blood from the dynamically exercising limbs. The idea of respiratory muscles “stealing” blood from the exercising limb muscles is therefore questionable. Regarding the brain, cerebral blood flow increases with exercise intensity up to \(~60\)% of \( \dot{V}O_2^{\text{peak}} \), after which it decreases towards baseline values (140-142); numerically, cerebral blood flow is between \(~0.7\) and \(~0.9\) L/min during various exercise intensities.

Blood flow to the above-mentioned more active tissues during acute dynamic exercise is redistributed from less active tissues such as skin, kidneys, splanchnic organs (22,41,42), less active muscles (143,144), and bones (145). Blood flow to the skin is reduced with the onset of mild-to-moderate exercise intensities (146,147) and also remains minimal unless the cutaneous vascular bed is forced to dilate for the purposes of thermoregulation as a response to elevated core temperature (41,42,148). In addition, blood flow of kidneys and splanchnic organs can both fall to \(~25\)% of their resting levels during acute dynamic exercise (149-152), albeit \( O_2 \) utilization in these tissues is preserved by elevated \( O_2 \) extraction (149-151). Moreover, after having initially increased, blood flow to less active muscles (143,144) and bones (145) levels off with increasing exercise intensities.
Figure 5: Effects of increasing exercise intensity (i.e., increasing percentage of peak pulmonary O$_2$ uptake [VO$_{2peak}$]) on distribution of systemic blood flow (cardiac output [Q]) to all tissues (A) and to all tissues except skeletal muscles (B). Modified from Laughlin et al. (42).

The key point in regulating this blood flow redistribution during acute dynamic exercise lies in a competition between two fundamental physiological needs (22,41,42). First, in an integrated manner, pulmonary, cardiovascular, and muscular systems strive for meeting the increased metabolic demands of the exercising musculature. Second, appropriate maintenance of MAP is simultaneously needed to ensure there is adequate perfusion pressure to all vital organs such as the brain. From a systemic point of view, these two coinciding challenges are faced by sympathetic nerve activity, which begins to rise when cardiac vagal withdrawal is nearly complete at an HR of ~100 bpm (148,153). A dramatic illustration of the importance of sympathetic activity in the integrated hemodynamic response to acute exercise in humans is the observation that cerebral hypoperfusion may evoke loss of consciousness within one minute of exercise in patients with autonomic failure since they cannot maintain their MAP (154).

The origins of the increase in sympathetic activity are not firmly established, but likely involve the action and interaction of the following neural inputs: central command, arterial baroreflex, and reflex responses to activation of mechano- and metabosensitive afferents within the active skeletal muscle (41,153,155). Central command refers to the idea that volitional activation of motor systems causes parallel stimulation of brain stem cardiovascular regulatory centers, which causes changes in autonomic nerve activity (156). Interestingly, as early as 1893, Johansson (157) suggested that the cardiovascular response to exercise was partly caused by neurological activation of the heart in parallel with the central neural drive to the exercising muscles. Central command is a positive, feedforward reflex contrary to
negative feedback reflexes such as the arterial baroreflex (156). The arterial baroreflexes are stretch-sensitive afferent receptor systems located primarily in the aortic arch and carotid sinus, and they suppress sympathetic activity as part of the reflex MAP-lowering responses to the afferent signals (158). For a long time, the arterial baroreflex was thought to be turned off during exercise as both HR and MAP increase. However, a vast amount of evidence since the 1960s has shown that, instead of being turned off, it is reset to a higher pressure during exercise (159-161). The mechano- and metabosensitive afferents originating from skeletal muscles are group III and IV fibers sensing increases in tension as well as increases in the interstitial concentration of chemicals released during increased metabolism (162). Activation of these fibers can cause large increases in sympathetic output, MAP, HR, and $Q\ddot{}$ (41).

Taken together, the central cardiovascular control mechanisms described above tune the autonomic nervous system so that the increased demands for blood flow and particularly $O_2$ delivery to the exercising musculature can, for their part, be faced without a decrease in MAP. Importantly, they are employed simultaneously with local vascular control mechanisms, designed primarily for the maintenance of tissue homeostasis in the periphery (22,41,42). The following sections, also providing a brief insight into these local regulatory mechanisms, will cover blood flow, $O_2$ extraction, and $O_2$ utilization in skeletal muscles and cerebral tissue during acute dynamic exercise. NIRS, a method to evaluate tissue-specific (im)balance between $O_2$ delivery and utilization, will also be presented.

2.1.2.2 Skeletal muscle blood flow and oxygen extraction and utilization

Skeletal muscle blood flow

At rest, skeletal muscle $O_2$ utilization and blood flow account for ~20% of whole-body $O_2$ utilization and $Q\ddot{}$, respectively (22,42). However, as introduced in the previous section and illustrated in Figure 5, metabolic demands in the periphery and hence systemic $O_2$ delivery along with blood flow to the exercising skeletal muscles increase considerably during acute dynamic exercise. Data from one-leg knee-extensor exercise have shown that blood flow can rise from a few mL/min/100 g muscle at rest to well above 300 mL/min/100 g muscle (103,104). Consequently, as skeletal muscle typically comprises ~50% of lean and ~35-40% of whole-body mass in normal-weight humans (22), it has been concluded that if all the muscles were activated maximally, their blood flow would be at least 2-3 times higher than the capacity of the heart to deliver blood (102-104). Particularly in terms of whole-body dynamic exercise, this numerical discrepancy has given rise to the notion that skeletal muscle is a “sleeping giant” whose blood flow must be under tonic vasoconstrictor constraint if hypotension is to be averted (163). While sympathetic vasoconstriction hereby invariably affects muscle blood flow during dynamic exercise, it must be, however, overcome by local vascular control mechanisms since blood flow within exercising muscles is able to increase along with the increased $O_2$ demand (97,103,164).

The local mechanisms participating in the control of skeletal muscle blood flow include mechanical effects of contraction, shear stress-induced vasodilatation, conducted vasodilatation, myogenic control, metabolic control, and erythrocyte-
mediated vasodilatation (42,165). Particularly Laughlin et al. (42) have comprehensively reviewed the current knowledge of these mechanisms, and only the basics are presented here. First, mechanical contractions per se can lead to the local release of vasoactive compounds such as ATP (166) and nitric oxide (NO) (167). Second, blood flowing through a vessel applies a frictional force onto the endothelial cells lining the lumen of all blood vessels. This physical force, termed shear stress, leads to endothelial formation of NO and thus to vasodilatation particularly in large conduit arteries (168). Third, conducted vasodilatation refers to vasodilatation, which initiates at the microvascular level as a result of hyperpolarization of endothelial and smooth muscle cells and conducts to upstream arteries (42). Fourth, myogenic control is evidence of the autoregulatory ability of smooth muscle cells, particularly in resistance arteries, to constrict as a response to increased transmural pressure and to dilate when transmural pressure is reduced (169). Fifth, metabolic control refers to the effect of any compound (e.g., adenosine, ATP, CO2, hydrogen, NO, potassium) that mediates vasodilatation by acting on receptors of cells such as endothelial or skeletal muscle cells, leading to the formation of vasodilatators (e.g., NO, prostacyclin). This metabolic control is regarded as a major component in tight local matching of peripheral O2 delivery to O2 demand (42). Sixth, erythrocyte-mediated vasodilatation is to date the only mechanism known to couple the need for O2 with vasodilatator formation; when erythrocytes off-load O2 to the tissue, they simultaneously release ATP and NO (170).

Functional sympatholysis refers to a modulation of the effect of sympathetic activity induced by locally formed compounds (particularly ATP (171)), thereby reducing the effect of sympathetic vasoconstriction on contracting skeletal muscles (172). Joyner and Casey (22) have thoroughly presented the key findings on this over 50-year-old concept and concluded that functional sympatholysis does exist at high exercise intensities and classically demonstrates the interplay between the central and local cardiovascular control mechanisms: during near-maximal exercise conditions when 80-90% of Q̇ is directed to contracting skeletal muscles, sympatholysis in the smallest arterioles ensures adequate flow to the most metabolically stressed muscle regions, while vasoconstriction is retained in larger blood vessels. However, all this must happen under the tight control of the central mechanisms (i.e., the neural reflexes) (22).

Figure 6 summarizes the central and local cardiovascular control mechanisms that work in concert. As a consequence of the illustrated entity, blood flow to exercising skeletal muscles is closely matched to changing metabolic demands during acute dynamic exercise (97,103,164), as repeatedly mentioned here. However, at the microvascular level of exercising muscles (i.e., arterioles, capillaries, and venules) where vasodilatation and diffusional gas exchange occurs (173,174), the relationship between local blood flow and O2 utilization is not perfectly linear (175-177); muscle blood flow increases immediately after the first contraction reflected as a rapid increase in microvascular blood flow. This rapid increase results from increasing erythrocyte flux and velocity in muscle capillaries (178) and tissue pressure causing vascular smooth muscle relaxation and consequent vasodilatation after pressure release (179). After the first few contractions, metabolic vasodilatation takes over and accounts for most of the rise in muscle blood flow (179). If/when acute dynamic exercise leads to HR of near ~100 bpm, muscle sympathetic nerve activity begins to increase.
(77,180,181). From this point onwards, the interplay between the sympathetic stimulus and local vascular control mechanisms determines muscle blood flow. The sympathetic activation is likely demonstrated as a period during which the flow increases only modestly, and this period is eventually followed by one in which the increase in microvascular blood flow is again accelerated (175-177). This final acceleration of blood flow is accompanied by a plateau in local muscle O$_2$ extraction, highlighting that any observable increase in local O$_2$ utilization necessitates rising blood flow (175-177,182-186). The acceleration of active muscle blood flow may be due to increased sympathetic vasoconstriction in less active tissues (148) but also to functional sympatholysis (22).

The above-described nonlinear profile of microvascular blood flow leads us to the following section dealing with skeletal muscle O$_2$ extraction and utilization.

**Figure 6**  Simplified summary of key relationships between cardiac pump function, local factors contributing to blood flow rise in contracting skeletal musculature, and need to regulate arterial blood pressure to guarantee perfusion of central nervous system and other vital organs. Modified from Joyner and Casey (22).
**Skeletal muscle oxygen extraction and utilization**

The Fick principle shows that VO$_2$ comprises of $Q$ and C(a-v)O$_2$, the latter of which is the difference between CaO$_2$ and CvO$_2$ (Equation 3). During acute dynamic exercise, not only $Q$ but also C(a-v)O$_2$ increases. However, the increase in C(a-v)O$_2$ along with intensity is not linear, but hyperbolic; C(a-v)O$_2$ rises until reaching ~40-60% of VO$_2$peak, after which it begins to plateau (41,81,107,175). Eventually, C(a-v)O$_2$ rises from ~50 mL O$_2$/L at rest to peak values of ~140-150 mL O$_2$/L (22,41,107,175). However, far higher values, over 180 mL O$_2$/L, have been reported in cardiac patients with low $Q$ (187) and in physically trained individuals (188,189). When these are expressed as per-cent-age values of CaO$_2$, C(a-v)O$_2$ on average rises from 25% at rest to 75-85% at peak exercise (22,148), while even 90% of CaO$_2$ may be extracted by cardiac patients (187) and physically trained individuals (188,189).

As already indicated, CaO$_2$ remains nearly constant through various exercise intensities (41). Hence, the exercise-induced increase in C(a-v)O$_2$ is mainly attributable to a decrease in CvO$_2$. Two factors account for the decreasing CvO$_2$. First, sympathetically mediated vasoconstriction diminishes blood flow in less active regions such as kidneys, splanchnic regions, and less active muscles (143,144,149-152), so that their local O$_2$ extraction increases to guarantee adequate O$_2$ utilization (41). Second, local O$_2$ extraction rises within the exercising skeletal musculature.

The widening of O$_2$ extraction in the active muscle during acute dynamic exercise is likely explained by “longitudinal capillary recruitment”, presented by Poole et al. in two extensive reviews using the supporting-opposing approach (178,190). This theory lies on evidence that, in many preparations, a majority of the muscle capillaries are perfused already at rest. Thus, instead of recruitment of new flowing capillaries, capillary surface area available for O$_2$ diffusion is widened via exercise-induced increases in erythrocyte flux and velocity and decreases in intramyocyte O$_2$ pressure. Simultaneously, a rise in capillary Hb content substantially elevates O$_2$ diffusion capacity (178,190). This theory of “longitudinal capillary recruitment” challenges the older idea of exercise-induced recruitment of new flowing capillaries presented by Nobel Prize recipient August Krogh in 1919 (191,192). The issue has been a matter of some debate in the paper of Clark et al. (193) and subsequent point-counterpoint discussion.

Several studies using NIRS have shown that local microvascular O$_2$ extraction within exercising skeletal muscles follows a nonlinear, S-shaped profile from low intensities to peak exercise: muscle O$_2$ extraction remains relatively constant at low intensities, increases in a steeper manner during moderate exercise, and typically (but not always) reaches a plateau at higher intensities (175-177,182-186). This extraction profile reflects the nonlinear relationship between local microvascular blood flow and O$_2$ utilization, described in the previous section, and is thus explained by changes in a dynamic balance between local O$_2$ delivery and utilization (175-177). However, these responses are heterogeneous between exercising muscle groups: there is a rightward shift in the S-shaped O$_2$ extraction profile in deeper muscles compared to more superficial ones (185,186), which is likely due to higher vascularization, blood flow, and/or proportion of oxidative muscle fibers in deep muscles (194). Nevertheless, little heterogeneity seems to exist in the ratio of regional VO$_2$ and blood flow (164), indicating again that blood certainly goes where it is needed.
After traveling from the atmosphere to the myocytes of the exercising skeletal muscles, O₂ is utilized in a mitochondrial process of oxidative phosphorylation to produce energy (i.e., ATP) in accordance with Equation 1 (23). Mitochondrial capacity to utilize O₂ has been shown to exceed \( \dot{V}O_{2\text{peak}} \) achieved during whole-body dynamic exercise (106). This would explain why recruiting more active muscles at or near \( \dot{V}O_{2\text{peak}} \) does not typically lead to further increases in \( Q \) or \( \dot{V}O_{2} \) (22,41), as mentioned earlier, and thus supports the idea that systemic O₂ delivery governs \( \dot{V}O_{2\text{peak}} \). However, Gifford et al. (195) have recently demonstrated how mitochondrial capacity to utilize O₂ does exceed whole-body \( \dot{V}O_{2\text{peak}} \) in trained but not untrained individuals. Thus, it is possible that, in certain circumstances, the heart can supply enough O₂ to meet the maximal demands of the mitochondria for O₂. In other words, muscular capacity to utilize O₂ may determine \( \dot{V}O_{2\text{peak}} \) in such cases. This is definitely the case in patients with McArdle’s disease (196) or mitochondrial myopathies (197), in which intracellular metabolism sets detrimental limitations on \( \dot{V}O_{2\text{peak}} \). Mitochondrial oxidative metabolism may determine \( \dot{V}O_{2\text{peak}} \) also in sedentary individuals without any specific myopathy. Their \( \dot{V}O_{2} \) has been shown to increase nonlinearly along with increasing O₂ fraction of inspired air (198,199), contrary to trained individuals, in whom the corresponding increase is linear (200,201). This agrees with the above-mentioned recent findings of Gifford et al. (195). Such a peripheral limitation to \( \dot{V}O_{2\text{peak}} \) has also been suggested by studies in which reduced peak \( C(a-v)O_{2} \) has been observed in subjects with obesity (202) or type 2 diabetes (203). All of these findings emphasize the integrated nature of the Fick principle (Equation 3); each component of the principle truly affects the pathway for O₂ from the atmosphere to the mitochondria.

### 2.1.2.3 Cerebral blood flow and oxygen extraction and utilization

Total cerebral blood flow is ~0.7-0.9 L/min during various exercise intensities, as seen in Figure 5. Some older studies, such as that of Scheinberg et al. from 1954 (204), suggested that the effect of exercise on cerebral blood flow is minimal. However, that particular study (204) compared cerebral blood flow during upright exercise with that at supine rest, thus ignoring the influence of the upright posture-induced decrease in cerebral blood flow (205,206). It is now known that the response of cerebral blood flow to acute dynamic exercise follows an intensity-dependent trend: cerebral blood flow rises until ~60% of \( \dot{V}O_{2\text{peak}} \), after which it decreases towards baseline values (140-142). In detail, depending on the method used and the area examined, cerebral blood flow at its peak is ~10-50% higher during exercise than at rest (42,141). Thus, the magnitude of the increase is relatively modest in terms of the amount of systemic blood flow. It is of note that cerebral blood flow shows regional distribution, paralleling changes in the regional activity and metabolism of the brain, as initially suggested by Lassen et al. in the 1970s (207): For example, mean flow velocity rises only in the contralateral middle cerebral artery during one-side handgrip exercise (208) and only in the contralateral anterior cerebral artery during one-side foot movements (209). However, since cerebral blood flow decreases at high intensities as mentioned above, it must be regulated to a greater extent by other mechanisms than neuronal activity and metabolism (210,211).
NIRS, which will be closely examined in the following section, is a method to evaluate a regional balance between tissue-specific O₂ delivery and utilization. NIRS studies have collectively shown that regional microvascular blood volume in the prefrontal cerebral cortex increases from low to moderate exercise intensities, which is then followed by a decrease in untrained individuals, but an increase in trained ones (212). Accordingly, regional microvascular O₂ extraction within the prefrontal cortex follows different profiles in untrained and trained subjects; in untrained subjects, prefrontal O₂ extraction first increases from low to moderate intensities, then plateaus, and again rises at high intensities. Contrarily, in trained subjects, prefrontal O₂ extraction remains relatively constant at low intensities, increases in a steeper manner during moderate exercise, and then begins plateauing at higher intensities (212). Hence, fitness status seems to affect regional cerebral O₂ delivery (~blood flow) and utilization. However, from a methodological viewpoint, it must be recognized that when no light-absorbing tracers are used, only regional blood volume but not flow can be estimated by NIRS.

Different mechanisms participating in the regulation of cerebral blood flow aim at maintaining MAP so that cerebral perfusion pressure remains adequate at any exercise intensity (22,41,42). Cerebral blood flow during acute dynamic exercise is strongly regulated by PaCO₂ and cerebral autoregulation. Increasing PaCO₂ leads to cerebral vasodilatation, whereas decreasing PaCO₂ causes cerebral vasoconstriction (213). The following offers a potential explanation for the above-mentioned difference in exercise-induced blood volume response between untrained and trained individuals: lower chemosensitivity and lower submaximal $\dot{V}_E$ of trained individuals attenuate PaCO₂ reduction at high intensities thus resulting in less cerebral vasoconstriction (214). The respiratory chemoreflex interacts with cerebrovascular CO₂ reactivity so that they together strive to maintain cerebral CO₂ homeostasis during exercise (215). Cerebral autoregulation is a term describing the ability of the cerebral circulation to maintain cerebral blood flow within a narrow range over the MAP range of 60-150 mmHg (216). It has been demonstrated that cerebral autoregulation is maintained during dynamic exercise and seems to compensate for exercise-induced hemodynamic and CO₂ responses (217). However, acute exercise may also impair this autoregulatory ability to regulate cerebrovascular tone, but this area warrants further investigation (211). In addition to PaCO₂ and autoregulation, a variety of other factors, such as PaO₂, Q, the arterial baroreflex, and endothelium-mediated mechanisms, may take part in the regulation of cerebral blood flow during exercise, but integrative physiological research exploring the complex interactions of these mechanisms is lacking (42,210,211).

2.1.2.4 Near-infrared spectroscopy (NIRS) as a method to evaluate tissue-specific (im)balance between oxygen delivery and utilization

When O₂ has bound to Hb, the Hb molecule is more reddish. Instead, the color of Hb without O₂ is more bluish. This “oxygenation vs. deoxygenation” effect on the color of Hb was observed in 1875 by German physician Karl von Vierordt (218). About 50 years later Glenn Millikan began metering blood oxygenation in vitro and was the first person to coin the term “oximeter” (219). In 1977, Frans Jöbsis reported that a
relatively high degree of biological tissue transparency in the range of NIR light (700-1300 nm) enables real-time noninvasive detection of Hb oxygenation using transillumination spectroscopy (220). Jöbsis’s observations made him the founder of in vivo medical NIRS and launched the development of NIRS methodology, which enables noninvasive metering of tissue-specific oxygenation status. In other words, NIRS is a method to evaluate local tissue-specific (im)balance between O₂ delivery and utilization. In clinical research, NIRS was first used to transilluminate the newborn head in 1985 (221).

As a result of its broad applicability, in vivo NIRS has been validated by numerous studies focusing on different tissues and using varied experimental modalities as presented by the comprehensive review of Ferrari et al. (222). While there is no other method quantifying exactly the same variables as NIRS does, the validation studies regarding NIRS have largely been based on examining correlations between NIRS variables and other methods. For example, Wilson et al. (maximal incremental cycling exercise) (223) and Vogiatzis et al. (submaximal graded cycling exercise) (164) have observed very strong correlations (e.g., r = -0.97 with a range of -0.97 - -0.98 in the study of Wilson et al.) between NIRS-derived (de)oxygenation status in the active vastus lateralis muscle and directly measured venous O₂ saturation. However, NIRS truly has inherent methodological weaknesses that must always be considered when one performs NIRS experiments or interprets NIRS data. These methodological issues will be detailed at the end of this section, after first presenting the physico-mathematical principles of NIRS in the following paragraphs.

The physical principles of NIRS are based on the propagation of light through biological tissue. The propagation depends on reflection, absorption, and scattering. Reflection is determined by the angle of the light beam in relation to the tissue surface. Absorption of light by tissue causes light attenuation and depends on certain compounds known as chromophores (224). Each chromophore has its absorption spectrum in which the specific extinction coefficient is expressed as a function of wavelength (224). At least the following oxygenation-dependent chromophores are present in biological tissue: oxygenated Hb (O₂Hb), deoxygenated Hb (HHb), myoglobin (in muscle only), and cytochrome oxidase (222). Light attenuation is measured in optical density and can be quantified using the Beer-Lambert law (225):

\[
OD = \log \left( \frac{I_0}{I} \right) = \alpha \times c \times d
\]

where OD is dimensionless optical density of the medium (e.g., biological tissue), \(I_0\) is incident light intensity, \(I\) is transmitted light intensity, \(\alpha\) is the specific extinction coefficient of the chromophore, \(c\) is the concentration of the chromophore, and \(d\) is the distance between light entry and exit points.

Scattering, which is the third factor affecting light propagation through biological tissue, is the deviation of the light beam straight trajectory due to particulate matter in the sample. Scattering results in an increased optical pathlength traveled by the light beam in tissue, thus causing light attenuation via increased light absorption; ~80% of the total attenuation of NIR light is due to scattering (224). Hence, scattering is the biggest problem when quantifying optical density, and since the Beer-Lambert law was
originally intended for use in a nonscattering medium, the law has been modified to take this issue into account (226):

\[
OD = \log \left( \frac{I_0}{I} \right) = \alpha \times c \times d \times DPF + G
\]

where DPF refers to the differential pathlength factor and \( G \) to the \( \text{O}_2 \) independent light losses due to scattering in the medium. DPF depends on age and the tissue of interest (227-229) and accounts for the scattering-induced increase in the optical pathlength, whereas \( G \) is assumed to have the same value for any chromophore in the medium and thus cleared. Furthermore, as DPF and the geometrical distance \( d \) are known and remain constant during any measurement period, quantitative data on changes in the concentration of any chromophore can be derived as follows (224):

\[
\Delta c = \frac{\Delta OD}{\alpha \times d \times \text{DPF}}
\]

where \( \Delta \) refers to a difference between two time points during any measurement period. The basic idea of Equations 9-11 is illustrated in Figure 7.
Several different techniques to physically carry out NIRS measurements have been developed, applied, and extensively described in numerous reviews (e.g., 222,224,230). The main focus here is on continuous wave instruments and the parameters based on the measurement of O$_2$Hb and HHb.

Continuous wave NIRS instruments have been perhaps the most widely used NIR spectrophotometers to date. Continuous wave means that only changes in light intensity are measured, and the concentration changes of the chromophores of interest (e.g., O$_2$Hb and HHb) are to be resolved based on Equations 9-11. The number of the chromophores of interest determines the number of required light wavelengths. This continuous wave method usually relies on the DPF method described above and only allows the continuous quantification of relative values (222,224,230). A source-detector distance of $>2.5$ cm is recommended to achieve a nearly constant DPF in all tissues (228). The continuous wave method is inexpensive and relatively robust, although it assumes that coupling and light scattering over time are constant, and that changes in absorption are only due to blood (222,224,230).

Other NIRS methods include spatially resolved spectroscopy and time resolved spectroscopy, among others (222,224,230). Spatially resolved spectroscopy, also called multidistance spectroscopy, is based on light intensity being measured at several different source-detector distances (231,232). This method assumes that the light coupling between the optodes and the tissue is the same for different source-detector distances, and, by measuring the light attenuation as a function of the distance, determines a parameter that is independent of the coupling (231). This allows the determination of an O$_2$Hb/(O$_2$Hb+HHb) ratio, which is known as the tissue saturation index (TSI). Time resolved spectroscopy, also known as time domain spectroscopy, is a technique measuring not only the light intensity but also the time the light takes to pass through the tissue. This eventually allows the measurement of absolute concentrations of the chromophores of interest (224,230,233). Time resolved spectroscopy usually requires large and expensive instrument, which limits its usability (224,230).

NIRS evaluates tissue (de)oxygenation, reflecting the regional (im)balance between O$_2$ delivery and utilization, as repeatedly noted here. It does so by monitoring either relative concentration changes or absolute concentrations of oxygenation-dependent chromophores within the microvasculature (i.e., arterioles, capillaries, and venules) of the examined tissue (222,234). Regional blood flow can also be quantified or assessed by NIRS: by using NIRS and a light-absorbing tracer, such as indocyanine green, tissue blood flow can be quantified by regionally applying the Fick principle (139,164,235). Furthermore, blood flow in the vastus lateralis muscle can be mathematically assessed without tracers by a method also relying on the regional application of the Fick principle (see Section 5.1.5.3 for details) (175-177). In addition, regional blood volume but not flow can be evaluated by total Hb (tHb = O$_2$Hb + HHb) (222,234). Altogether, NIRS can provide important information regarding the profiles of regional vascular responses and fractional O$_2$ extraction within the skeletal muscle (175-177,182-186,233,236-238) and/or cerebral (236,239-242) tissue, for example, during acute dynamic exercise.

The following methodological issues have to be considered with NIRS: First, Hb and myoglobin have similar absorption spectra within the range of NIR wavelengths,
thus being indistinguishable with NIRS. In fact, muscle myoglobin may occupy from ~10% (243,244) to ≥50% (245) of the NIRS light absorption signal. However, Davis and Barstow (245) have summarized existing data on similar kinetics of muscle deoxygenation (Hb and myoglobin) and muscle arterial-venous O₂ difference ((a-v)O₂) and have suggested that, despite its potentially great contribution to the NIRS signal, myoglobin does not invalidate the interpretation that particularly HHb data reflect regional O₂ (im)balance also in muscle tissue. Second, muscle measurements may be confounded by subcutaneous adipose tissue; the maximum measurement depth of NIRS is approximately half the source-detector distance in muscle tissue (246) as well as in the cerebrum (247), meaning that an exceptionally thick layer of fat (and/or skin) prevents a substantial amount of light from passing through the muscle tissue. This further leads to the underestimation of muscle O₂ metabolism as adipose tissue is metabolically more passive (248). Third, NIRS experiments may be affected by local changes in skin blood flow when performed under conditions inducing cutaneous vasodilatation (e.g., local warming, high-intensity or prolonged exercise). According to the most recent literature, this is likely the case when monitoring O₂Hb or tHb in muscle (249-251) and cerebral (252,253) tissue. On the contrary, the same studies have consistently shown that HHb and TSI responses are highly insensitive to changes in skin blood flow (249-253). Fourth, the spatial heterogeneity of local blood flow and O₂ extraction profiles between deep and superficial muscles (185,186,194), described earlier, must be considered when interpreting NIRS data in muscle experiments.

2.1.3 PEAK OXYGEN UPTAKE (\(\dot{V}O_{2\text{peak}}\))

2.1.3.1 \(\dot{V}O_{2\text{peak}}\) as a concept

\(\dot{V}O_{2\text{peak}}\) represents the highest achievable level of whole-body oxidative metabolism (Equation 1) and is formed according to the Fick principle (Equation 2). The highest pulmonary O₂ uptake, typically measured during a maximal incremental CPET involving large muscle groups (20,21), is traditionally expressed as \(\dot{V}O_{2\text{max}}\) referring to maximal \(\dot{V}O_{2}\). This “max” term refers to almost 100-year-old observations of an English Nobel Prize physiologist Archibald V. Hill and his colleagues, who made careful experiments of \(\dot{V}O_{2}\) on one subject running around a grass track (254,255). Hill et al. (255) found that the highest \(\dot{V}O_{2}\) attained was similar at the speed of 243 m/min and at higher speeds of up to 282 m/min. Based on these findings of plateauing \(\dot{V}O_{2}\) (254,255), Hill et al. stated that, “...however much the speed (or work rate) be increased beyond this limit, no further increase in oxygen intake occur” (254). Later, these observations about body’s maximal ability (or rate) to utilize O₂ were confirmed, for example, in the classic studies by Taylor et al. (256) and Åstrand and Saltin (257). However, only up to ~50% of individuals demonstrate a \(\dot{V}O_{2}\) plateau before symptom limitation when stressed to maximal effort (56-58). Consequently, the term \(\dot{V}O_{2\text{peak}}\) is often used as an appropriate estimate of \(\dot{V}O_{2\text{max}}\) to describe the absence of the plateau phenomenon (21,258).

Since plateauing of \(\dot{V}O_{2}\) may or may not be demonstrable in any given individual, different end criteria for verifying maximal effort have been presented (56,259) and
used widely for several decades (258). These alternative criteria particularly include a respiratory exchange ratio (RER = \( \dot{V}CO_2 / \dot{V}O_2 \)) and blood lactate concentration (56,259). The rises of RER and lactate concentration at high exercise intensities are due to an imbalance between the formation and elimination of acids, particularly lactic acid, induced by increases in anaerobic energy metabolism (260). Recently, a study on a large random Norwegian population has presented recommended age- and sex-related reference values of RER and lactate concentration to be used when defining maximal physical effort (56).

Before presenting \( \dot{V}O_2 \text{peak} \) as an integrated physiological model, three important aspects, providing a framework for the interpretation of any \( \dot{V}O_2 \text{peak} \) observations, are noteworthy. First, the heritability of \( \dot{V}O_2 \text{peak} \), even when adjusted for age, sex, and anthropometrics, is at least 40% (261). Second, \( \dot{V}O_2 \text{peak} \) has a relatively low day-to-day variability of 2-4% (41). Third, \( \dot{V}O_2 \text{peak} \) and its components are important, but by no means the only factors determining endurance exercise performance; for example, motivation, tolerance for pain and dyspnea, development of neuromuscular fatigue, and the intensity of exercise that can be attained before a significant rise in lactate concentration, all fundamentally affect endurance performance (25,28).

### 2.1.3.2 \( \dot{V}O_2 \text{peak} \) as an integrated model

The integrated nature of the pathway for \( O_2 \) from the atmosphere to mitochondria has been repeatedly emphasized here. Furthermore, the pathway, containing serial steps of \( \dot{V}A \), alveolar-to-capillary diffusion, circulation, and muscle diffusion, has been detailed step by step together with references to the Fick principle (Equation 2). Eventually, \( \dot{V}O_2 \text{peak} \) truly forms as an integrated model and depends on all of the serial steps presented (23-30). In addition to its serial nature, this model, which has mainly been theorized by Peter D. Wagner over the last three decades (23-27), relies on a principle that \( O_2 \) throughput at each step must conserve \( O_2 \) mass. The model ends up replacing the standard question of “what limits \( \dot{V}O_2 \text{peak}? \)” with the more precise question of “how important are various independent variables to \( \dot{V}O_2 \text{peak}? \)”

The conceptual basis for the integrated pathway for \( O_2 \) from the atmosphere to the mitochondria is illustrated by Figure 8. Figure 8 describes the coexistent processes of circulatory \( O_2 \) convection (~blood flow) to and diffusion within muscle; these can be explained by two equations. The first equation is the previously mentioned Fick principle (Equation 3), and the second equation underlies the Fick law of diffusion (23-25,27):

\[
\dot{V}O_2 = D \times (P_{capO2} – P_{mitO2})
\]

where \( D \) is muscle \( O_2 \) diffusional capacity (or conductance), \( P_{capO2} \) reflects mean capillary \( O_2 \) pressure between the artery and vein and in effect serves to integrate \( O_2 \) diffusion along the capillary length, and \( P_{mitO2} \) refers to mitochondrial \( O_2 \) pressure.
Conceptual basis for the coexistent processes of circulatory O₂ convection (i.e., blood flow provided by cardiac output [Q̇]) and O₂ diffusion from erythrocytes to muscle mitochondria. O₂ mass must be conserved through these processes; therefore, the convection must obey the Fick principle \( \dot{V}_O₂ = Q \times (C_{aO₂} - C_{vO₂}) \), and the diffusion must obey the Fick law of diffusion \( \dot{V}_O₂ = D \times (P_{capO₂} - P_{mitO₂}) \). In the latter equation, D is muscle O₂ diffusional capacity, while \( P_{mitO₂} \) refers to mitochondrial O₂ pressure. See text for details. Modified from Wagner (27).

Mean capillary O₂ pressure is ~40-50 mmHg (262), whereas mitochondrial O₂ pressure must be less than 3-4 mmHg, which is the measured value of the O₂ pressure associated with myoglobin in the cytoplasm during strenuous exercise (263). In consequence, as mitochondrial O₂ pressure is very low relative to mean capillary O₂ pressure, it is taken to be zero and hence neglected. With these approximations, Equation 12 may be rewritten as follows (23-25,27):

\[
\dot{V}_O₂ = D \times P_{capO₂} = D \times k \times P_{vO₂}
\]

where \( k \) is a constant and \( P_{vO₂} \) is partial pressure of muscle venous O₂.

The Fick principle (Equation 3) and Equation 13 embody the same undisputed law: O₂ mass is conserved during the pathway for O₂ from the atmosphere to the mitochondria. Herewith, both equations must yield the same \( \dot{V}_O₂ \) and \( P_{vO₂} \). Since both Equations 3 and 13 relate \( \dot{V}_O₂ \) to venous O₂ levels, they can be plotted on one diagram with \( \dot{V}_O₂ \) on the y-axis and \( P_{vO₂} \) on the x-axis. In this diagram, which is shown in Figure 9A, the intersection point of two plots is the only point where conservation of O₂ mass exists, therefore indicating the value of \( \dot{V}_{O₂peak} \) for the given values of \( Q \), \( C_{aO₂} \), and muscle O₂ diffusional capacity. This is where the integrated, in-series nature of \( \dot{V}_{O₂(peak)} \) is etched; if any of \( Q \), \( C_{aO₂} \), or diffusional capacity is changed, the plots in Figure 9A will shift, yielding a different intersection point (i.e., different \( \dot{V}_{O₂peak} \)) (23-25,27). Figures 9B-F detail how such changes in independent key variables would affect \( \dot{V}_{O₂peak} \) and \( P_{vO₂} \); changing inspired O₂ pressure and therefore \( P_{aO₂} \), \( S_pO₂ \), and \( C_{aO₂} \) will produce a linear and proportional relationship between \( \dot{V}_{O₂peak} \) and \( P_{vO₂} \) (Figure 9B) (201,262). Figures 9C and 9D contrast the effects of reduced \( Q \) (and/or muscle
Figure 9  Plot of pulmonary O\(_2\) uptake (\(\dot{V}O_2\)) against partial pressure of muscle venous O\(_2\) (PvO\(_2\)) showing the two mass conservation equations describing circulatory O\(_2\) convection into the muscle microcirculation (Fick principle), and subsequent O\(_2\) diffusion from the microcirculation to the mitochondria (Fick law of diffusion) (A). Effects of progressive reduction of partial pressure of arterial O\(_2\) (B), reduced cardiac output (\(\dot{Q}\)) (and/or muscle blood flow) (C), reduced muscle O\(_2\) diffusional capacity (D), high (E) and low (F) mitochondrial metabolic capacities to utilize O\(_2\) on \(\dot{V}O_{2\text{peak}}\) are described. See text for further details. Modified from Wagner (27).
blood flow) versus impaired muscle O$_2$ diffusional capacity, respectively; the former reduces PvO$_2$ as VO$_{2\text{peak}}$ is lowered, whereas the latter reduces VO$_{2\text{peak}}$ but increases PvO$_2$ (23,25,27). Furthermore, Figures 9E and 9F illustrate the effects of mitochondrial metabolic capacity to utilize O$_2$ on the model; metabolic capacity to utilize O$_2$ has been demonstrated to exceed whole-body VO$_{2\text{peak}}$ (Figure 9E) (106), but as already mentioned earlier, this is not necessarily the case (Figure 9F) in sedentary/untrained individuals (195,198,199), let alone patients with McArdle’s disease (196) or mitochondrial myopathies (197).

2.1.3.3 VO$_{2\text{peak}}$ as a predictor of cardiovascular disease and mortality

In 1859, Charles Darwin published his theory of evolution as a constant struggle among individuals with different degrees of fitness within a species (264). In this regard, studies on a wide range of biological organisms have to date shown that oxidative metabolism has strong associations with overall physical capacity and health, which presumably is a product of the pivotal role of O$_2$ in our evolutionary history (265). VO$_{2\text{peak}}$ represents the highest achievable level of whole-body oxidative metabolism, thereby reflecting so-called cardiorespiratory fitness. Today, over 150 years after Darwin’s writings, in the era of evidence-based medicine and rigorous scientific methods, when VO$_{2\text{peak}}$ is measured and study subjects are followed for years, the data supporting Darwin’s concept of “survival of the fittest” are strong and compelling.

Some of the most essential findings on VO$_{2\text{peak}}$ and its associations with cardiovascular disease and mortality, mainly published over the last three decades, are briefly presented here. Blair et al. (266) published a seminal study in the field in 1989: After measuring VO$_{2\text{peak}}$ in 13 344 individuals (77% men) and then following them for over eight years, Blair et al. concluded that the higher the initial level of VO$_{2\text{peak}}$, the lower the subsequent death rate from cardiovascular disease and cancer. These findings closely paralleled an earlier follow-up study among 4276 asymptomatic and clinically healthy men, in which a lower physical fitness, estimated by exercise time and submaximal HR responses, was reported to be associated with a higher risk of death from cardiovascular causes (267). More recent studies have substantiated these findings in asymptomatic women (268,269). Furthermore, these observations have been confirmed in numerous clinical populations such as in patients with clinically proven cardiovascular disease (270-272), overweight or obesity (273), diabetes (270), or impaired fasting glucose (274). A carefully adjusted meta-analysis, involving 33 studies and nearly 103 000 individuals, has numerically demonstrated that each 1-MET (i.e., a metabolic equivalent corresponding the resting VO$_2$ of 3.5 mL/min/kg) increment in VO$_{2\text{peak}}$ is independently associated with 13% lower risk of cardiovascular event or mortality and 15% lower risk of all-cause mortality (19). In addition, individuals with low VO$_{2\text{peak}}$ of <7.9 METs have 56% and 70% higher risks of cardiovascular event/mortality and all-cause mortality, respectively, than those with higher VO$_{2\text{peak}}$ of ≥10.9 METs (19). Herewith, VO$_{2\text{peak}}$ is certainly an independent predictor of cardiovascular health and mortality (19).

The above-mentioned findings are accompanied and/or characterized by a few important details. First, the greatest health benefits, such as a reduction in risk of
cardiovascular disease, have consistently been observed between the least fit and the next least fit groups (Figure 10B) (266,270,272,274,275). Second, the degree of health risk associated with low $\dot{V}O_2$peak is similar to or in some reports even stronger than (270,272) that associated with more traditional cardiovascular risk factors such as smoking, hypertension, lipid profile, or blood glucose. Additionally, some studies have shown low $\dot{V}O_2$peak to be a more powerful predictor of cardiovascular or all-cause mortality than exercise-induced ST-segment depression (268,270) or arrhythmias (270). In consequence, Myers et al. (276) have recently stated that “... no matter what an individual's health status (i.e., the traditional cardiovascular risk factors), higher levels of physical activity and cardiorespiratory fitness improve the overall cardiovascular risk profile”. Figure 10A illustrates this statement. Third, compared with physical activity, cardiorespiratory fitness is likely a more powerful, and thus, more clinically meaningful prognostic measure of cardiovascular risk (Figure 10B) (275). In summary, these observations support the previous conclusion; in terms of overall risk stratification, $\dot{V}O_2$peak is a strong and independent predictor of cardiovascular health and mortality.

Figure 10  Illustration of essential roles of physical activity and peak pulmonary O₂ uptake ($\dot{V}O_2$peak ~ cardiorespiratory fitness) in relation to traditional risk factors in determining cardiovascular disease risk (A). Decrease in relative cardiovascular risk along with percentiles of increasing physical activity or $\dot{V}O_2$peak (B). See text for more detailed interpretation. Modified from Myers et al. (276) (A), and Franklin and McCullough (277) and Williams (275) (B).
2.1.4 EFFECTS OF ENDURANCE TRAINING ON OXYGEN DELIVERY AND UTILIZATION DURING ACUTE DYNAMIC EXERCISE

More than 100 years ago, the effect of exercise training on peak $\dot{Q}$ was perhaps first described by Henschen (278), who used only a basic physical examination with careful percussion to identify enlargement of the heart due to athletic activity in cross-country skiers. Current knowledge of the effects of regular exercise training on the various organ systems participating in $O_2$ delivery and utilization is mainly based on scientific observations made since the 1960s (40,41). The focus of the following Sections 2.1.4.1-2.1.4.2 is on endurance-type exercise training and adaptations in both the different components of systemic $O_2$ delivery and skeletal muscles. Section 2.1.4.3 will then cover these adaptations in light of the Fick principle and the Fick law of diffusion (Equations 2, 3, and 13), describing the integrated pathway for $O_2$ from the atmosphere to the mitochondria.

2.1.4.1 Effects of endurance training on systemic oxygen delivery

Endurance training reduces $\dot{V}E$ at a given submaximal $\dot{V}O_2$ level (214,279). This lowers the percentage of the total exercise $O_2$ cost attributable to pulmonary function and may thus be beneficial for two reasons. First, fatiguing exercise effects on ventilatory musculature (280) are reduced, which attenuates the vasoconstrictive influence of reflexly increased sympathetic activation on skeletal muscle vasculature (281). Second, any $O_2$ freed from use by the ventilatory muscles becomes available to the exercising skeletal muscles (137). In terms of integrated exercise responses at peak exercise, problems may be caused by the fact that training has little effect on lung structure (282): positive training adaptations outside the lungs (i.e., particularly in the heart and the skeletal musculature) enable higher endurance performance, and thus, lead to increasing ventilatory requirements at peak exercise, concomitantly leading to increased peak pulmonary blood flow facilitated by a training-elicited rise in peak $\dot{Q}$ (41). This can result in $V_A/Q_m$ mismatch, which may at least partly cause EIAH, hence setting limitations on increases in peak systemic $O_2$ delivery and $\dot{V}O_2peak$ (41,69). Consequently, trained athletes in particular may undergo EIAH during exercise (69-71), as mentioned earlier.

Dynamic endurance training elicits both structural and functional cardiac adaptations. The findings presented here are mainly based on observations of the left ventricle, acknowledging that at least structural remodeling of the right ventricle resembles that of the left one (283). Both cross-sectional and longitudinal studies have shown training-induced structural adaptations to include increases in left ventricular diameter (90,91,93), wall thickness (89-91), and mass (89,90,92,93). Importantly, training also leads to enhanced ventricular compliance, resulting in larger EDV via a greater use of the Frank-Starling mechanism (89,94). Functionally, endurance training leads to more rapid diastolic filling (85-87), whereas data on changes in early diastolic relaxation rate are controversial (92,284). Collectively, the diastolic (and structural) adaptations lead to increased EDV and hence larger SV during acute exercise. Contrarily, systolic function, typically evaluated by EF, has a minor role in training-
induced functional cardiac remodeling (283), although occasional cross-sectional studies have reported both greater contractility (95,96) and longer ventricular ejection time (86,87) in individuals with pronounced SV. Likely secondary to the training-induced increase in SV, resting and submaximal HR lower after endurance training (40), whereas training has little effect on peak HR (22,48). Thus, the training-elicited rise in peak $Q$, which further leads to increases in peak systemic $O_2$ delivery and $V\dot{O}_{2peak}$, is due to the adaptations enabling increased peak SV (40,41).

Both BV and tHb-mass may be up to 50% higher in endurance athletes with high $V\dot{O}_{2peak}$ than in untrained individuals (125). By contrast, $[\text{Hb}]$, formed as a ratio of “coincidingly” increased BV and tHb-mass, has no associations with $V\dot{O}_{2peak}$ (125,127).

Endurance training leads to the hypervolemic expansion of BV mainly via three mechanisms: first and second, an aldosterone-sodium retention mechanism together with increasing plasma albumin content lead to expanded plasma volume (285). This expansion of body water begins immediately after a single exercise session mainly due to the activation of a renin-angiotensin-aldosterone cascade as a response to acute exercise-induced reduction of plasma volume (41,124,285). Third, erythrocyte volume and therefore tHb-mass begin increasing BV after ~10-14 days of training, or in other words, after the plasma volume expansion has first peaked and then plateaued above the initial level (286). The exact mechanisms of such training-induced erythropoiesis under normoxic conditions are not fully understood (287). Importantly, the training-induced expansion of BV does not stimulate a feedback diuresis, as CVP is reset to a higher operational range allowing greater intravascular volume (288). Overall, both studies employing acute BV expansion by intravenous colloid infusions (86) and studies re-establishing BV to a pretraining level by phlebotomy after a training intervention (289) have demonstrated the importance of expanded BV; increased BV increases cardiac preload, ventricular filling, and SV, thereby increasing peak systemic $O_2$ delivery and $V\dot{O}_{2peak}$.

2.1.4.2 Effects of endurance training on skeletal muscle blood flow and oxygen extraction and utilization

In individuals with no vascular dysfunction at the onset of any training period or intervention, endurance training seems to lead to lower blood flow within the exercising skeletal musculature during submaximal dynamic exercise (40). This is mainly allowed by a training-induced increase in absolute local $O_2$ extraction (290). At peak exercise, skeletal muscle blood flow rises as a consequence of endurance training (22,40). The rise in peak muscle blood flow is partly due to increased peak $Q$ and an increased proportion of that peak $Q$ to be distributed to active muscles (41). In addition, endurance training results in several adaptations within both the skeletal muscle vasculature and the mitochondria, which underlies the above-mentioned local changes in submaximal and peak responses.

Vascular adaptations to endurance training are both structural and functional. Training constitutes a powerful stimulus for capillary proliferation, and hence, the elevation of muscle capillary density (291). This importantly leads to increased mean blood transit time (102), during which more $O_2$ can be extracted (290) and diffused
from the microvasculature to the myocytes, allowing the above-mentioned reduction of muscle blood flow during submaximal exercise. Local $O_2$ extraction may further be enhanced by training-induced decreases in blood flow heterogeneity (290), possibly demonstrated as improved matching of local $O_2$ delivery and utilization during acute dynamic exercise (292). Endurance training also enlarges the luminal diameters of arterioles as well as their respective conduit arteries, which is favorable in terms of increasing blood flow and $O_2$ delivery capacity throughout the arterial vasculature (293). This enlargement particularly occurs in response to elevated blood flow, which first increases flow-mediated shear stress on the endothelium, thereby stimulating both NO production and bioavailability in the endothelial wall. Further, the increase in functional NO bioactivity leads to the vessel enlargement if exercise training continues. Arterial vessels in other words grow in size to normalize shear stress (293,294). Accordingly, NO has a substantial role in structural and functional vascular adaptations, although it is not the sole local factor mediating beneficial health effects on the vasculature (295); Hellsten and Nyberg (40) have recently presented other training-induced functional adaptations of the vasculature in their extensive review.

Endurance training also improves the oxidative capacity of the skeletal musculature. This occurs via increases in mitochondrial content (296,297), mitochondrial inner membrane surface (297), and activities of mitochondrial oxidative enzymes (296,298). In addition, the mitochondrial adaptations are accompanied by selective muscle fiber hypertrophy and fiber type transformation to a more oxidative direction (299). If an individual’s metabolic capacity to utilize $O_2$ exceeds $O_2$ delivery capacity and thus whole-body $\dot{V}O_2$peak as earlier illustrated in Figure 9E, its further training-induced increase does not further improve the individual’s $\dot{V}O_2$peak but does have beneficial influences on overall endurance performance: for example, improved oxidative metabolism delays the prominence of anaerobic metabolism during submaximal exercise (300), in addition to which muscle glycogen is spared longer as energy production can rely more on $\beta$-oxidation of free fatty acids (301). This demonstrates that while $\dot{V}O_2$peak and its components are important, they are by no means the only factors determining endurance exercise performance, as pointed out earlier.

The vascular and mitochondrial adaptations mentioned in this section are revealed in the NIRS profiles of local muscle microvascular $O_2$ extraction during maximal incremental dynamic exercise. A cross-sectional study has suggested that training leads to a rightward shift of the profile, when microvascular $O_2$ extraction during maximal incremental dynamic exercise is examined in relation to maximal extraction capacity (177); in other words, relative muscle $O_2$ extraction remains constant longer at low intensities, begins increasing later during moderate exercise, and also reaches a plateau later at higher intensities. Another cross-sectional study described how NIRS-derived microvascular $O_2$ extraction is higher from submaximal to peak exercise (302).
2.1.4.3 Endurance training effects in the context of Fick’s theories

In 1968, Saltin et al. (188) published their landmark “Dallas Bedrest” study describing the alterations in \( \dot{V}O_2 \text{peak} \) resulting from two sequential interventions, which were 20 days of bed rest and almost eight weeks of training. In terms of training adaptations and the Fick principle (Equation 2), Saltin et al. (188) concluded that their observation of training-induced increase in \( \dot{V}O_2 \text{peak} \) (+18.5%) was explained by increases in both peak \( \dot{Q} \) (+14%) (or actually peak SV) and peak C(a-v)O2 (+4.5%). This kind of interpretation fails, however, to acknowledge the integrated relationship between training-induced elevations of systemic blood flow (i.e., peak \( \dot{Q} \)) and increased muscle \( O_2 \) diffusional capacity. Such a failure was recently highlighted by Wagner (303), who reanalyzed the data of the “Dallas Bedrest” study in light of the Fick law of diffusion (Equation 13). Wagner (303) observed that the great majority of the improvement of \( \dot{V}O_2 \text{peak} \) was actually attributed to the increase in muscle \( O_2 \) diffusional capacity (+23.4%), and only a small amount due to elevated peak \( \dot{Q} \). This reanalysis of demonstrates that the only way to observe training-induced increases simultaneously in both peak blood flow (\(~\text{peak} \dot{Q}\)) and peak C(a-v)O2 is when muscle \( O_2 \) diffusional capacity increases relatively more than peak \( \dot{Q} \). In other words, if peak \( \dot{Q} \) was increased without any change in diffusional capacity, peak C(a-v)O2 would be compromised by the reduction in mean blood transit time through the microcirculation. Unfortunately, Wagner had no data to identify whether the elevation of muscle diffusional capacity stems from increased muscle capillarity, greater subsarcolemmal mitochondrial content, or some other factor (303). It is noteworthy that this above-mentioned importance of the balance between increases in muscle \( O_2 \) delivery and \( O_2 \) diffusional capacity was interestingly expressed by Saltin himself (102) ~20 years after the publication of the “Dallas Bedrest” study.

When interpreting endurance training effects and improved \( \dot{V}O_2 \text{peak} \) in general but especially in light of Fick’s theories (Equations 2, 3, and 13), one must recognize three important aspects. First, as the described reanalysis of Wagner (303) points out, when physically trained individuals are reported to reach either significant increases in \( \dot{V}O_2 \text{peak} \) (188) or high peak C(a-v)O2 values (189), it is most likely due to the relative dominance of peripheral muscle adaptations, although endurance training obviously also elicits significant cardiac adaptations. Second, while the rise in muscle \( O_2 \) diffusional capacity (if greater than a simultaneous rise in peak systemic blood flow) leads to a decrease in peak CvO2 (188), the possible training-induced development of EIAH (69-71) is demonstrated as a decrease in CaO2 and thus affects the interpretation of C(a-v)O2 and the Fick principle. Third, time courses of different endurance training-induced adaptations vary considerably; while cardiac and systemic circulatory changes (particularly structural adaptations in cardiac morphology) progress rather slowly, within months or years, peripheral muscle adaptations (particularly increased capillary density and functional flow-mediated vasodilatation) take place more rapidly, within the first three months of any training period/intervention (40,41). Eventually, regarding training-induced increase in \( \dot{V}O_2 \text{peak} \), cumulative evidence shows that the most prominent increase in \( \dot{V}O_2 \text{peak} \) is seen within the first two or three months of training, after which it continues to increase but in a more moderate manner (40), given that training is executed appropriately.
2.2 POLYCYSTIC OVARY SYNDROME (PCOS)

2.2.1 DEFINITION AND EPIDEMIOLOGY

PCOS is a syndrome and as such no single diagnostic criterion is sufficient for clinical diagnosis. Ever since Stein and Leventhal first described a series of women with oligo- or anovulation and polycystic ovaries in 1935 (304), different definitions of PCOS have been widely used: In 1990, the National Institute of Health Conference recommended that the major criteria for PCOS should include clinical and/or biochemical hyperandrogenism and chronic oligo- or anovulation (305). In 2003, the Rotterdam consensus expanded the diagnosis of PCOS to be based on the presence of at least two of the following three features: clinical and/or biochemical hyperandrogenism, chronic oligo- or anovulation, and polycystic ovaries (306). In 2006, the Androgen Excess Society recommended that PCOS be defined by clinical and/or biochemical hyperandrogenism, with either chronic oligo- or anovulation or/and polycystic ovaries (307). All three diagnostic definitions also included the exclusion of other confounding endocrine disorders such as hypothyroidism, hyperprolactinemia, and hyperandrogenism of some other etiology (305-307). Today, several recent international clinical guidelines and reviews (e.g., 308-310) recommend using the Rotterdam 2003 criteria (306), which are also applied in Finland (311).

Worldwide, depending on the population studied, PCOS approximately affects from 6% to 15% of women according to the 1990 NIH criteria (4-6) and even twice as much according to the broader Rotterdam criteria (6,7). The prevalence of PCOS is in the middle of these two extremities, when the 2006 Androgen Excess Society criteria are applied (6). PCOS is consequently one of the most common human disorders and the most common single endocrinopathy in reproductive-aged women (1). Up to 70% of women with PCOS are either overweight (25 kg/m^2 ≤ body mass index [BMI] <30 kg/m^2) or obese (BMI ≥30 kg/m^2) (312), although the number varies along with the population studied and averages ~50% (313).

2.2.2 PATHOGENESIS

PCOS is a complex endocrinopathy, and both genetic and environmental factors and possibly also epigenetic changes in fetal life contribute to its pathogenesis. However, the pathophysiology of the syndrome is not fully understood. Hence, while several scholarly papers have reviewed the accumulated evidence in the field (e.g., 1-3), only the most important and well-defined links between pathogenesis and clinical manifestations are presented here.

IR drives different phenotypic features of women with PCOS, and thus, has an essential role in the syndrome (1-3). Recently, the first study to use the “gold standard” method of the hyperinsulinemic-euglycemic clamp to define the prevalence of IR among PCOS women indicated that 75% of lean and 95% of overweight PCOS women have pronounced IR (314), whereas earlier studies using non-clamp techniques had suggested somewhat lower prevalences of ~50-70% (315). A recent meta-analysis of clamp studies further verified that PCOS does involve intrinsic syndrome-specific IR.
in addition to well-identified extrinsic BMI-related IR of overweight and obese women (316).

IR interacts with hyperandrogenism, and this interplay largely forms the basis of the PCOS pathophysiology. The molecular mechanisms underlying IR in women with PCOS remain elusive (1). However, primary defects in insulin signaling pathways particularly in skeletal muscle (317-319) but also in adipose tissue (320) have been reported. In addition, pronounced hepatic glucose production has been observed in obese PCOS women (320). Subsequently impaired glucose metabolism leads to compensatory hyperinsulinemia, which strives to maintain normal glucose levels. Hyperinsulinemia contributes to hyperandrogenism particularly via direct stimulation of ovarian androgen secretion and inhibition of hepatic sex hormone-binding globulin production (SHBG); the inhibition of SHBG production worsens hyperandrogenism by increasing the proportion of unbound, and thus, biologically active testosterone in the circulation. Androgen excess is also exacerbated by itself as it increases hypothalamic release of gonadotropin-releasing hormone, which leads to a rise in pituitary release of luteinizing hormone, and hence, to a further elevation of ovarian androgen secretion, but also to menstrual dysfunction. It is of note, however, that despite the major pathogenetic role of hyperandrogenism, “only” up to 80% of women with PCOS have elevated circulating androgen levels (1-3). The clinical manifestations of these mechanistic derangements are discussed in the following section.

2.2.3 CLINICAL MANIFESTATIONS

PCOS is a chronic condition manifesting across the lifespan (14). Women with PCOS exhibit clinical manifestations related to reproductive and hormonal, psychosocial, and metabolic and cardiovascular disturbances. Excess weight is an important environmental factor contributing to all of these derangements (1-3,12).

Reproductive and hormonal abnormalities form the basis of the diagnostic criteria (305-307) and are primarily caused by IR-driven hyperandrogenism. First, hyperandrogenism particularly causes hirsutism but also acne and alopecia. Second, hyperandrogenism interacts with hyperinsulinemia to disrupt follicle growth, which is then demonstrated as menstrual irregularity, anovulatory subfertility, accumulation of small antral follicles within the periphery of the ovary (i.e., “polycystic ovaries” on ultrasound), and an increased risk of endometrial cancer (1-3).

Psychosocial manifestations in PCOS are various: Women with PCOS face challenges in feminine identity and body image due to obesity, hirsutism, acne, and infertility (12), in addition to which their long-term health-related concerns compromise quality of life and adversely affect mood and psychological well-being (12,321).

Metabolic and cardiovascular disturbances in women with PCOS involve increased risks of several health complications. With IR being a prominent feature of the syndrome, PCOS is associated with ~two- to fourfold increases in risks of impaired glucose tolerance, type 2 diabetes, and metabolic syndrome (13), as well as gestational diabetes (322). The risks of impaired glucose tolerance or diabetes may be evident already during adolescence (323). Vast evidence suggests that lifelong metabolic
dysfunction in women with PCOS results in exaggerated risk of cardiovascular disease with aging. Impaired glucose metabolism is accompanied by an adverse lipid profile, pronounced centralized adiposity, carotid intima-media thickening, coronary artery calcification, and an increased prevalence of obstructive sleep apnea, which rather consistently but not explicitly are dependent on the severity of hyperandrogenism and obesity. However, evidence of increased risk of cardiovascular morbidity and mortality in PCOS is surprisingly limited and remains to be established (1,2,14). A few other subclinical cardiac and vascular defects observed in women with PCOS are integrated in the following section covering acute exercise responses and PCOS.

2.2.4 PCOS AND EXERCISE

2.2.4.1 Oxygen delivery and utilization during acute dynamic exercise in PCOS

Since the above-mentioned clinical manifestations elevate cardiovascular and health risks in PCOS, and $\dot{V}O_2\text{peak}$ is known to be an independent predictor of cardiovascular and all-cause mortality in women (268,269), it would be tempting to hypothesize that women with PCOS demonstrate reductions in $\dot{V}O_2\text{peak}$ and its subcomponents. However, responses to acute dynamic exercise have been sparsely studied in women with PCOS. Data on measured $\dot{V}O_2\text{peak}$ in PCOS are slightly contradictory. The largest cross-sectional studies (i.e., 45-90 PCOS women vs. 45-90 non-PCOS women) in the field have been conducted in Italy and observed even 48-63% lower $\dot{V}O_2\text{peak}$ in overweight and obese women with PCOS than in age- and BMI-matched healthy women (31-33). By contrast, smaller studies (i.e., 8-11 PCOS women vs. 6-14 non-PCOS women) from the Commonwealth countries have reported similar $\dot{V}O_2\text{peak}$ between the groups with similar IR profiles (34,35). As the magnitude of IR has also been shown to have a strong inverse association with $\dot{V}O_2\text{peak}$ in PCOS women (31), IR has been suggested to be the leading pathophysiological feature affecting $\dot{V}O_2\text{peak}$ in overweight and obese women with PCOS.

Little is known about the mechanisms affecting exercise responses of O\textsubscript{2} delivery and utilization, and therefore, $\dot{V}O_2\text{peak}$ in women with PCOS. At rest, both diastolic (324-326) and systolic (326,327) dysfunction, associated with IR (324-327), as well as normal cardiac pump function (328-330) have been reported in women with PCOS in case-control echocardiographic studies. Theoretically, greater exercise sweating observed in overweight and obese women with PCOS (331) may also have a negative impact on plasma volume, and thus, cardiac filling at least during prolonged exercise performed in a warm environment. Taken together, cardiac dysfunction and concomitantly impaired $Q$ and systemic O\textsubscript{2} delivery are potential factors affecting $\dot{V}O_2$ response to acute dynamic exercise in PCOS.

Peripheral O\textsubscript{2} delivery and utilization during exercise may also be influenced by PCOS. Women with PCOS exhibit endothelial dysfunction manifested as impaired NO-mediated vasodilatation (35,332), which has the potential to impair muscle blood flow, and hence, exercise tolerance in disease (e.g., chronic heart failure) (333). While it remains to be elucidated whether endothelial dysfunction is associated with IR status in PCOS (332), it is well acknowledged that endothelial dysfunction and IR have a
reciprocal relationship in various diseases (334). An exaggerated systolic blood pressure response to maximal exercise has also been reported in PCOS women (335). This probably reflects exaggerated SVR and, on a larger scale, increased sympathetic nerve activity characteristic of PCOS (336). Defects in skeletal muscle insulin signaling pathways (317,318) and expression of genes involved in mitochondrial oxidative metabolism (319) have also been observed in PCOS women with pronounced IR, possibly reducing responsiveness to glucose and O₂ utilization within the skeletal musculature. These findings provide some PCOS-specific evidence of mitochondrial dysfunction, which is on large scale linked with insulin-resistant metabolic derangements (337). However, any potential role of mitochondrial dysfunction behind reduced VO₂peak of PCOS women is controversial since intact gene expression (338) and function (339,340) of skeletal muscle mitochondria have also been found in PCOS.

In conclusion, slightly contradictory evidence suggests that VO₂peak may be lower in women with PCOS than in age- and anthropometry-matched women without the syndrome. At least, this is likely the case in women with PCOS and pronounced IR. In more detail, appropriate VO₂ response to exercise may potentially be affected by both systemic and peripheral limitations set by PCOS. However, responses of different components of systemic O₂ delivery or peripheral O₂ delivery and utilization to acute dynamic exercise have not yet been studied in this patient group.

2.2.4.2 Effects of exercise training in PCOS

Based on the limited data available, regular exercise has beneficial effects on overall health risks of PCOS women. Exercise-induced improvements in IR, menstrual cyclicity, ovulation, self-esteem, quality of life, depression, body composition, and VO₂peak have been reported (341,342). Hence, regular exercise is recommended to be an essential part of healthy lifestyle of PCOS women, particularly in those with excess weight (308,309).

2.3 TYPE 1 DIABETES

2.3.1 DEFINITION AND EPIDEMIOLOGY

Diabetes is a group of systemic conditions characterized by a chronic state of hyperglycemia. In ancient times, Greek and Roman physicians used the term “diabetes” to describe a finding of a large urine volume, whereas the words “diabetes mellitus” referred to the sweet taste of the urine (51); the word diabetes comes from the Greek word “diabainein” meaning “to pass through”, whereas the subsequent word mellitus originates from the Latin word “mel” referring to honey. The criteria for diabetes diagnosis are an increased fasting plasma glucose of ≥7.0 mmol/L or a 2-h plasma glucose of ≥11.1 mmol/L during an oral glucose tolerance test or glycosylated Hb A₁c (HbA₁c) of ≥6.5% (≥48 mmol/mol), or a random plasma glucose of ≥11.1 mmol/L in conjunction with classic hyperglycemic symptoms of polyuria, polydipsia,
and weight loss, accompanied by life-threatening ketoacidosis in some cases (9,343,344).

Diabetes is etiologically classified into four categories: type 1 diabetes, type 2 diabetes, other specific types (e.g., types induced by genetic defects, pancreatic diseases, drugs, etc.), and gestational diabetes. The etiology of hyperglycemia in diabetes lies in either decreased production of insulin from the pancreatic β-cells or diminished effect of insulin on target tissues (i.e., IR), or in variable combinations of these two. Type 2 diabetes, which is the most common diabetes type accounting for ~90-95% of those with diabetes, is caused by such a combination of relative insulin deficiency and IR, and has strong associations with genetic predisposition and sedentary lifestyle. By contrast, type 1 diabetes, previously encompassed by the terms juvenile-onset or insulin-dependent diabetes, is a type caused by a more total cellular-mediated autoimmune destruction of the insulin-secreting pancreatic β-cells (9). Notably, significant overlap exists between these two types, which thus have been suggested to merely be different ends of the spectrum of one and the same disease (345,346). However, the lack of evidence of genetic overlap between type 1 and type 2 diabetes (346) justifies the concept of two separate diseases.

Worldwide, 382 million people were estimated to have diabetes in 2013, and the prevalence is expected to reach 592 million people by 2035 (8). Type 1 diabetes accounts for 5-10% of all diabetes patients (9), and is typically diagnosed before the age of 15 years (10), albeit immune-mediated diabetes can occur at any age, even in 70–80-year-olds (9). Type 1 diabetes is one of the most common chronic diseases of childhood, and its incidence varies markedly depending on the population examined; the highest incidence is observed in Finland (~64/100 000/year below the age of 15) (11), while the incidence is at its lowest in Venezuela and China (0.1/100 000/year below the age of 15) (347). The incidence of type 1 diabetes has been increasing rapidly over the past decades; in Finland, the incidence rate doubled nonlinearly from the early 1980s until 2005, after which it has shown signs of plateauing (11).

2.3.2 PATHOGENESIS

Type 1 diabetes is caused by an autoimmune attack directed against β-cells of the islets of Langerhans in the pancreas. The attack, mediated by T-cells, results in selective destruction of the insulin-secreting β-cells, eventually leading to a complete dependency on exogenous insulin (348). The rate of the β-cell destruction is variable, typically being rapid in childhood and slower in adults (9). Eventually, at least 80% of the β-cells have been estimated to be destroyed at presentation of clinical disease (348). As humoral markers of the autoimmune activity, diabetes-associated autoantibodies (i.e., antibodies to the 65 kD isoform of glutamic acid decarboxylase, insulin autoantibodies, and classical islet cell, protein tyrosine phosphatase-related IA-2A, and zinc transporter 8 antibodies (349)) can be detected from the circulation in ~90% patients with newly diagnosed type 1 diabetes (350). Persistent positivity for two or more of these autoantibodies is highly predictive of progression to clinical diabetes, whereas positivity for a single autoantibody may reflect harmless β-cell autoimmunity (348).
The cascade leading to the autoimmune activity and subsequent β-cell destruction in type 1 diabetes involves both genetic and environmental factors. The most essential genes contributing to disease susceptibility are located in the human leukocyte antigen region of chromosome 6. These genes explain ~50% of the susceptibility for the disease, whereas the other ~50% is caused by over 50 other polymorphisms (346,349). Nevertheless, only a relatively small portion (i.e., ~10%) of individuals with human leukocyte antigen-conferred diabetes susceptibility progress to clinical disease (348). Furthermore, it has been shown that only 2-17% of children with newly diagnosed type 1 diabetes have family history of the disease (351). In addition, abundant scientific evidence from twin, incidence rate, and migrant studies support the view that additional environment-related factors are needed to both trigger and contribute to the β-cell destruction in genetically predisposed individuals (349). In fact, since the proportion of high-risk human leukocyte antigen genotypes has decreased among newly diagnosed type 1 diabetes patients, environmental factors have perhaps even increased their role in the disease development (352). Over time, environmental factors implicated in the etiology of type 1 diabetes have particularly included different viruses (e.g., Coxsackie B1 and other enteroviruses) and dietary factors (e.g., vitamin D deficiency, short duration of breastfeeding) (349), in addition to which the role of the intestinal microbiota in mediating the progression from autoantibody positivity to clinical disease has recently been under investigation (353). However, identifying the precise roles of known and unknown environmental factors in the pathogenesis of type 1 diabetes is in its early beginnings and thereafter requires further research (349).

It further warrants mention that in addition to the above-described factors contributing to the autoimmune β-cell destruction, the β-cells are also stressed by IR of variable magnitude, which may act as an environmental trigger or contributor, thus speeding up the pathogenesis of type 1 diabetes. This “accelerator hypothesis” has particularly been linked to excess weight, which especially nowadays may be a problem in type 1 diabetes as it is in type 2 diabetes (345). IR also has an influence on cardiovascular risk in type 1 diabetes (see Section 2.3.3.2).

2.3.3 CLINICAL MANIFESTATIONS

Clinical manifestations of diabetes can be categorized into micro- and macrovascular complications. The microvascular complications comprise diabetic retinopathy, nephropathy, and neuropathy. The macrovascular complications consist of coronary artery, cerebrovascular, and peripheral artery diseases. Simply put, while microvascular disease can lead to premature mortality but particularly to substantial morbidity diminishing the quality of life, by far the greatest cause of death in patients with diabetes is macrovascular disease. Chronic hyperglycemia can also lead to diabetes-specific myocardial dysfunction known as diabetic cardiomyopathy.
2.3.3.1 Microvascular complications

Results from randomized controlled trials, such as the seminal Diabetes Control and Complications Trial (354), have demonstrated that the risk of all microvascular complications can be reduced by intensive glycemic control. In addition, other modifiable (e.g., blood pressure, lipid profile, smoking status) and nonmodifiable (e.g., male predominance, diabetes duration) risk factors also affect the development and progression of microvasculature-related manifestations (355-357), naturally adjusted by genetic traits.

Diabetic retinopathy is characterized by gradually progressing alterations in the retinal microvasculature and can lead to severe and permanent visual loss. In type 1 diabetes, retinopathy is present in some of its forms in at least 80% of patients with diabetes of 15 years’ duration (355).

Diabetic nephropathy is clinically characterized by renal protein leakage to the urine, decreased glomerular filtration rate, and hypertension (358). Diabetic nephropathy has also been suggested to be a marker of overall (micro)vascular dysfunction (359), and accordingly, type 1 diabetes patients with nephropathy have a 10-fold risk of cardiovascular disease relative to patients without nephropathy (360). Up to ~30% of type 1 diabetes patients develop diabetic nephropathy after 20 years of diabetes, albeit the incidence seems to be decreasing (356).

Diabetic neuropathy can be divided into sensory polyneuropathies, focal/multifocal mononeuropathies, and autonomic neuropathy. Poly- and mononeuropathies manifest themselves as various sensorimotor symptoms. Manifestations of diabetic autonomic neuropathy particularly include cardiovascular autonomic neuropathy (e.g., exercise intolerance, orthostatic hypotension) but also several other disorders, the examples of which are gastroparesis and hypoglycemic autonomic failure. Prevalence of diabetic autonomic neuropathy depends on the tests used and the population studied, thus ranging widely from 1.6% to 90% within diabetes patients (357).

2.3.3.2 Macrovascular complications

Atherosclerosis is a common feature in individuals with diabetes and leads to severe macrovascular complications such as coronary artery, cerebrovascular, and peripheral artery diseases. These macrovascular complications are hence the predominant cause of death also in patients with type 1 diabetes (361). A study of Livingstone et al. (362) was one of the recent extensive population studies in the field and concluded that while the relative risks of macrovascular disease and total mortality seem to have declined in type 1 diabetes patients over time, the risks are still at least twofold compared to individuals without diabetes; findings in type 2 diabetes studies have recently been relatively similar (363).

Risk factors for macrovascular disease include dyslipidemia, hypertension, nephropathy, obesity, and smoking, are largely modifiable, and overlap between diabetes types. In addition, female sex, IR, and inflammation increase the cardiovascular risk in both types (361). The potential of intensive glycemic control to
reduce macrovascular complications has been less clearly defined, suggesting that glycemic control may be more important for micro- than macrovascular disease (364). However, the 17-year follow-up results of the Diabetes Control and Complications Trial have demonstrated that, compared with conventional therapy, intensive 6.5-year glycemic treatment was able to reduce the incidence of macrovascular complications over 40% and it did so independently of nephropathy (365).

2.3.3.3 Diabetic cardiomyopathy

A concept of diabetic cardiomyopathy was first postulated in 1972 by Rubler et al. (17), who reported post-mortem data on four diabetes patients with glomerulosclerosis and dilated left ventricles, but without other common causes. Accordingly, diabetic cardiomyopathy is today defined as myocardial dysfunction in the absence of coronary artery disease, hypertension, and significant valvular disease (16).

Prominent functional manifestations of diabetic cardiomyopathy progress gradually from diastolic to systolic dysfunction. Diastolic dysfunction is hence observed first and characterized by impairment of relaxation and passive filling of the left ventricle, whereas systolic dysfunction emerges at a later stage to accompany the diastolic derangements and is expressed as reduced EF. Hyperglycemia is regarded as a central driver in the pathophysiology of diabetic cardiomyopathy since it has the potential to trigger several adaptations characteristic of the phenomenon (15,16). In detail, Tillquist and Maddox (15) have summarized that the effects of chronic hyperglycemia on the myocardium can be mediated by micro- and macrovascular disease, lipotoxicity, inflammation, autonomic neuropathy, mitochondrial dysfunction, epigenetic changes, and advanced glycation end products causing myocardial stiffness. However, the pathogenesis is overall poorly understood (15).

Data on the prevalence of diabetic cardiomyopathy are limited. Two small echocardiography studies involving 100 and 157 patients have shown that diastolic dysfunction was evident in 27% and 36% of type 1 diabetes adults, respectively, with no signs of other contributors such as coronary artery disease or hypertension (366,367). Importantly, a recent 8-year follow-up study of Rosengren et al. (18) showed that a cohort of 33,402 type 1 diabetes patients with a mean baseline age of 35 years had a fourfold increase in the risk of being admitted to hospital with heart failure when compared with 166,228 well-matched controls without diabetes. The risk was evident despite a near absence of other risk factors for the development of heart failure, and also with glycemic control within target or with no signs of nephropathy (18). Furthermore, this study (18) together with an earlier observational study (368) reported a steep increase in the risk of heart failure with worsening glycemic control. Taken together, these findings suggest that diabetic cardiomyopathy seems to have clinical consequences and may likely be modified by appropriate glycemic treatment.

Related to diabetic cardiomyopathy, diabetes-specific defects in cardiac function along with diabetes-specific vascular dysfunction are discussed in an integrated manner in the following section (Section 2.3.4) covering acute exercise responses and adaptations to exercise training in type 1 diabetes.
2.3.4 TYPE 1 DIABETES AND EXERCISE

2.3.4.1 Oxygen delivery and utilization during acute dynamic exercise in type 1 diabetes

As in the case of women with PCOS, it would be tempting to hypothesize that also type 1 diabetes patients demonstrate reductions in \( \dot{V}O_2^{\text{peak}} \) and its components due to both the disease-related cardiovascular manifestations and the ability of \( \dot{V}O_2^{\text{peak}} \) to independently predict cardiovascular disease and mortality. Available cross-sectional data on \( \dot{V}O_2^{\text{peak}} \) in type 1 diabetes are presented in Table 1. Table 1 shows that \( \dot{V}O_2^{\text{peak}} \) is rather consistently 8-21% lower in children and adolescents with type 1 diabetes than in their matched counterparts without diabetes (369-374). By contrast, the data on the relation between type 1 diabetes and \( \dot{V}O_2^{\text{peak}} \) in adults at first glance appear contradictory, but are consistent after strict interpretation; both reduced (236,375-378) and similar (379-386) \( \dot{V}O_2^{\text{peak}} \) have been observed in type 1 diabetes adults in comparison with healthy subjects. However, HbA1c of diabetes patients has been 6.4-7.7% \( (\text{i.e.}, \text{glycemic control within or at least close to target HbA1c of 7.0%) in all studies reporting intact \( \dot{V}O_2^{\text{peak}} \) (379,381-386) except for that of Benbassat et al. (380). Additionally, studies that have examined \( \dot{V}O_2^{\text{peak}} \) separately in diabetes patients with good or poor glycemic control have repeatedly found \( \dot{V}O_2^{\text{peak}} \) reduction only in the latter group (387-390). Moreover, several studies (369-371,385,391) have observed an inverse correlation between HbA1c and \( \dot{V}O_2^{\text{peak}} \); although the absence of such an association has also been reported in smaller studies (236,372,374,376,380,382). Stubbe et al. (378) recently published the largest study in the field, and, while they did report reduced \( \dot{V}O_2^{\text{peak}} \) in type 1 diabetes adults, they did not detect a significant association between HbA1c and \( \dot{V}O_2^{\text{peak}} \). The latter may be explained by relatively good level and relatively narrow range of glycemic control in the population with diabetes \( (\text{i.e., HbA1c of 7.4\% with 25th and 75th percentiles of 6.7\% and 8.1\%, respectively}) \) (378) and indicates that \( \dot{V}O_2^{\text{peak}} \) is not severely affected by glycemic control until HbA1c attains levels (far) above 8.1%. Taken together, the studies referred to in Table 1 reflect two main points: First, children and adolescents with type 1 diabetes seem to have reduced \( \dot{V}O_2^{\text{peak}} \) relative to individuals without diabetes, suggesting that the onset of the disease affects the integrated whole-body pathway for O\(_2\) rather rapidly. Second, adults with type 1 diabetes are capable of attaining \( \dot{V}O_2^{\text{peak}} \) similar to that of healthy adults, but this most likely depends on them maintaining good glycemic control, as previously concluded by Baldi and Hofman (36).

Defects in each component of systemic O\(_2\) delivery have been observed in type 1 diabetes patients. Regarding pulmonary function, reduced peak \( V_E \), associated with \( \dot{V}O_2^{\text{peak}} \) (370), as well as impaired lung diffusion capacity (378,382,389) have been reported in individuals with type 1 diabetes. In addition, reductions of tHb-mass and BV in type 1 diabetes (376) as well as lower BV also in type 2 diabetes (392) have been observed, reflecting impaired blood O\(_2\) carrying capacity. These derangements have the potential to set limitations on CaO\(_2\) and \( \dot{Q} \), and hence, on \( \dot{V}O_2^{\text{peak}} \).

Cardiac response to acute exercise may be deteriorated via mechanisms associated with diabetic cardiomyopathy, thus limiting systemic O\(_2\) delivery. Reduced EDV during submaximal exercise (371) as well as limitations of exercise SV and \( \dot{Q} \).
Table 1  
Cross-sectional studies on VO_{2peak} in type 1 diabetes. See text for interpretation.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Mean age (years)</th>
<th>Mean diabetes duration (years)</th>
<th>Sex</th>
<th>Matched for physical activity</th>
<th>VO_{2peak} (or VO_{2max})</th>
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<tr>
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<tr>
<td>Austin et al. 1993</td>
<td>D: 59, C: 18</td>
<td>D: 16, C: 14</td>
<td>8</td>
<td>F and M</td>
<td>No</td>
<td>F: ↔, M: -21%</td>
</tr>
<tr>
<td>Komatsu et al. 2005</td>
<td>D: 72, C: 46</td>
<td>D: 16, C: 16</td>
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<td>F and M</td>
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</tr>
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<td>D: 15, C: 15</td>
<td>6</td>
<td>F</td>
<td>No</td>
<td>-17%</td>
</tr>
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<td>D: 15, C: 16</td>
<td>8</td>
<td>F and M</td>
<td>Yes</td>
<td>-19%</td>
</tr>
<tr>
<td>Gusso et al. 2012</td>
<td>D: 53, C: 22</td>
<td>D: 16, C: 17</td>
<td>6</td>
<td>F and M</td>
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<td>-8%</td>
</tr>
<tr>
<td>Bjornstad et al. 2015</td>
<td>D: 69, C: 13</td>
<td>D: 16, C: 15</td>
<td>6</td>
<td>F and M</td>
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<td>D: 14, C: 14</td>
<td>5</td>
<td>F and M</td>
<td>Yes</td>
<td>↔ a, -21% b</td>
</tr>
<tr>
<td><strong>Mean age &gt;18 years:</strong></td>
<td></td>
<td></td>
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<td>D: 42, C: 36</td>
<td>18</td>
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<td>-26% c, -33% d</td>
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<td>D: 39, C: 31</td>
<td>21</td>
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<td>D: 34, C: 34</td>
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<td>M</td>
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<td>↔</td>
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<tr>
<td>Item et al. 2011</td>
<td>D: 9, C: 29</td>
<td>D: 27, C: 24</td>
<td>13</td>
<td>M</td>
<td>Yes</td>
<td>↔</td>
</tr>
<tr>
<td>Wheatley et al. 2011</td>
<td>D: 12, C: 18</td>
<td>D: 40, C: 34</td>
<td>?</td>
<td>F and M</td>
<td>No</td>
<td>↔</td>
</tr>
<tr>
<td>Hägglund et al. 2012</td>
<td>D: 10, C: 11</td>
<td>D: 34, C: 31</td>
<td>11</td>
<td>M</td>
<td>Yes</td>
<td>-20%</td>
</tr>
<tr>
<td>Peltonen et al. 2012</td>
<td>D: 10, C: 10</td>
<td>D: 33, C: 32</td>
<td>11</td>
<td>M</td>
<td>Yes</td>
<td>-19%</td>
</tr>
<tr>
<td>Koponen et al. 2013</td>
<td>D: 12, C: 23</td>
<td>D: 33, C: 33</td>
<td>4 – 27 a</td>
<td>M</td>
<td>Yes</td>
<td>-20%</td>
</tr>
<tr>
<td>Tagougui et al. 2015a</td>
<td>D: 18, C: 18</td>
<td>D: 28, C: 27</td>
<td>8</td>
<td>F and M</td>
<td>Yes</td>
<td>↔ a, -14% b</td>
</tr>
<tr>
<td>Tagougui et al. 2015b</td>
<td>D: 23, C: 23</td>
<td>D: 26, C: 26</td>
<td>8</td>
<td>F and M</td>
<td>Yes</td>
<td>↔ a, -16% b</td>
</tr>
<tr>
<td>Stubbe et al. 2016</td>
<td>D: 73, C: 292</td>
<td>D: 50; C: 51</td>
<td>?</td>
<td>F and M</td>
<td>No</td>
<td>-13%</td>
</tr>
<tr>
<td>Waclawowsky et al. 2016</td>
<td>D: 14, C: 5</td>
<td>D: 30, C: 27</td>
<td>14</td>
<td>M</td>
<td>Yes</td>
<td>↔</td>
</tr>
</tbody>
</table>

D, type 1 diabetes patients; C, healthy controls; F, female; M, male.

* a good glycemic control; b poor glycemic control; c no autonomic neuropathy; d autonomic neuropathy; a range.

(371,372,385,389) have been observed in both adolescents and adults with type 1 diabetes, while findings on faster active muscle deoxygenation in type 1 diabetes patients may also reflect limited ability to increase O_2 delivery during increasing O_2 demand (236). Reduced BV is likely one component of diabetes-related diastolic dysfunction as discussed earlier (see Sections 2.1.1.3 and 2.1.4.1). Further, diastolic dysfunction may be partly due to prolonged isovolumic relaxation, decreased ventricular compliance, or their combination, since Regensteiner et al. (393) have observed pronounced ventricular filling pressure during incremental exercise in adults
with uncomplicated type 2 diabetes. To highlight the significance of glycemic control, HbA1c has been shown to have inverse relationships with these exercise SV and/or Q̇ responses in type 1 diabetes (371,384,385), indirectly being in line with the earlier referenced registry studies (18,368) reporting pronounced heart failure risk in patients with poorer glycemic control.

Diabetes-specific deterioration of cardiac pump function both at rest and during exercise is associated with cardiovascular autonomic neuropathy and nephropathy. Reduced peak HR has been reported in type 1 diabetes (370,372,391), pronounced in individuals with autonomic neuropathy (391). In detail, the attenuated peak HR response may be explained by a reduction of β-adrenoceptor sensitivity (394). Furthermore, Pop-Busui et al. (395) have shown an association between autonomic neuropathy and diastolic dysfunction and also observed compensatorily elevated resting HR and Q̇ in the type 1 diabetes patients of their study. To summarize this, Baldi (396) presented a “vicious cycle hypothesis”, according to which diminished cardiac volumes would lead to exaggerated resting/exercise sympathetic activity, which in the long run would particularly desensitize cardiac β-adrenoceptors, diminishing cardiac reserve and attenuating cardiac response to exertion, thereby predisposing diabetes patients to heart failure. Baldi et al. (397) have very recently reviewed this cascade in more detail by focusing mainly on existing evidence on type 2 diabetes. Nephropathy may also have a role in these complex interactions since significant associations between renal function and VO2peak have been reported in type 1 diabetes patients (373,398), and renal function has been shown to be diminished in patients with autonomic neuropathy (395). However, while the role of renal function in determining cardiovascular risk is highly pronounced in type 1 diabetes (361), underlying pathophysiological mechanisms shared by nephropathy and overall cardiovascular dysfunction remain largely unclear.

Defects in not only systemic but also peripheral O2 delivery are evident in type 1 diabetes. Type 1 diabetes is characterized by vascular dysfunction manifested as endothelial dysfunction (399), reduced arterial compliance (374,400,401), decreased capillary-to-muscle fiber ratio (402,403), and impairments in microvascular muscle blood flow and O2 diffusional capacity (403-405). All of this evidence emerging from both animal and human studies shows potential limitations on peripheral O2 delivery also during acute dynamic exercise. During exercise, defects in both systemic and peripheral O2 delivery are tried to be compensated by pronounced local active muscle O2 extraction and utilization if type 1 diabetes is in good glycemic control (236). However, patients with poor glycemic control exhibit blunted exercise-induced active muscle O2 extraction (387), possibly owing to exaggerated O2 affinity of highly glycosylated Hb (406). In terms of O2 extraction at the whole-body level, no reductions of exercise C(a-v)O2 have been observed in patients with type 1 diabetes (372,384).

In summary, type 1 diabetes may affect several steps of the integrated pathway for O2 from the atmosphere to the muscle mitochondria, and overall the diabetes-related derangements are dependent on glycemic control. However, the contribution of the different integrated steps to VO2 response to acute dynamic exercise has not simultaneously been studied in this patient group.
2.3.4.2 Effects of exercise training in type 1 diabetes

After the discovery of insulin in the early 1920s, fundamental exogenous insulin treatment has transformed type 1 diabetes from a fatal illness into a chronic systemic disease. While optimal insulin treatment in combination with smoking cessation, and dietary and medical treatment of other modifiable risk factors (i.e., blood pressure, lipid profile) strive for better glycemic control and reduction of diabetes-related cardiovascular manifestations, it is generally recommended that patients with type 1 diabetes also engage in regular physical activity (39). Physical activity importantly improves insulin sensitivity (407-410), lipid profile (407,408,411-414), and also well-being (415) in those with the disease. However, the main focus is here on current evidence of effects of physical activity and exercise training on glycemic control, micro- and macrovascular complications, and acute exercise responses in type 1 diabetes, which is briefly covered below.

Existing literature on effects of physical activity and exercise training on glycemic control is inconclusive. Epidemiological evidence has shown mainly favorable effects of physical activity. A large cross-sectional study reported a beneficial association between physical activity frequency and HbA1c in 23,251 ≤18-year-old type 1 diabetes patients (416). In addition, physical activity was shown to be beneficial with respect to HbA1c in a recent cross-sectional study involving 18,028 adults with type 1 diabetes (417). Wadén et al. (418) furthermore showed with their cross-sectional analyses of the Finnish Diabetic Nephropathy (FinnDiane) Study that low levels of leisure-time physical activity (LTPA) were associated with poor glycemic control in women with type 1 diabetes but not in men. Meta-analyses, in turn, have provided more conflicting results. A recent meta-analysis, including only randomized controlled trials, indicated an absolute -0.85% reduction in HbA1c in ≤18-year-old type 1 diabetes patients after physical activity interventions (419). In adult patients with type 1 diabetes, an absolute -0.78% decrease in HbA1c was observed after exercise interventions by another meta-analysis, involving only randomized controlled trials but being substantially affected and possibly biased by a single study with a relatively large population (420). By contrast, a meta-analysis that included both randomized and nonrandomized controlled trials was unable to reveal any significant glycemic benefits of exercise training in children, adolescents, or adults with type 1 diabetes (421). In addition, Table 2, which presents effects of exercise training interventions on \( \dot{V}O_{2\text{peak}} \) but also on HbA1c, shows that evidence for glycemic benefit of exercise in type 1 diabetes is not consistent at all. This uncertainty in the existing evidence has been speculated to be due to excessive energy consumption around the time of physical activity (to meet energetic requirements but particularly to avoid hypoglycemia) and/or training-induced reduction of insulin requirements (415). In conclusion, while physical activity conclusively has a beneficial influence on glycemic control in type 2 diabetes (422), it remains unresolved whether this is the case in type 1 diabetes.

Since effects of physical activity on glycemic control remain inconclusive in type 1 diabetes as described above, but the magnitude of hyperglycemia is a major risk factor for cardiovascular disease (18,354,365,368), it is not surprising that current literature only barely supports physical activity-related benefits for micro- and macrovascular complications. The Pittsburgh IDDM Morbidity and Mortality Study suggested an
inverse association between physical activity and the occurrence of microvascular complications (423), whereas results of the Diabetes Control and Complications Trial indicated no such benefit (424). Regarding macrovascular complications and mortality, prospective (425,426), retrospective (427), and cross-sectional (425) settings have all suggested (but not fully proved) a favorable influence of physical activity. The results of the FinnDiane Study have further specified that health benefits of physical activity in type 1 diabetes likely depend on its intensity level and frequency of performance: low intensity has been shown to be associated with both micro- and macrovascular disease (cross-sectional analysis) (428), whereas high intensity and high frequency have been suggested to prevent development and progression of diabetic nephropathy (429) and to reduce the risk of adverse cardiovascular events (430) (longitudinal analyses). However, prospective randomized controlled trials examining effects of exercise training intervention on micro- and macrovascular complications in type 1 diabetes patients are lacking. The Look AHEAD Trial was such a randomized controlled trial observing the issue in type 2 diabetes, but concluded that one year of intensive lifestyle (diet+exercise) intervention had no effect on cardiovascular or all-cause mortality during a ~10-year follow-up of 2570 type 2 diabetes patients despite several other health-promoting effects (431). It may thus be that, at least in terms of “hard” end points, cardiovascular adaptations to recreational-like, low-to-moderate intensity exercise training are somewhat deficient in individuals with diabetes, as suggested by the review of Baldi et al. (397) as well as the most recent and above-mentioned findings of the FinnDiane Study (430).

Table 2 details effects of exercise training interventions on $\dot{V}O_2$peak. Aerobic training programs of six weeks to five months have consistently resulted in 5-27% increases in $\dot{V}O_2$peak in both youth (410,411,432-434) and adults (407-409,412-414,435-439) with type 1 diabetes. Furthermore, Table 2 shows that a reduction of HbA1c is not directly a prerequisite for or a consequence of an improvement of $\dot{V}O_2$peak, although vast evidence from cross-sectional studies suggests an obvious association between $\dot{V}O_2$peak and glycemic control, as discussed earlier (see Section 2.3.4.1). Three studies that have compared training-induced increases in $\dot{V}O_2$peak between individuals with and without type 1 diabetes have observed no differences in $\dot{V}O_2$peak improvements between the groups (411,435,436). With respect to integrated subcomponents of $\dot{V}O_2$peak, endurance-type exercise training has been shown to improve cardiac pump function in animal models of type 1 diabetes (440,441), whereas human studies examining training effects on systemic O2 delivery in type 1 diabetes are sparse. A 10% increase in peak O2 pulse (i.e., a surrogate for peak SV (20,123)) has been reported after three months of aerobic training in type 1 diabetes adults (412). In addition, studies on type 2 diabetes patients have produced contradictory findings; both training-induced improvements (442,443) and no improvements (444,445) in echocardiography parameters describing myocardial function have been observed, with high training intensity possibly leading to more pronounced effects (443). In terms of peripheral O2 delivery, aerobic endurance training enhances endothelial function (434,439) and leads to muscle capillary neoformation (407) in type 1 diabetes patients, and has also been reported to increase the expression of pro-angiogenic genes in mice with type 1 diabetes (402). However, animal (402), cross-sectional (401), and short-term training intervention (436) studies have showed that these vascular adaptations are deficient.
compared with those on individuals without diabetes. Instead, subjects with and without type 1 diabetes have displayed similar training-induced rises in enzymatic capacity to utilize O$_2$ \cite{435,436}.

In summary, evidence of favorable effects of physical activity and exercise training on glycemic control and microvascular complications is inconclusive. Instead, epidemiological data more consistently support benefits of regular exercise for macrovascular complications, albeit the strongest evidence provided by large prospective randomized controlled trials is fully absent. As regards the integrated pathway for O$_2$ from the atmosphere to the mitochondria, three small and short-term studies \cite{411,435,436} suggest that VO$_{2\text{peak}}$ increases “normally” in individuals with type 1 diabetes. However, no adaptations of the integrated O$_2$ pathway to long-term (i.e., >4-5 months) exercise training interventions have been studied in type 1 diabetes. In addition, type 1 diabetes studies of any duration examining training-induced adaptations of different components of systemic O$_2$ delivery are almost completely lacking, while peripheral vascular adaptations, which seem to be deficient in type 1 diabetes, have also been sparsely studied.
Table 2  
*Intervention studies on effects of exercise training on V̇O₂peak and HbA1c in type 1 diabetes.*

<table>
<thead>
<tr>
<th>Authors</th>
<th>Trained type 1 diabetes subjects</th>
<th>Mean age (years)</th>
<th>Mean diabetes duration (years)</th>
<th>Sex</th>
<th>RCT</th>
<th>Intervention</th>
<th>V̇O₂peak (or V̇O₂max)</th>
<th>HbA1c</th>
</tr>
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<tbody>
<tr>
<td>Mean age &lt;18 years:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campagne et al. 1984</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>F and M</td>
<td>Yes</td>
<td>AeTr, 30 min x 3/wk, 12 wk</td>
<td>↑: +8%</td>
<td>↓: 12.5%→11.3%</td>
</tr>
<tr>
<td>Landt et al. 1985</td>
<td>9</td>
<td>16</td>
<td>7</td>
<td>F and M</td>
<td>Yes</td>
<td>AeTr, 45 min x 3/wk, 12 wk</td>
<td>↑: +9%</td>
<td>↔</td>
</tr>
<tr>
<td>Huttunen et al. 1989</td>
<td>16</td>
<td>12</td>
<td>5</td>
<td>F and M</td>
<td>Yes</td>
<td>AeTr, 60 min x 1/wk, 3 mo</td>
<td>↑: +10%</td>
<td>↑: 9.8%→10.5%</td>
</tr>
<tr>
<td>Mosher et al. 1998</td>
<td>10</td>
<td>17</td>
<td>&gt;2</td>
<td>M</td>
<td>No</td>
<td>AeTr+ReTr, 45 min x 3/wk, 12 wk</td>
<td>↑: +11%</td>
<td>↓: 7.7%→6.8%</td>
</tr>
<tr>
<td>Seeger et al. 2011</td>
<td>7</td>
<td>11</td>
<td>3</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 50-60 min x 2/wk, 18 wk</td>
<td>↑: +5%</td>
<td>?</td>
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<tr>
<td>Mean age &gt;18 years:</td>
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<td></td>
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<tr>
<td>Costill et al. 1979</td>
<td>12</td>
<td>21</td>
<td>0.25 – 11 a</td>
<td>M</td>
<td>No</td>
<td>AeTr, 30 min x 5/wk, 10 wk</td>
<td>↑: +11%</td>
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<tr>
<td>Wallberg-Henriksson et al. 1982</td>
<td>9</td>
<td>35</td>
<td>12</td>
<td>M</td>
<td>No</td>
<td>AeTr, 60 min x 2-3/wk, 16 wk</td>
<td>↑: +8%</td>
<td>↔</td>
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<tr>
<td>Wallberg-Henriksson et al. 1984</td>
<td>10</td>
<td>32</td>
<td>14</td>
<td>M</td>
<td>No</td>
<td>AeTr, 45 min x 3/wk, 8 wk</td>
<td>↑: +13%</td>
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<tr>
<td>Yki-Järvinen et al. 1984</td>
<td>7</td>
<td>26</td>
<td>7</td>
<td>F and M</td>
<td>Yes</td>
<td>AeTr, 60 min x 4/wk, 6 wk</td>
<td>↑: +8%</td>
<td>↔</td>
</tr>
<tr>
<td>Zinman et al. 1984</td>
<td>13</td>
<td>30</td>
<td>14</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 45 min x 3/wk, 12 wk</td>
<td>↑: +20%</td>
<td>↔</td>
</tr>
<tr>
<td>Wallberg-Henriksson et al. 1986</td>
<td>6</td>
<td>36</td>
<td>14</td>
<td>F</td>
<td>Yes</td>
<td>AeTr, 20 min x 7/wk, 5 mo</td>
<td>↑: +9%</td>
<td>↔</td>
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<tr>
<td>Lehmann et al. 1997</td>
<td>20</td>
<td>33</td>
<td>11</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 45 min x ≥3/wk, 3 mo</td>
<td>↑: +6%</td>
<td>↔</td>
</tr>
<tr>
<td>Laaksonen et al. 2000</td>
<td>20</td>
<td>33</td>
<td>14</td>
<td>M</td>
<td>Yes</td>
<td>AeTr, 20-60 min x 3-5/wk, 12-16 wk</td>
<td>↑: +6%</td>
<td>↔</td>
</tr>
<tr>
<td>Rigla et al. 2000</td>
<td>14</td>
<td>26</td>
<td>6</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 50-60 min x ≥3/wk, 3 mo</td>
<td>↑: +14%</td>
<td>↔</td>
</tr>
<tr>
<td>Fuchsjaeger-Mayrl et al. 2002</td>
<td>18</td>
<td>42</td>
<td>20</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 60 min x 2-3/wk, 4 mo</td>
<td>↑: +27%</td>
<td>↔</td>
</tr>
<tr>
<td>Ramalho et al. 2006</td>
<td>AeTr: 7</td>
<td>20</td>
<td>7</td>
<td>F and M</td>
<td>No</td>
<td>AeTr, 40 min x 3/wk, 12 wk</td>
<td>↑: +6%</td>
<td>↑: 8.7%→9.8%</td>
</tr>
<tr>
<td></td>
<td>ReTr: 6</td>
<td>21</td>
<td>8</td>
<td>F and M</td>
<td>No</td>
<td>ReTr, 40 min x 3/wk, 12 wk</td>
<td>↑: +16%</td>
<td>↔</td>
</tr>
</tbody>
</table>

RCT, randomized controlled trial; F, female; M, male; AeTr, aerobic training; wk, week(s); mo, months; ReTr, resistance training. For other abbreviations, see the Abbreviations section.

*a range.*
3 AIMS OF THE STUDY

The main aim of this thesis was to study O₂ delivery and utilization during acute dynamic exercise in both healthy individuals and individuals with PCOS or type 1 diabetes. In addition, a particular interest was in the adaptations induced by long-term exercise training in individuals with type 1 diabetes. Acute dynamic exercise was hence used as a physiological probe to identify early signs of cardiovascular dysfunction in the patient groups of interest (i.e., PCOS and type 1 diabetes), whereas exercise training was employed to demonstrate whether it alleviates such early dysfunctional signs associated with type 1 diabetes.

Specific aims of the study in accordance with four original publications were as follows:

I To simultaneously investigate alveolar gas exchange and imbalance between local O₂ delivery and utilization within active muscle, less active muscle, and cerebral tissues during maximal incremental treadmill exercise in healthy adult men. Moreover, associations of blood O₂ carrying capacity with both alveolar gas exchange and local tissue-specific O₂ delivery and utilization were examined.

II To explore the contribution of systemic O₂ delivery and C(a-v)O₂ to whole-body V̇O₂ response during maximal incremental cycling exercise in adult overweight and obese women with and without PCOS.

III To simultaneously examine the contribution of systemic O₂ delivery and local active muscle O₂ delivery and utilization to whole-body V̇O₂ response during maximal incremental cycling exercise in adult men with and without type 1 diabetes. Furthermore, associations of BV with cardiac responses to exercise were determined.

IV To investigate the adaptations of V̇O₂peak, peak O₂ pulse, local active muscle O₂ extraction, and glycemic control to a 1-year individualized exercise training intervention in adult men with and without type 1 diabetes.
4 SUBJECTS AND STUDY DESIGN

Studies I-IV are part of a Canadian-Finnish research collaboration entitled “ARTEMIS – Innovation to Reduce Cardiovascular Complications of Diabetes at the Intersection of Discovery, Prevention and Knowledge Exchange”. ARTEMIS was designed to identify, prevent, and treat early markers of cardiovascular dysfunction in patients at risk for, or with, diabetes, and to develop best practices for the prevention and reversal of diabetes-related cardiovascular complications (446).

4.1 STUDY I

Study I was conducted as a collaborative effort between the Department of Sports and Exercise Medicine (University of Helsinki), the Clinic for Sports and Exercise Medicine (Foundation for Sports and Exercise Medicine), and the Centre of Excellence for Health and Work Ability (Finnish Institute of Occupational Health). The experiments were performed between February and May in 2009.

Twenty-nine male volunteers were assessed for inclusion in Study I. The subjects aged 22-37 years, were nonathletes but physically active, nonsmoking, had no history of cardiovascular, endocrine, musculoskeletal, neurological, or respiratory diseases, and were free of medication. The subjects visited the laboratory twice: One visit consisted of CPET accompanied by a medical examination and supplementary pre-exercise experiments, whereas another visit included the determination of tHb-mass and BV. Eventually, the data on 22 subjects were fully available, characterized by no artifacts, and thus included in final analyses.

4.2 STUDY II

Study II was a cross-sectional substudy of a collaboration between the Department of Sports and Exercise Medicine (University of Helsinki), the Clinic for Sports and Exercise Medicine (Foundation for Sports and Exercise Medicine), and the Department of Obstetrics and Gynecology (Helsinki University Hospital and Helsinki University). Study II combined data from two different study projects of the collaboration: “Well-Being of WOMen during pregnancy: focus on individualized EXercise training” (WEBWOMEX) and “Autonomic Nervous System and EXercise in gestational diabetes” (ANS-EXE) (ClinicalTrials.gov: NCT01675271). These projects have mainly been designed to identify effects of individualized exercise training on those cardiovascular, inflammatory, and autonomic nervous system functions that contribute to infertility in women with PCOS and predict, prevent, or promote gestational diabetes. The experiments of the two projects were performed between March 2010 and September 2015. However, the statistical analyses for Study II were finished in April 2015; therefore, data gathered until the end of March 2015 were evaluated to be included in Study II.
A total of 122 female volunteers were assessed for eligibility for Study II: all 62 women from the WEBWOMEX population, and those 60 women from the ANS-EXE population who had reported to the project until the end of March 2015. Thirty of all 122 women had met the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria for a diagnosis of PCOS (i.e., Rotterdam criteria) (306). PCOS women were recruited from the patient population of the Department of Obstetrics and Gynecology (Helsinki University Hospital), whereas women without PCOS were mainly recruited among the employees and students of the University of Helsinki. The exclusion criteria of Study II were as follows: age of <18 or >40 years, BMI of <25 or >40 kg/m², pregnancy, androgen-secreting tumors, congenital adrenal hyperplasia, Cushing’s syndrome, anemia, current diabetes, hypertension, antiandrogen medication, β-blocker medication, medication influencing glucose homeostasis, use of oral contraceptives, substance abuse, physical disability, smoking, and other cardiovascular, endocrine, musculoskeletal, neurological, or respiratory diseases that could have affected any outcome of interest. Thus, four over 40-year-old women, 44 women with BMI <25 kg/m², one woman with BMI >40 kg/m², five pregnant women, one woman with cortisone treatment for asthma, two women using oral contraceptives, two smokers, and one woman with hypothyroidism were excluded from the analyses. In addition, seven irregularly menstruating (i.e., menstrual period not occurring every 23-32 days (447)) women without PCOS, two women with no North European background, and three women showing artifacts in their PhysioFlow data (i.e., impedance cardiography data on cardiac pump function), were not included in the analyses. Furthermore, one woman stopped her CPET at submaximal work rates, and 19 women withdrew prior to the commencement of any study experiments. Thus, a final sample of 30 women, 15 of whom had a diagnosis of PCOS, were included in the eventual analyses of Study II.

The data for Study II were collected during two visits to the laboratory. One visit comprised CPET along with a medical examination and supplementary pre-exercise experiments, and the other visit consisted of drawing fasting venous blood. For the women who moved on to the lifestyle interventions of the WEBWOMEX or ANS-EXE projects, the data collected before any intervention were used for the analyses of Study II.

4.3 STUDY III

Study III was a cross-sectional subanalysis of an “Exercise, Diet and GEnes in T1D” (EDGE) Helsinki project. The EDGE subjects with type 1 diabetes were recruited from the patient pool of the FinnDiane Study (418), whereas the subjects without diabetes were mainly recruited from the employees and students of the University of Helsinki. The EDGE experiments were performed between May 2009 and December 2013.

In addition to the EDGE subjects, the subjects of Study III also included participants from a Finnish television series entitled Finnautti. In Finnautti, five individuals (one woman, four men) underwent a diverse set of space flight-related tests including CPET and determination of tHb-mass and BV in the Department of Sports and Exercise Medicine (University of Helsinki) and the Clinic for Sports and Exercise
Medicine (Foundation for Sports and Exercise Medicine). The Finnautti experiments were performed in May 2010.

All 42 male volunteers participating in EDGE and four male volunteers taking part in Finnautti were assessed for inclusion in Study III. Fifteen EDGE subjects had type 1 diabetes. The exclusion criteria of Study III were age of <18 or >45 years at baseline; previous diagnosis or previous clinical evidence of microvascular complications (i.e., retinopathy, nephropathy, neuropathy), hypertension, or any chronic disease other than diabetes for the diabetes patients; β-blocker medication; medication influencing glucose homeostasis except for multiple daily insulin injections of those with diabetes; physical disability; substance abuse; smoking; and elite athlete status. Eleven EDGE subjects with and 19 without diabetes, who were not excluded based on the exclusion criteria, went through all experiments both before (i.e., baseline) and after (i.e., post) individualized exercise training- or no training intervention, as shown in Figure 11, which details the design and flow of Study IV. However, one of the 19 subjects without diabetes had only completed baseline experiments by the time that the statistical analyses of Study III were performed in June and July 2013. In addition, four subjects with and two without type 1 diabetes performed experiments only at baseline, and three ≤45-year-old Finnautti men performed experiments just once. Furthermore, a PhysioFlow device (used for estimating cardiac pump function) was not available until January 2010, before which 10 eligible men with and 13 without diabetes had already performed their baseline experiments. In consequence, full data on 16 subjects’ baseline experiments (five with and 11 without diabetes) and 29 subjects’ post experiments (11 with and 18 without diabetes) were assessed for inclusion in Study III. After further exclusion of 15 CPETs showing artifacts in PhysioFlow data (six with and nine without diabetes), one diabetic man with hypertension medication during post experiments, and one nondiabetic man with anemic [Hb] during post experiments, all required baseline or post data of only seven men with type 1 diabetes were eventually available for Study III. A numerically convenient sample of 10 age-, anthropometry-, and LTPA-matched men without diabetes was included in Study III so that those included were subsequently checked to match for age, anthropometry, LTPA, and VO2peak with all eligible subjects without diabetes (0.255 < P < 0.925). Thus, final samples of seven men with and 10 without type 1 diabetes were eventually analyzed for Study III.

Collecting the data for Study III required three visits to the laboratory per subject. One visit consisted of drawing fasting venous blood, one visit was composed of CPET along with a medical examination and supplementary pre-exercise experiments, and one visit included the determination of tHb-mass and BV.

4.4 STUDY IV

Study IV was a main part of EDGE (for details, see Section 4.3) and consisted of a prospective, nonrandomized, and controlled exercise training intervention. The exclusion criteria of Study IV were the same as those of Study III (see Section 4.3).

The study flow chart presented in Figure 11 illustrates the design and flow of Study IV. After the enrollment, exclusions, nonrandomized allocation (i.e., training- or no
intervention), and discontinuations, eight adult men with type 1 diabetes and 13 healthy subjects completed a 1-year individualized exercise training intervention. To completely match these two groups for baseline age, anthropometry, and VO\textsubscript{2peak}, five healthy subjects with the highest baseline VO\textsubscript{2peak} values were excluded from further analyses. Thus, eight men with and eight without type 1 diabetes were included in between-group analyses evaluating the effects of the training intervention.

In addition, five healthy men served as Reference group. At baseline and after a 1-year time period, they went through the same clinical experiments as the training groups, but were only instructed to maintain their lifestyle, particularly regarding diet and physical activity, during that period. Age and VO\textsubscript{2peak} differed substantially from those of the training groups (see Sections 6.1.4 and 6.2.4, respectively), and the sample size of Reference group was small (n = 5). Thus, to avoid confounding the between-group analyses (i.e., the analyses between the training groups) but to demonstrate the repeatability of clinical experiments, Reference group was analyzed separately from the training groups.

For Study IV, the subjects with diabetes visited the laboratory twice both at baseline and after their 1-year training intervention, whereas the subjects without diabetes visited the laboratory once at baseline and twice after the 1-year periods of training or no interventions. The first visits comprised of drawing fasting venous blood, and the second visits consisted of CPET, a medical examination, and supplementary pre-exercise experiments.

**Figure 11** Study IV flow chart. * Five trained healthy subjects with the highest peak pulmonary O\textsubscript{2} uptake (VO\textsubscript{2peak}) values at baseline were excluded from further between-group analyses to match two training intervention groups also for baseline VO\textsubscript{2peak}. 

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4.5 ETHICAL APPROVAL

All subjects gave written informed consent prior to their participation in the study. All study projects conformed to the Declaration of Helsinki and were approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa, Helsinki, Finland.
5 METHODS

5.1 CLINICAL EVALUATION AND EXPERIMENTS

5.1.1 MEDICAL EXAMINATION

In Studies I-IV, a physician specialized or specializing in sports medicine examined the subjects before any CPETs to ensure their suitability for exercise testing. The examination comprised a personal health and medical history (including interpretation of a preliminary questionnaire), clinical status, and interpretation of a 12-lead resting electrocardiography, resting blood pressure, and the other pre-exercise experiments described below. Regarding the preliminary questionnaire, those subjects with type 1 diabetes who participated in a 1-year training intervention of Study IV reported their insulin doses within a 3-day period around the performed experiments.

5.1.2 PRE-EXERCISE EXPERIMENTS

5.1.2.1 Anthropometry

In Studies I-IV, the subjects’ weight and height were measured, and body composition (i.e., fat-free mass [FFM], body fat percentage) was determined using the bioimpedance method (InBody 720, Biospace Co., Ltd., Seoul, South Korea). Subjects’ waist and hip circumferences were also measured and waist-to-hip ratio was calculated in Studies II-IV. Furthermore, thickness of subcutaneous fat was measured with skinfold calipers at NIRS recording sites particularly in Studies III and IV, in which NIRS data were compared between men with and without type 1 diabetes.

5.1.2.2 Flow-volume spirometry

In Studies I-IV, flow-volume spirometry (Medikro Spiro 2000, Medikro Oy, Kuopio, Finland) was one part of the pre-exercise experiments. Levels of forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were evaluated as both absolute values and percentual values of the reference data on the Finnish population (448).

5.1.2.3 Leisure-time physical activity (LTPA)

In Studies II-IV, LTPA was registered as one part of the preliminary questionnaire concerning personal health and medical history and interpreted by a physician. For this, the following single question was asked: “If you think about your past three months and physical activity sessions lasting more than 20 min in all settings (e.g.,
commuting, walking a dog, recreation, sport), how many times a week and how long at a time have you engaged in physical activity?” This question meets the general minimum recommendations (i.e., frequency, duration, all settings) for enquiring about LTPA (449).

5.1.3 BLOOD SAMPLES

At a separate visit, the subjects reported to the laboratory after overnight fast and their antecubital venous blood was drawn for measurement of either lipid profile (i.e., high-density lipoprotein, low-density lipoprotein, and total cholesterol, and triglycerides), plasma glucose, and serum insulin (Study II), or HbA1c (Studies III and IV).

In Study II, fasting plasma glucose and serum insulin were determined by the hexokinase method and the immunochemiluminometric assay, respectively, and homeostasis model assessment of IR (HOMA-IR) was calculated as follows (450):

\[
\text{HOMA-IR} = \frac{\text{fasting plasma glucose} \times \text{fasting serum insulin}}{22.5}
\]

where the concentrations of fasting plasma glucose and serum insulin are in mmol/L and μU/mL, respectively.

Serum testosterone and SHBG concentrations of PCOS women were not determined separately for Study II, but as part of the patients’ clinical care by the mass spectrometry assay and the immunochemiluminometric assay, respectively. Free androgen index was determined as follows:

\[
\text{free androgen index} = \frac{\text{serum testosterone}}{\text{serum SHBG}} \times 100
\]

where the concentrations of serum testosterone and SHBG are in nmol/L.

5.1.4 BLOOD OXYGEN CARRYING CAPACITY

The components of [Hb] (i.e., tHb-mass [Study I] and BV [Study III]) were determined by the optimized CO-rebreathing method (132) (SpiCO, BloodTech, Bayreuth, Germany), previously described in Section 2.1.1.3. The experiments were performed at separate visits and preceded by abstinence from physical exercise for at least 12 h and alcohol for at least 24 h. Capillary blood was drawn from a fingertip to analyze [HbCO] and [Hb] by a blood gas analyzer (ABL725, Radiometer, Copenhagen, Denmark). [Hb] was similarly determined also in Studies II and IV. According to duplicate tests, a typical error (451) of the tHb-mass measurements in the laboratory of the Department of Sports and Exercise Medicine (University of Helsinki) has been reported to be 1.7% with a 95% confidence interval of 1.3-2.4% (376).

In Study I, tHb-mass was examined as both absolute and body weight-adjusted values. In addition, the magnitude of change in VO₂peak versus a given change in tHb-mass was determined by plotting VO₂peak values against tHb-mass values and
interpreting the slope of this linear regression line. In Study III, BV was examined as both absolute and FFM-adjusted values.

5.1.5 CARDIOPULMONARY EXERCISE TEST (CPET)

5.1.5.1 CPET protocols

In Studies I-IV, the subjects performed CPETs and underwent a medical examination and the pre-exercise measurements described above. Before CPETs, the subjects reported to the laboratory 2-3 h after their ordinary meal. The CPET protocols used in Studies I-IV differed slightly from each other, as detailed below. However, all of the protocols were incremental and used 3-min steps, which was based on VO₂ kinetics: the time constant of VO₂ is ~30 s, implying that it takes ~30 s to reach 63% of the remaining difference between the VO₂ value at each time point and steady-state VO₂ value. Thus, it takes 5-6 time constants (i.e., ~3 min) to reach a steady state after each transition from the previous intensity to the next one. Notably, at intensities above AT, the slow component of VO₂ is added to comprise the overall response to reach steady state. The slow component is calculated to be 3-6 min after transition from the previous intensity to the next one, but since it is affected by exercise intensity and interindividual variability (e.g., VO₂peak) it cannot be standardized (452).

In Study I, each subject performed their CPET on a treadmill (Juoksumatto OJK-1, Telineyhtymä, Kotka, Finland). The following step incremental protocol was used: 3-min rest, while the subjects stood on the treadmill, preceded 5-min baseline walking at a speed of 5 km/h, which was then followed by incremental exercise (+1 km/h / 3 min) initiated with a speed of 8 km/h. The subjects continued exercising until volitional exhaustion. The incline of the treadmill was 0.5° throughout the test to mimic air resistance during outdoor running.

In Studies II-IV, CPETs were performed on a mechanically braked cycle ergometer (Monark Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden). The tests began with 5-min rest, while the subjects sat on the ergometer. The rest period was followed by 5-min baseline unloaded cycling, after which a step incremental protocol (+30 W / 3 min in women [Study II], +40 W / 3 min in men [Studies III and IV]) was initiated with a work rate of 30 W (Study II) or 40 W (Studies III and IV). The subjects continued exercising until volitional exhaustion.

In Studies III and IV, capillary blood was drawn from a fingertip to analyze blood glucose concentration (Glucocard x-meter, Arkray Factor, Inc., Shiga, Japan) before CPET; subjects with diabetes had glucose levels of 5.6-16.7 mmol/L with no signs of ketosis, according to the published guidelines (39).

All somatic measurements during CPETs are described in Sections 5.1.5.2-5.1.5.3. In addition, rating of perceived exertion was obtained in Studies II-IV using the Borg scale of 6-20 (453) at the end of each work rate.
5.1.5.2 Systemic oxygen delivery

In addition to the determination of [Hb], information on systemic O\textsubscript{2} delivery was obtained by measuring several parameters of pulmonary and cardiac pump functions. Equation 3 was applied to calculate and analyze systemic O\textsubscript{2} delivery particularly for this thesis.

Pulmonary function

Breath-by-breath $V_E$ was measured by a low-resistance turbine (Triple V, Jaeger Mijnhardt, Bunnik, The Netherlands) to determine inspiratory and expiratory flows and volumes during each CPET. The turbine was calibrated prior to each CPET using a syringe of 3.00 L (Hans Rudolph, Inc., Kansas City, MO, USA). Inspired and expired gases were continuously sampled at the mouth and analyzed for concentrations of O\textsubscript{2}, CO\textsubscript{2}, N\textsubscript{2}, and Ar by mass spectrometry (AMIS 2000, Innovision A/S, Odense, Denmark) (21) after calibration with precision-analyzed gas mixtures. Breath-by-breath data were collected as raw data, which were transferred to a computer to determine gas delays for each breath. The gas concentrations were herewith aligned with volume data, and the profile of each breath was built. Breath-by-breath alveolar gas exchange was thereafter calculated with the AMIS algorithms, a moving average of individual test data was calculated over 5-s periods to lessen inherent breath-by-breath variability, and the data was eventually interpolated to obtain second-by-second data. In Study I, AT and RC were determined using the V-slope method (60). RER was traditionally calculated as a ratio of $V\dot{CO}_2$ and $V\dot{O}_2$.

To monitor SpO\textsubscript{2} during CPETs, fingertip pulse oximetry (Nonin 9600, Nonin Medical, Inc., Plymouth, MA, USA) was employed. Furthermore, CaO\textsubscript{2} was calculated (Equation 3).

Cardiac pump function

In Studies I-IV, the electrical activity of the heart and HR were continuously monitored by electrocardiography (PowerLab, ADInstruments, Oxford, United Kingdom) during CPETs. In Studies II and III, a PhysioFlow impedance cardiograph device (Manatec Biomedical, Paris, France) was employed to continuously evaluate cardiac pump function (i.e., particularly SV and hence $Q$ [Studies II and III], but also EDV and EF [Study III]). The PhysioFlow method has been extensively described in Section 2.1.1.2. In Study IV, peak O\textsubscript{2} pulse was calculated as a quotient of $\dot{V}O_2$peak and peak HR to provide a surrogate for peak SV (Equation 7) (20,123).

In Studies II-IV, systolic and diastolic arterial blood pressures were measured automatically (Tango+, SunTech Medical, Morrisville, NC, USA) from the left brachial artery at seated rest and at the end of each work rate. MAP was calculated by the standard equation: $MAP = \left(\text{systolic arterial blood pressure} + 2 \times \text{diastolic arterial blood pressure}\right) / 3$. In Studies II and III, where PhysioFlow data on $Q$ were available, SVR was determined applying Darcy’s law (Equation 4) and assuming that CVP is ~0 mmHg from rest to peak exercise (97). In Study II, peak cardiac power output (CPO), relating changes in systemic blood flow and afterload, was furthermore calculated from
the values of $\dot{Q}$ and MAP at peak exercise to reflect the hydraulic power of the heart (454):

\[(16) \quad \text{CPO} = \dot{Q} \times \text{MAP} \times K\]

where $K$ is a conversion factor ($2.22 \times 10^{-3}$) into watts.

**Scaling of cardiopulmonary data**

In Studies I-IV, $\dot{V}O_2$ was expressed as values of L/min but also as values scaled to body weight (mL/min/kg) and particularly to FFM (mL/min/kg FFM) (455). The importance of the latter method is particularly highlighted when examining overweight and obese individuals (456). Similarly, to further avoid ignoring any between-group differences in body size or composition we scaled EDV, SV, $\dot{Q}$, $O_2$ pulse, and CPO to FFM (93), whereas SVR was multiplied by FFM. These scaled cardiovascular variables are referred to as the indices: EDVi, SVi, $\dot{Q}_i$, $O_2$ pulsei, CPOi, and SVRi, respectively.

In Study I, $\dot{V}O_2$ at AT and RC were examined as absolute values (L/min; mL/min/kg; mL/min/kg FFM), but also as relative values (% of $\dot{V}O_2$ reserve = $[\text{measured } \dot{V}O_2 - \text{resting } \dot{V}O_2] / [\dot{V}O_2_{\text{peak}} - \text{resting } \dot{V}O_2] \times 100\%$).

**5.1.5.3 Peripheral oxygen delivery, extraction, and utilization**

**NIRS experiments**

A continuous wave NIRS device (Oxymon Mk III Near-Infrared Spectrophotometer, Artinis Medical Systems, Zetten, The Netherlands) was employed to monitor exercise-induced changes in tissue-specific (de)oxygenation in Studies I, III, and IV. Thus, data on local tissue-specific (im)balance between O2 delivery and utilization, or in other words, local fractional O2 extraction, were acquired. A methodological principle of NIRS has been comprehensively presented in Section 2.1.2.4.

In Study I, local (de)oxygenation profiles were monitored simultaneously from active leg muscle, less active arm muscle, and cerebral tissue. In Studies III and IV, the interest was only in active leg muscle deoxygenation. For leg muscle monitoring, a NIRS probe was placed over the right vastus lateralis muscle at mid-thigh level and parallel to the long axis of the muscle. To monitor arm muscle, a NIRS probe was positioned longitudinally on the right biceps brachii muscle above the elbow joint and lateral to the middle line. For cerebral monitoring, a NIRS probe was placed over the right prefrontal cortex, about 2 cm above the right eyebrow and as laterally as possible to the longitudinal fissure of the cerebrum. This prefrontal site projects to the premotor cerebral areas, and thus, participates in planning (457) and pacing (458) strategies during voluntary movements. Oxygenation status of the prefrontal cortex has also been shown to reflect muscle force-generating capacity (459). The optically dense plastic NIRS probes were attached to the skin with double-sided adhesive tape and covered by an elastic tape.

The NIRS probes consisted of three transmitting and one receiving optode operating at wavelengths of 860 and 765 nm corresponding to the specific extinction
coefficients of \(O_2\text{Hb}\) and \(HHb\) (224), respectively. The interoptode distance was set to 35-50 mm so that a good signal quality was achieved before any experiments. Relative concentration changes (\(\Delta\mu\text{mol/L}\)) from the resting baseline of \(O_2\text{Hb}\) (\(\Delta[O_2\text{Hb}]\)) (Study I) and particularly of \(HHb\) (\(\Delta[HHb]\)) (Studies I, III, and IV) were analyzed. In addition, TSI as well as a relative concentration change of \(t\text{Hb}\) (\(\Delta[t\text{Hb}]\)) were examined in Study I. The DPF values used were 5.51 and 4.16 for leg and arm (227), respectively, whereas DPF for cerebral tissue was calculated according to the manufacturer’s guidelines (i.e., \(\text{DPF} = 4.99 + 0.067 \times \text{age}^{0.814}\)). Sampling frequencies of 50 Hz (Study I) or 10 Hz (Studies III and IV) were used for collecting NIRS data. The obtained NIRS data were averaged to give values in second-by-second intervals and time-aligned with cardiopulmonary data. In Studies III and IV, the averaged \(\Delta[HHb]\) data were further normalized (%\(\Delta[HHb]\)) so that 0% represents the mean steady-state value of the last 30 s of unloaded cycling (Study III) or of any work rate (Study IV), while 100% represents the highest mean value of the last 30 s of any work rate. The rationale for this, instead of comparing values to resting values, was that muscle pump action, expelling blood from muscles towards the heart at the onset of exercise (460), is expected to induce rapid temporary changes in NIRS data (238).

In Study I, particular tissue-specific NIRS inflection points (NIP) were determined as follows:

1. Second-by-second data of \(\Delta[O_2\text{Hb}], \Delta[HHb]\), and TSI were plotted against time starting from locomotion at 8 km/h until the end of exercise. The plottings were performed for all three tissues of interest (m. vastus lateralis, m. biceps brachii, prefrontal cerebral cortex) in every subject.
2. The sizes and axes of the plotted figures were scaled to be similar and comparable with each other (see Appendix 1).
3. Trend lines were drawn and adjusted to reflect trends throughout the scatter plots.
4. When an angle of \(\geq 15^\circ\) was observed between consecutive trend lines, the intersection of the two trend lines was regarded as an NIP and included in further analyses.
5. A vertical line cutting both the intersection and X-axis (time) was drawn to determine the timing of the NIP.
6. \(\dot{V}O_2\) at each NIP was determined as a 30-s average (i.e., time ± 15 s) from second-by-second \(\dot{V}O_2\) data.

Any determined NIP was included in further analyses on the following condition: an angle of \(\geq 15^\circ\) between consecutive trend lines had to be observed at the same time point in all three parameters (\(\Delta[O_2\text{Hb}], \Delta[HHb]\), and TSI). The change of this magnitude was interpreted to reflect permanent changes in tissue (de)oxygenation trend. In addition, as regards cerebral tissue, inflection points with a rise only in \(\Delta[HHb]\) were also included in further analyses. NIPs were named as follows: NIP\(_{\text{LegAT}}\) (i.e., NIP observed in leg muscle and being the closest to AT), NIP\(_{\text{LegRC}}\), NIP\(_{\text{ArmAT}}\), NIP\(_{\text{ArmRC}}\), NIP\(_{\text{CerAT}}\), and NIP\(_{\text{CerRC}}\), respectively.

In Study III, %\(\Delta[HHb]\) data were converted to reflect local microvascular blood flow in the active vastus lateralis muscle (\(Q_{\text{VL}}\)) as previously described (175-177): local (a-
v)O₂ was estimated from the pattern of %Δ[HHb] using published values for muscle (a-v)O₂, which was hence assumed to equal 10 mL per 100 mL blood during unloaded cycling (461) and 18 mL per 100 mL blood at peak exercise (55). Subsequently, normalized change in %Δ[HHb] was converted to peripheral (a-v)O₂ as follows:

\[
\text{Peripheral (a-v)O}_2 = 10 + \left( \frac{\% \Delta [\text{HHb}]}{100\%} \right) \times 8
\]

where 10 corresponds to the assumption of (a-v)O₂ during unloaded cycling and 8 reflects the assumed change in (a-v)O₂ from unloaded cycling to peak exercise.

Second-by-second \(Q_{VL}\) was then calculated for each subject of Study III as the quotient of \(\dot{V}O_2\) (measured) and peripheral (a-v)O₂ (Equation 17); this calculation was thus based on a regional application of the Fick principle. Notably, the \(\dot{V}O_2\) values used in this context were time-aligned with %Δ[HHb] data by left-shifting the \(\dot{V}O_2\) signal by 20 s to account for the circulatory transit delay between the muscle and the lungs (175). Although this 20-s value may not precisely match the circulatory time delay in every subject, it represents a reasonable estimate for the subjects tested (462).

**Systemic arterial-venous oxygen difference**

In Studies II and III, where PhysioFlow data on \(Q\) were available, C(a-v)O₂ was derived according to the Fick principle (Equation 2).

### 5.2 EXERCISE TRAINING INTERVENTION (STUDY IV)

A 1-year individualized exercise training intervention in Study IV was performed in a real-world setting. After their baseline experiments, the subjects allocated to training groups (Figure 11) were given a 30-min lecture containing research-based justification for general principles and effects of endurance and resistance training, and general instructions on adjusting carbohydrate and insulin consumption according to physical activity. The lectures were given by exercise physiologists to 1-3 subjects at a time and also included face-to-face discussions aimed at individual goal setting. The overall goal of the intervention was to increase and improve exercise training in real-world circumstances according to an individual’s desires and goals, which were set based on the individual results of the baseline experiments.

Throughout the intervention, the subjects were instructed to use HR monitors (Suunto t6c, Suunto Oy, Vantaa, Finland; or Polar RS800CX, Polar Electro Oy, Kempele, Finland) to collect data on every exercise session in their individual training diaries. The collected data included information on duration, energy expenditure, exercise modes, and mean HR of the performed exercise sessions. The subjects emailed these individual training diaries monthly to the researchers, who subsequently emailed individual prescriptive feedback to the subjects. The monthly feedback focused on frequency, energy expenditure, duration, modes, intensity, and progression of performed exercise training. An exercise mode was considered 1) endurance training if it included various dynamic aerobic and/or anaerobic activities performed with a large mass of contracting muscles (e.g., walking, jogging, running, cycling, ball games) (22), and 2) resistance training if it aimed at improvements in or maintenance of muscular
strength, power, and/or endurance (463). Exercise intensity was examined as % of HR reserve (% of HR reserve = [mean HR – resting HR] / [peak HR – resting HR] × 100%) (463). In this regard, resting HR was the lowest nocturnal HR obtained by Firstbeat Bodyguard (Firstbeat Technologies Oy, Jyväskylä, Finland) during the night following the baseline CPET, whereas peak HR was the peak HR during the baseline CPET.

5.3 STATISTICAL ANALYSES

Data were computed with PASW Statistics 18.0 (IBM Corporation, Somers, NY, USA) (Studies I-III) or IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY, USA) (Study IV). All data are expressed as mean ± standard deviation (SD), and statistical significance was set at \( P < 0.05 \). Shapiro-Wilk test was used to check normality and data were log-transformed where appropriate. Relationships between any key variables were examined by Pearson’s correlation coefficients.

As regards CPET data, the mean values of the last 30 s at rest, during baseline walking (Study I) or unloaded cycling (Studies II-IV), at each speed (Study I) or work rate (Studies II-IV), and at peak exercise were included in further analyses. However, \( \dot{V}O_{2\text{peak}} \) was defined as the highest value of a 60-s moving averaging \( \dot{V}O_2 \) interval.

Further details of the statistical analyses of Studies I-IV are presented below.

5.3.1 STUDY I

NIRS data obtained during CPETs were compared with the values during the last 30 s of the 5-min baseline walking. For this purpose, repeated-measures ANOVA with Sidak post hoc analysis was used. Temporal coincidence of NIPs with AT and RC was tested with paired samples \( t \)-test. In addition, particularly for this thesis, one-way ANOVA with Games-Howell post hoc analysis was used to compare the extent of exercise-induced deoxygenation (i.e., exercise-induced change in \( \Delta[Hb] \) from baseline walking to peak exercise) between leg muscle, arm muscle, and cerebral tissue.

5.3.2 STUDY II

One-way ANOVA was used to compare descriptive characteristics, cardiovascular function at seated rest, profile of glucose metabolism, lipid profile, and CPO between PCOS women and controls. Two-way repeated-measures ANOVA was used to evaluate whether there were differences in responses to CPET between PCOS women and controls: Group×Exercise interactions were assessed, while Group (i.e., PCOS women vs. controls) was a between-subjects factor, and Exercise (i.e., unloaded cycling [0 W], work rates accomplished by each subject [30 W, 60 W, 90 W, 120 W], peak exercise) was a within-subject factor. In a separate analysis, relative intensities (i.e., % of \( \dot{V}O_{2\text{peak}} \) during unloaded cycling [0 W], 25% of \( \dot{V}O_{2\text{peak}} \), 50% of \( \dot{V}O_{2\text{peak}} \), 75% of \( \dot{V}O_{2\text{peak}} \), 100% of \( \dot{V}O_{2\text{peak}} \)) were used as a within-subject factor. Multivariate ANOVA with Bonferroni
post hoc analysis was performed to further identify the differences in CPET responses between PCOS women and controls.

The response of Q̇ to acute incremental exercise was determined as follows: the means of Q̇ and V̇O₂ at six data points (i.e., unloaded cycling [0 W], 30 W, 60 W, 90 W, 120 W, peak exercise) were calculated separately for PCOS women and controls, after which ΔQ/ΔV̇O₂ slopes (i.e., β coefficients) for both subject groups were determined by performing a linear regression of Q̇ over V̇O₂ at the six data points. The ΔQ/ΔV̇O₂ slopes were further compared between PCOS women and controls by using the method of dummy variables and interaction terms (464): a dummy variable named Syndrome was first created so that it took the value 0 for controls and 1 for PCOS women. To test whether PCOS influenced the linear regression of Q̇ over V̇O₂, an interaction term Syndrome×V̇O₂ was created and then included in an additional multiple linear regression model, where Q̇ was the outcome and Syndrome, V̇O₂, and Syndrome×V̇O₂ were the predictors. Eventually, the contribution of the Syndrome×V̇O₂ interaction to the model indicated whether the ΔQ/ΔV̇O₂ slopes differed between PCOS women and controls.

5.3.3 STUDY III

Two-tailed independent samples t-test was overall used to compare men with and without type 1 diabetes. Repeated-measures ANOVA with Sidak post hoc analysis was used to compare the within-group values of %Δ[HHb] and Q̇VL at each work rate with those during unloaded cycling.

5.3.4 STUDY IV

One-way ANOVA was used for between-group comparisons regarding descriptive characteristics and CPET responses at baseline as well as characteristics of exercise training. One-way repeated-measures ANOVA was used to evaluate whether within-group changes from baseline to post occurred in two training groups or Reference group. Two-way repeated-measures ANOVA was used to assess whether there were between-group differences in changes from baseline to post between the training groups: Group×Time interactions were assessed with Group (i.e., men with type 1 diabetes vs. men without diabetes) as a between-subjects factor and with Time (i.e., Baseline, Post) as a within-subject factor. t-test with Bonferroni post hoc analysis was then used, if a significant interaction was observed. In case of nonnormally distributed data despite log transformation, nonparametric Mann-Whitney U and Wilcoxon signed-rank tests were used for between-group and within-group analyses, respectively. To evaluate magnitudes of any observed within-group training-induced changes, standardized effect sizes (ES) were calculated with threshold values of ≤0.2 trivial, >0.2 small, >0.6 moderate, and >1.2 large (465).
5.3.5 STATISTICAL POWER CALCULATION (STUDIES II-IV)

The main outcome in Studies II-IV was $\dot{V}O_{2peak}$ (mL/min/kg FFM), based on which the adequacy of statistical power was evaluated. Two Web-enabled interfaces were used for statistical power calculations: One was provided by Decision Support Systems, LP (Studies II and III) (466) and the other by Clinical & Translational Science Institute, University of California, San Francisco (Study IV) (467).
6 RESULTS

6.1 DESCRIPTIVE CHARACTERISTICS AND BLOOD OXYGEN CARRYING CAPACITY

Ages, anthropometric profiles, LTPA, hematologic variables, and flow-volume spirometry data of the subjects of Studies I-III are summarized in Table 3. Table 3 also presents the data on cardiovascular function at seated rest in Studies II and III. Table 3 importantly demonstrates that in both Studies II and III PCOS women and men with type 1 diabetes, respectively, were matched with their healthy controls for age, anthropometry, and LTPA. In addition, no defects (Studies I-III) or between-group differences (Studies II and III) were observed in [Hb] or any flow-volume spirometry experiments.

Sections 6.1.1-6.1.3 are complementary to Table 3 and detail the descriptive characteristics as well as blood O₂ carrying capacity of the subjects of Studies I-III, whereas Section 6.1.4 presents the descriptive characteristics of Study IV subjects.

6.1.1 STUDY I

The subjects of Study I had a tHb-mass of 928 ± 105 g corresponding to 11.6 ± 1.2 g/kg. When individual \( \dot{V}O_{2\text{peak}} \) values were plotted against individual tHb-mass data and a linear regression line was fitted, a tHb-mass change of 1 g was associated with a 3.6 mL/min change in \( \dot{V}O_{2\text{peak}} \) (\( r = 0.83, P < 0.001 \)).

6.1.2 STUDY II

Of 15 PCOS women included, four had clinical (hirsutism) and/or biochemical (serum testosterone > 2.0 nmol/L) hyperandrogenism, all showed oligo- or anovulation, and all showed polycystic ovaries on transvaginal ultrasound, in accordance with the diagnostic Rotterdam criteria (306).

Waist (99 ± 6 vs. 95 ± 11 cm, \( P = 0.246 \)) and hip (115 ± 6 vs. 114 ± 12 cm, \( P = 0.817 \)) circumferences as well as waist-to-hip ratio (0.86 ± 0.07 vs. 0.83 ± 0.08, \( P = 0.279 \)) were similar between PCOS women and controls, respectively.

No differences between PCOS women and controls were observed for HR, SV, SVi, or systolic or diastolic arterial blood pressures at rest, whereas PCOS women had higher \( \dot{Q} \) but similar \( \dot{Q}_i \) at rest relative to controls (Table 3).

Table 4 summarizes the profile of glucose metabolism and lipid profile of the Study II subjects: fasting glucose and insulin, HOMA-IR, low-density lipoprotein and total cholesterols, and triglycerides were similar between the groups, while PCOS women had lower high-density lipoprotein cholesterol than controls.

Serum testosterone was 1.4 ± nmol/L, serum SHBG was 40 ± 17 nmol/L, and free androgen index was 4.3 ± 4.0 in PCOS women.
Table 3  Descriptive characteristics of the subjects of Studies I-III.

<table>
<thead>
<tr>
<th></th>
<th>STUDY I (men)</th>
<th>STUDY II (women)</th>
<th>STUDY III (men)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects (n=22)</td>
<td>PCOS (n=15)</td>
<td>Controls (n=15)</td>
<td></td>
<td>T1D (n=7)</td>
<td>Controls (n=10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.2 ± 5.8</td>
<td>29.3 ± 4.0</td>
<td>31.1 ± 5.5</td>
<td>0.326</td>
<td>34.8 ± 6.0</td>
<td>34.0 ± 7.0</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 ± 8</td>
<td>94 ± 8</td>
<td>86 ± 17</td>
<td>0.121</td>
<td>86 ± 9</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180 ± 8</td>
<td>171 ± 6</td>
<td>167 ± 10</td>
<td>0.166</td>
<td>182 ± 7</td>
<td>183 ± 8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 ± 2.1</td>
<td>32.0 ± 2.0</td>
<td>30.6 ± 3.9</td>
<td>0.208</td>
<td>25.9 ± 3.4</td>
<td>24.5 ± 1.9</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>69 ± 8</td>
<td>55 ± 5</td>
<td>52 ± 8</td>
<td>0.106</td>
<td>73 ± 5</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>14 ± 5</td>
<td>41 ± 4</td>
<td>39 ± 6</td>
<td>0.386</td>
<td>15 ± 6</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>LTPA (h:min/week)</td>
<td>-</td>
<td>2:22 ± 1:02</td>
<td>2:33 ± 1:19</td>
<td>0.691</td>
<td>5:01 ± 1:35</td>
<td>5:25 ± 2:28</td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Hb] (g/L)</td>
<td>140 ± 6</td>
<td>132 ± 9</td>
<td>134 ± 7</td>
<td>0.685</td>
<td>146 ± 9</td>
<td>149 ± 6</td>
</tr>
<tr>
<td>HbA&lt;sub&gt;c&lt;/sub&gt; (% (mmol/mol))</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.4 ± 0.9 (57 ± 10)</td>
<td>5.3 ± 0.2 (35 ± 3)</td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (L)</td>
<td>6.1 ± 0.7</td>
<td>4.2 ± 0.7</td>
<td>4.3 ± 0.7</td>
<td>0.743</td>
<td>5.9 ± 0.5</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>FVC (% of reference value)</td>
<td>108 ± 7</td>
<td>96 ± 8</td>
<td>103 ± 14</td>
<td>0.105</td>
<td>101 ± 8</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (L)</td>
<td>4.8 ± 0.6</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.5</td>
<td>0.623</td>
<td>4.6 ± 0.5</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (% of reference value)</td>
<td>101 ± 11</td>
<td>92 ± 8</td>
<td>95 ± 11</td>
<td>0.352</td>
<td>97 ± 10</td>
<td>97 ± 12</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (%)</td>
<td>79 ± 7</td>
<td>82 ± 6</td>
<td>80 ± 7</td>
<td>0.296</td>
<td>79 ± 6</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>CV function at seated rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>-</td>
<td>86 ± 12</td>
<td>79 ± 12</td>
<td>0.120</td>
<td>72 ± 9</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>-</td>
<td>73 ± 11</td>
<td>66 ± 12</td>
<td>0.073</td>
<td>79 ± 14</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>SV&lt;sub&gt;i&lt;/sub&gt; (mL/kg FFM)</td>
<td>-</td>
<td>1.33 ± 0.22</td>
<td>1.29 ± 0.25</td>
<td>0.655</td>
<td>1.08 ± 0.17</td>
<td>1.08 ± 0.08</td>
</tr>
<tr>
<td>Q̇ (L/min)</td>
<td>-</td>
<td>6.2 ± 0.8</td>
<td>5.2 ± 1.1</td>
<td>0.006</td>
<td>5.7 ± 0.9</td>
<td>5.5 ± 1.1</td>
</tr>
<tr>
<td>Q̇&lt;sub&gt;i&lt;/sub&gt; (mL/min/kg FFM)</td>
<td>-</td>
<td>114 ± 12</td>
<td>101 ± 18</td>
<td>0.071</td>
<td>78 ± 8</td>
<td>77 ± 8</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>-</td>
<td>116 ± 8</td>
<td>116 ± 17</td>
<td>0.979</td>
<td>126 ± 19</td>
<td>120 ± 19</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>-</td>
<td>82 ± 8</td>
<td>76 ± 10</td>
<td>0.078</td>
<td>77 ± 7</td>
<td>81 ± 12</td>
</tr>
</tbody>
</table>

Data are means ± SD. T1D, men with type 1 diabetes; CV function, cardiovascular function, SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure. For other abbreviations, see the Abbreviations section.

<sup>a</sup>Between-group difference is evaluated within a study; <sup>b</sup>n = 7.
Table 4  
Metabolic profile and cardiovascular risk factors of the subjects of Study II.

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 15)</th>
<th>Controls (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.4 ± 0.4 a</td>
<td>5.5 ± 0.6</td>
<td>0.814</td>
</tr>
<tr>
<td>Fasting insulin (μU/mL)</td>
<td>12.1 ± 6.9 a</td>
<td>10.0 ± 4.4</td>
<td>0.356</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0 ± 1.8 a</td>
<td>2.5 ± 1.3</td>
<td>0.442</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>0.043</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L) b</td>
<td>3.0 ± 0.8</td>
<td>3.0 ± 0.9</td>
<td>0.764</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.6 ± 0.7</td>
<td>4.7 ± 0.9</td>
<td>0.604</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>0.496</td>
</tr>
</tbody>
</table>

Data are means ± SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein. For other abbreviations, see the Abbreviations section.

a n = 14; b Log-transformed for statistical analysis due to nonnormally distributed data.

6.1.3 STUDY III

Diabetes duration was 15.0 ± 8.6 years (range: 6.0-28.0 years) in men with diabetes. Neither waist (89 ± 8 vs. 88 ± 8 cm, \( P = 0.776 \)) and hip (101 ± 7 vs. 97 ± 7 cm, \( P = 0.312 \)) circumferences nor waist-to-hip ratio (0.88 ± 0.07 vs. 0.90 ± 0.07, \( P = 0.542 \)) differed between men with and men without diabetes, respectively. Similar thickness of subcutaneous fat of the vastus lateralis muscle (8 ± 3 vs. 8 ± 3 mm, \( P = 0.984 \)) was measured in men with diabetes and controls, respectively. In addition, men with diabetes had higher HbA1c than controls (Table 3). At seated rest, HR, SV, SVi, \( \dot{Q} \), \( \dot{Qi} \) as well as systolic and diastolic arterial blood pressures were similar between men with diabetes and controls (Table 3).

The difference in absolute BV between men with and without diabetes failed to reach statistical significance (6810 ± 382 vs. 7269 ± 904 mL, respectively, \( P = 0.175 \)). However, when BV was scaled to FFM, men with diabetes had lower BV than controls (94.0 ± 5.8 vs. 103.0 vs. 5.0 mL/kg FFM, respectively, \( P = 0.004 \)).

6.1.4 STUDY IV

Table 5 presents the descriptive characteristics of the training groups both at baseline and after the 1-year training intervention: at baseline, the presented characteristics were similar between men with diabetes and controls. Training decreased waist-to-hip ratio in controls, increased [Hb] in men with diabetes, increased absolute FVC in both groups, increased FVC (% of reference value) in men with diabetes, decreased FEV1/FVC in controls, and decreased systolic arterial blood pressure at rest in controls. However, no Group×Time interactions were evident (Table 5).

In addition to the data in Table 5, thickness of subcutaneous fat of the vastus lateralis muscle (9 ± 4 vs. 13 ± 7 mm, \( P = 0.410 \)) as well as resting nocturnal HR (51 ±
Table 5  
Descriptive characteristics of the training groups of Study IV at baseline and after the intervention.

<table>
<thead>
<tr>
<th></th>
<th>T1D (n = 8)</th>
<th>Controls (n = 8)</th>
<th>P at Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.4 ± 6.3</td>
<td>34.3 ± 6.4</td>
<td>37.9 ± 7.1</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>10.5 ± 6.8</td>
<td>11.4 ± 6.9</td>
<td></td>
</tr>
<tr>
<td>(range: 4.0-24.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80 ± 11</td>
<td>80 ± 12</td>
<td>86 ± 13</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180 ± 11</td>
<td>180 ± 11</td>
<td>181 ± 6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 2.8</td>
<td>24.7 ± 3.1</td>
<td>26.3 ± 3.8</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>68 ± 10</td>
<td>67 ± 10</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16 ± 5</td>
<td>16 ± 6</td>
<td>21 ± 9</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>89 ± 6</td>
<td>88 ± 7</td>
<td>95 ± 11</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>95 ± 10</td>
<td>97 ± 5</td>
<td>98 ± 10</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.95 ± 0.07</td>
<td>0.91 ± 0.05</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Hb] (g/L)</td>
<td>144 ± 6</td>
<td>150 ± 8**</td>
<td>146 ± 3 c</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 ± 0.9 c</td>
<td>7.5 ± 1.1 c ‡</td>
<td>-</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>58 ± 10 c</td>
<td>59 ± 11 c ‡</td>
<td>-</td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (L)</td>
<td>5.5 ± 0.9</td>
<td>5.6 ± 0.9*</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>FVC (% of reference value)</td>
<td>97 ± 12</td>
<td>99 ± 11*</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>4.5 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>FEV₁ (% of reference value)</td>
<td>96 ± 13</td>
<td>96 ± 13</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>82 ± 7</td>
<td>79 ± 6</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>SAP at seated rest (mmHg)</td>
<td>133 ± 14</td>
<td>125 ± 16</td>
<td>128 ± 11</td>
</tr>
<tr>
<td>DAP at seated rest (mmHg)</td>
<td>86 ± 7</td>
<td>84 ± 14</td>
<td>79 ± 16</td>
</tr>
</tbody>
</table>

Data are means ± SD. T1D, men with type 1 diabetes; SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure. For other abbreviations, see the Abbreviations section.

a Between-group difference at baseline is evaluated with Group (T1D vs. CON) as a between-subjects factor; b Nonnormally distributed data: Nonparametric tests are used to compare the groups at Baseline (Mann-Whitney U) and Baseline vs. Post (Wilcoxon signed-rank); c n = 7.

* Significantly (P < 0.05) different from Baseline, ** Significantly (P < 0.01) different from Baseline, ‡ Significantly (P < 0.01) different from Controls.

9 vs. 47 ± 5 bpm, P = 0.251) were similar for men with diabetes and controls at baseline, respectively.

In men with diabetes, training decreased basal (baseline: 0.35 ± 0.19 IU/kg/d; change: -0.06 ± 0.06 IU/kg/d, ES = 1.0, moderate, P = 0.049) but not rapid-acting (baseline: 0.30 ± 0.09 IU/kg/d; change: 0.06 ± 0.07 IU/kg/d, P = 0.114) nor total
(baseline: 0.65 ± 0.26 IU/kg/d; change: -0.01 ± 0.09 IU/kg/d, \( P = 0.867 \)) daily insulin doses.

Reference group, which did not participate in the exercise training intervention but underwent the same clinical experiments as the training groups, was 28.6 ± 1.0 years old, and hence, younger than the training group without diabetes (\( P = 0.017 \)). Changes in anthropometry, hematology, or flow-volume spirometry were not observed in Reference group after one year (\( P > 0.05 \)).

### 6.2 Oxygen Delivery and Utilization During Acute Dynamic Exercise

#### 6.2.1 Responses to CPET in Healthy Men (Study I)

The data on speed and cardiopulmonary responses at peak exercise in the subjects of Study I are presented as part of Table 6. In addition, PETCO₂ and RER at peak exercise were 37.2 ± 4.9 mmHg and 1.09 ± 0.04, respectively.

The patterns of local (de)oxygenation during incremental exercise are presented in Figure 12. In terms of exercise-induced change in \( \Delta[\text{HHb}] \) from baseline walking to peak exercise, \( \Delta[\text{HHb}] \) increased 12.2 ± 5.8 \( \mu \text{mol/L} \) in leg muscle, 21.9 ± 12.7 \( \mu \text{mol/L} \) in arm muscle, and 6.2 ± 2.4 \( \mu \text{mol/L} \) in cerebral tissue. This implies that deoxygenation was greatest in arm muscle, then in leg muscle, and least in cerebral tissue (arm vs. leg, \( P < 0.01 \); arm vs. cerebral, \( P < 0.001 \); leg vs. cerebral, \( P < 0.001 \)).

An example of NIP determination is illustrated in Figure 13. For the 22 subjects of Study I, a total of 24 NIPs were observed in leg muscle, 36 in arm muscle, 64 in cerebral tissue. Of the 24 NIPs observed in leg muscle, seven reflected oxygenation (i.e., \( \Delta[\text{O}_2\text{Hb}] \uparrow, \Delta[\text{HHb}] \downarrow, \) and TSI \( \uparrow \)), one was indefinable (e.g., (\( \Delta[\text{O}_2\text{Hb}] \downarrow, \Delta[\text{HHb}] \downarrow \)), and 16 reflected deoxygenation (i.e., \( \Delta[\text{O}_2\text{Hb}] \downarrow, \Delta[\text{HHb}] \uparrow, \) and TSI \( \downarrow \)). In arm muscle, six NIPs reflected oxygenation and 30 reflected deoxygenation. In cerebral tissue, 63 NIPs reflected deoxygenation, while just one was indefinable.

The data on six tissue-specific NIPs being the closest to AT and RC are presented in Table 7. Table 7 reveals no differences between the NIPs closest to AT and AT (\( P > 0.05 \)). In terms of the NIPs closest to RC, NIP\(_{\text{LegRC}} \) did not differ from RC (\( P = 0.058 \)), whereas NIP\(_{\text{ArmRC}} \) (vs. RC, \( P < 0.001 \)) and NIP\(_{\text{CerRC}} \) (vs. RC, \( P < 0.05 \)) did.

The NIP reflecting acceleration of arm deoxygenation was observed in 10 subjects during last two minutes of CPET (63 ± 42 s before exhaustion). In nine of these subjects, an accelerated rise also occurred in \( \dot{V}_E \) 60 ± 72 s before the observed acceleration of arm deoxygenation.

Furthermore, a positive association (\( r = 0.64, P = 0.001 \)) between the extent of exercise-induced leg muscle deoxygenation (i.e., exercise-induced change in \( \Delta[\text{HHb}] \)) from baseline walking to peak exercise) and tHb-mass was found. By contrast, no such association was evident in arm muscle or cerebral tissue.
Table 6  Speed or work rates, and pulmonary and cardiovascular responses at peak exercise in Studies I-III.

<table>
<thead>
<tr>
<th>STUDY I (men)</th>
<th>STUDY II (women)</th>
<th>STUDY III (men)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n=22)</td>
<td>Controls (n=15)</td>
<td>Controls (n=10)</td>
</tr>
<tr>
<td>Speed (km/h)</td>
<td>14.9 ± 1.2</td>
<td>168 ± 22</td>
</tr>
<tr>
<td>Work rate (W)</td>
<td>-</td>
<td>177 ± 37</td>
</tr>
<tr>
<td>Work rate (W/kg FFM)</td>
<td>-</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>V̇O₂ (L/min)</td>
<td>3.95 ± 0.46</td>
<td>2.22 ± 0.27</td>
</tr>
<tr>
<td>V̇O₂ (mL/min/kg)</td>
<td>50 ± 6</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>V̇O₂ (mL/min/kg FFM)</td>
<td>58 ± 7</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>V̇E (L/min)</td>
<td>138 ± 18</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>93 ± 3</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>CaO₂ (mL O₂/L blood)</td>
<td>172 ± 8</td>
<td>172 ± 13</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>190 ± 7</td>
<td>179 ± 12</td>
</tr>
<tr>
<td>EDV (mL)</td>
<td>-</td>
<td>135 ± 28</td>
</tr>
<tr>
<td>EDV (mL/kg FFM)</td>
<td>-</td>
<td>2.46 ± 0.57</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>-</td>
<td>95 ± 13</td>
</tr>
<tr>
<td>SV (mL/kg FFM)</td>
<td>-</td>
<td>1.74 ± 0.32</td>
</tr>
<tr>
<td>EF (%)</td>
<td>-</td>
<td>72 ± 10</td>
</tr>
<tr>
<td>Q (L/min)</td>
<td>-</td>
<td>17.0 ± 2.2</td>
</tr>
<tr>
<td>Q (mL/min/kg FFM)</td>
<td>-</td>
<td>310 ± 55</td>
</tr>
<tr>
<td>QaO₂ (mL/min)</td>
<td>-</td>
<td>2927 ± 451</td>
</tr>
<tr>
<td>QaO₂ (mL/min/kg FFM)</td>
<td>-</td>
<td>53 ± 11</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>-</td>
<td>176 ± 18</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>-</td>
<td>83 ± 17</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>-</td>
<td>114 ± 15</td>
</tr>
<tr>
<td>SVR (mmHg/L/min)</td>
<td>-</td>
<td>6.8 ± 14</td>
</tr>
<tr>
<td>SVR (mmHg/L/min/kg FFM)</td>
<td>-</td>
<td>383 ± 99</td>
</tr>
<tr>
<td>C(a-v)O₂ (mL O₂/L blood)</td>
<td>-</td>
<td>132 ± 16</td>
</tr>
</tbody>
</table>

Data are means ± SD. %-diff, between-group difference in %; T1D, men with type 1 diabetes; QaO₂, systemic O₂ delivery; QaO₂, systemic O₂ delivery index; SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure. For other abbreviations, see the Abbreviations section.

* Significant between-group differences are quantified within a study; **PCOS (Study II) or T1D (Study III) are compared with Controls within a study; c n = 19; d n = 7.
Table 7  Data on the six tissue-specific NIRS inflection points being the closest to anaerobic threshold (AT) or respiratory compensation point (RC) (Study I). See text for interpretation.

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>VO₂ (mL/min/kg)</th>
<th>Subjects (n)</th>
<th>VO₂ (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>22</td>
<td>34 ± 5</td>
<td>RC</td>
</tr>
<tr>
<td>NIP₂₂LegAT</td>
<td>9</td>
<td>34 ± 5</td>
<td>NIP₂₂LegRC</td>
</tr>
<tr>
<td>NIP₂₂ArmAT</td>
<td>6</td>
<td>33 ± 3</td>
<td>NIP₂₂ArmRC</td>
</tr>
<tr>
<td>NIP₂₂CerAT</td>
<td>21</td>
<td>33 ± 4</td>
<td>NIP₂₂CerRC</td>
</tr>
</tbody>
</table>

Data are means ± SD. NIP₂₂LegAT, NIRS inflection point observed in leg muscle and being the closest to AT. The other five NIPs are named according to the same logic as NIP₂₂LegAT. For other abbreviations, see the Abbreviations section.

* Significantly (P < 0.05) different from RC, *** Significantly (P < 0.001) different from RC.

Figure 12  NIRS recordings from leg muscle (m. vastus lateralis; A-C), arm muscle (m. biceps brachii; D-F), and cerebral tissue (prefrontal cortex; G-I) during maximal incremental treadmill exercise (Study I). Relative concentration changes in deoxygenated (Δ[HHb]; A, D, G) and total hemoglobin (Δ[Hb]; C, F, I), and tissue saturation index (TSI; B, E, H) as a function of locomotion speed (n = 22). * Significantly (P < 0.05) different from baseline walking.
Figure 13  Example of the determination of NIRS inflection points (Study I). Relative concentration changes in oxygenated ($\Delta$[O$_2$Hb]) and deoxygenated hemoglobin ($\Delta$[HHb]), and tissue saturation index (TSI), as a function of time in cerebral tissue ($n = 1$). Baseline walking (5 km/h) begins at 180 s (dash-dot line). In this case, one NIRS inflection point (solid line) has been found between the start of locomotion at 8 km/h (dotted line) and the end of exercise (dashed line).
6.2.2 RESPONSES TO CPET IN WOMEN WITH VERSUS WITHOUT PCOS (STUDY II)

As regards Study II, the responses of the main components of the Fick principle (Equation 2) to maximal incremental cycling are illustrated in Figures 14A-C. Moreover, work rates as well as pulmonary and cardiovascular responses at peak exercise are detailed in Table 6.

A significant Group×Exercise interaction was found for \( \dot{V}O_2 \) \( (P = 0.014; \) Figure 14A). When scaled to FFM, both peak work rate and \( \dot{V}O_{2peak} \) were lower in PCOS women than in controls, whereas the differences in unscaled values failed to attain significance. At peak exercise, particularly RER (1.12 ± 0.05 vs. 1.12 ± 0.05, \( P = 0.845 \)) but also rating of perceived exertion (20 ± 1 vs. 19 ± 1, \( P = 0.346 \)) were similar between PCOS women and controls, respectively, indicating that both groups similarly made their maximal effort during CPET (56).

Table 6 shows how there were no differences between PCOS women and controls in variables comprising systemic \( O_2 \) delivery at peak exercise. The groups attained equivalent peak levels of \( V_E \), and both SpO2 and CaO2 were similarly maintained in the groups throughout CPET. In addition, similar profiles of HR, SVi, \( \dot{Q} \)i (Figure 14B), MAP, and SVRi as a function of work rate were seen in PCOS women and controls throughout CPET, while no significant Group×Exercise interactions in these analyses were observed. Neither were there between-group differences in HR, EDV, EDVi, SV, SVi, EF, \( \dot{Q} \), or \( \dot{Q} \)i at peak exercise (Table 6). Accordingly, calculated systemic \( O_2 \) delivery and systemic \( O_2 \) delivery scaled to FFM were similar in PCOS women and controls at peak exercise (Table 6). Furthermore, peak CPO (4.3 ± 0.7 vs. 4.0 ± 0.8 W, \( P = 0.213 \)) and CPOi (0.08 ± 0.01 vs. 0.08 ± 0.01 W/FFM, \( P = 0.733 \)) were equivalent in PCOS women and controls, respectively. Systolic and diastolic arterial blood pressures, MAP, SVR, and SVRi were also similar in the groups (Table 6).

Hyperbolic responses of \( C(a-v)O_2 \) to CPET were seen in PCOS women and controls (Figure 14C). Group×Exercise interaction for \( C(a-v)O_2 \) (\( P = 0.075 \); Figure 14C) suggested that there might be true group effects at single work rates regarding \( C(a-v)O_2 \). Consequently, peak \( C(a-v)O_2 \) was lower in PCOS women than in controls (Table 6). Moreover, the \( \Delta\dot{Q}/\Delta\dot{V}O_2 \) slope was steeper in PCOS women than in controls (\( \beta = 5.84 \) vs. \( \beta = 5.21 \), \( P = 0.004 \); Figure 14D).

In a pooled population including both women with and without PCOS, an inverse correlation between \( \dot{V}O_{2peak} \) (mL/min/kg FFM) and HOMA-IR was observed, but it did not reach statistical significance (\( r = -0.29, P = 0.066 \)). By contrast, there was a significant correlation between \( \dot{V}O_{2peak} \) and HOMA-IR in the pooled population (\( r = -0.34, P = 0.037 \)), when \( \dot{V}O_{2peak} \) was scaled to body weight. When examined separately in PCOS women and controls, the correlations between \( \dot{V}O_{2peak} \) (mL/min/kg FFM) and HOMA-IR were not significant (\( r = -0.29, P = 0.155; r = -0.19, P = 0.247 \); respectively).
Figure 14  Responses of the main components of the Fick principle (i.e., pulmonary O$_2$ uptake [$\dot{V}O_2$] [A], cardiac output index [$Q$] [B], and systemic arterial-venous O$_2$ difference [C(a-v)O$_2$] [C]) to maximal incremental cycling (Study II), and linear regression lines showing mean responses of cardiac output ($\dot{Q}$) to increasing $\dot{V}O_2$ during maximal incremental cycling (D) (Study II). White triangles ($\Delta$) = PCOS women ($n = 15$), black triangles ($\triangle$) = controls ($n = 15$). Presented work rates include unloaded cycling, work rates accomplished by every subject, and mean peak work rate. The $P$ values refer to either the results of two-way repeated-measures ANOVA (A–C; * Post hoc significantly [$P < 0.05$] different from PCOS women) or the significantly different $\Delta Q/\Delta \dot{V}O_2$ slopes between PCOS women ($\beta = 5.84$) and controls ($\beta = 5.21$) (D). See Section 5.3 for further statistical details.

6.2.3 RESPONSES TO CPET IN MEN WITH VERSUS WITHOUT TYPE 1 DIABETES (STUDY III)

$\dot{V}O_2$ and cardiovascular responses to CPET in the subjects of Study III are illustrated in Figure 15. Additionally, the Study III data on work rates as well as pulmonary and cardiovascular responses at peak exercise are shown in Table 6.

Men with type 1 diabetes attained lower peak work rate and $\dot{V}O_2$peak than controls. Similar RER ($1.20 \pm 0.06$ vs. $1.16 \pm 0.03$, $P = 0.072$) between men with diabetes and controls, respectively, indicate that the groups similarly made their maximal effort
during CPET (56). Peak rating of perceived exertion was also similar between the groups (20 ± 1 vs. 19 ± 1, \( P = 0.698 \)).

The responses of the variables describing pulmonary function did not differ between men with diabetes and controls. Peak \( \dot{V}_E \) was equivalent in the groups (Table 6), in addition to which \( \text{SpO}_2 \) and \( \text{CaO}_2 \) were similarly maintained in both groups throughout CPET (Table 6, Figure 15B).

At peak exercise, men with diabetes had lower \( SV_i \) and \( \dot{Q}_i \) as well as higher \( SVR_i \) than controls. Thus, calculated systemic O2 delivery scaled to FFM was also reduced in the group with diabetes (Table 6). By contrast, the differences in peak HR, EDV, \( EDV_i \), SV, EF, \( \dot{Q} \), systemic O2 delivery (when expressed as mL/min), systolic and diastolic arterial blood pressures, MAP, and SVR between men with diabetes and controls failed to reach significance (Table 6). Similar hyperbolic responses of \( C(a-v)O_2 \) were seen in the groups throughout CPET (Figure 15H, Table 6).

BV (mL) correlated with peak EDV (\( r = 0.45 \), \( P = 0.036 \)), peak SV (\( r = 0.85 \), \( P < 0.001 \)), and peak \( \dot{Q} \) (\( r = 0.78 \), \( P < 0.001 \)) in a pooled population containing both men with and without diabetes. Peak EDV/BV (24 ± 5 vs. 23 ± 3 mL/L, \( P = 0.709 \)) and peak SV/BV (16 ± 2 vs. 17 ± 1 mL/L, \( P = 0.816 \)) quotients were similar for men with diabetes and controls, respectively. In men with diabetes, HbA1c correlated inversely with peak \( SV_i \) (Figure 16A) and \( \dot{Q}_i \) (Figure 16B), whereas the association between HbA1c and \( \dot{V}O_2\text{peak} \) (mL/min/kg FFM) missed significance (\( r = -0.54 \), \( P = 0.107 \)), and HbA1c had no association with BV scaled to FFM (\( r = 0.08 \), \( P = 0.433 \)).

Figure 17 illustrates the NIRS profiles of leg muscle deoxygenation and \( \dot{Q}_{VL} \). Leg muscle \%\( \Delta [HHb] \) increased from unloaded cycling towards high work rates, where it reached a plateau, with the exception of one patient with diabetes and four controls. Each subject attained the highest \%\( \Delta [HHb] \) value (i.e., 100%) at peak exercise. \%\( \Delta [HHb] \) at moderate work rates tended to be higher in men with diabetes than in controls, but no significant differences between the groups were observed for \%\( \Delta [HHb] \) at any work rate. \( \dot{Q}_{VL} \) rose significantly in both groups from unloaded cycling towards high work rates; in detail, \( \dot{Q}_{VL} \) was similar between the groups at submaximal work rates but lower in men with diabetes than in controls at peak exercise (0.19 ± 0.02 vs. 0.22 ± 0.02, respectively, \( P = 0.042 \)).

Peak \( \dot{Q}_{VL} \) correlated positively with peak \( \dot{Q} \) in controls (\( r = 0.74 \), \( P = 0.008 \)) but not in men with diabetes (\( r = 0.27 \), \( P = 0.281 \)). In addition, HbA1c tended to correlate inversely with peak \( \dot{Q}_{VL} \) in men with diabetes (Figure 16C).
Figure 15  Pulmonary and cardiovascular responses to maximal incremental cycling (Study III). Pulmonary O₂ uptake (V̇O₂) (A), arterial O₂ content (CaO₂) (B), end-diastolic volume index (EDVi) (C), stroke volume index (SV) (D), heart rate (HR) (E), cardiac output index (Q̇) (F), systemic vascular resistance index (SVR) (G), and systemic arterial-venous O₂ difference (C(a-v)O₂) (H). White triangles (Δ) = men with type 1 diabetes (n = 7), black triangles (▲) = controls (n = 10). Presented work rates include seated rest, unloaded cycling, work rates performed by each subject, and mean peak work rate. * Significant (P < 0.05) difference between men with type 1 diabetes and controls.
Figure 16  Associations between glycosylated hemoglobin A1c (HbA1c) and stroke volume index (SVi) (A), HbA1c and cardiac output index (\(\dot{Q}_i\)) (B), and HbA1c and local microvascular blood flow in the vastus lateralis muscle (\(\dot{Q}_{VL}\)) (C) in men with type 1 diabetes (Study III).

Figure 17  NIRS recordings from leg muscle (m. vastus lateralis) during maximal incremental cycling (Study III). Normalized relative concentration changes in deoxygenated hemoglobin (%\(\delta[Hb]\)) (A) and microvascular blood flow (\(\dot{Q}_{VL}\)) (B) as a function of work rate. White triangles (\(\Delta\)) = men with type 1 diabetes (n = 7), black triangles (\(\▲\)) = controls (n = 10). Presented work rates include seated rest, unloaded cycling, work rates performed by each subject, and mean peak work rate. * Significant (\(P < 0.05\)) difference between men with type 1 diabetes and controls, ‡ Significantly (\(P < 0.01\)) different from unloaded cycling, § Significantly (\(P < 0.001\)) different from unloaded cycling.
Table 8 shows that in Study IV 1) exercise training was similar in the two training groups in terms of frequency, duration, energy expenditure, modes, and intensity; 2) training was on average composed of 3-4 endurance training sessions and one resistance training session per week; 3) average training intensity was moderate (463); and 4) no progression in training was observed within the two training groups.

At baseline before the training intervention, work rates as well as pulmonary and cardiovascular responses at peak exercise, including $\dot{V}O_{2\text{peak}}$ (L/min: $P = 0.451$; mL/min/kg: $P = 0.974$; mL/min/kg FFM: $P = 0.120$), were similar between men with type 1 diabetes and healthy controls ($P > 0.05$), apart from peak systolic arterial blood pressure, which was higher in men with diabetes (Table 9). Training elevated peak work rates and $\dot{V}O_{2\text{peak}}$ in both groups. Particularly, $\dot{V}O_{2\text{peak}}$ (mL/min/kg FFM) rose 10% ± 7% in men with diabetes (ES = 1.4, large, $P = 0.004$) and 8% ± 9% in controls (ES = 0.89, moderate, $P = 0.045$). In addition, peak O2 pulse increased 10% ± 11% in men with diabetes (ES = 0.91, moderate, $P = 0.032$) and 11% ± 10% in controls (ES = 1.1, moderate, $P = 0.018$). Group×Time interactions were not significant for any peak responses, whereas MAP at peak exercise was overall higher in men with diabetes ($P = 0.016$ for the Group effect).

In men with diabetes, the magnitude of training-induced changes in work rates or pulmonary or cardiovascular responses at peak exercise had no associations with different characteristics of exercise training ($P > 0.05$) (variables in Tables 8 and 9 were examined). By contrast, the associations were consistent in healthy controls: percentual change ($\Delta$) in peak work rate (W) vs. frequency ($r = 0.77, P = 0.027$), $\Delta$peak work rate (W) vs. duration ($r = 0.78, P = 0.022$), $\Delta$peak work rate (W/kg FFM) vs. duration ($r = 0.71, P = 0.048$), $\Delta$peak O2 pulse vs. endurance training frequency ($r = 0.83, P = 0.043$), $\Delta$peak O2 pulse vs. duration ($r = 0.72, P = 0.044$). The training-elicited changes in work rates or pulmonary or cardiovascular responses were not significantly associated with baseline $\dot{V}O_{2\text{peak}}$ (both groups), baseline HbA1c (men with diabetes), training-induced change in HbA1c (men with diabetes), or diabetes duration ($P > 0.05$).

At baseline, leg muscle $\%\Delta[\text{HHb}]$ and $\Delta[\text{HHb}]$ at any given work rate were similar in men with diabetes and controls ($P > 0.05$) (Figure 18). Figures 18A-B show that training decreased $\%\Delta[\text{HHb}]$ at submaximal work rates in controls but not in men with diabetes, and one significant Group×Time interaction was observed for $\%\Delta[\text{HHb}]$ at 80 W. Within-group changes and Group×Time interaction for $\%\Delta[\text{HHb}]$ at peak exercise were not significant ($P > 0.05$). Figures 18C-D demonstrate that training increased $\Delta[\text{HHb}]$ at peak exercise in controls (ES = 0.48, small, $P = 0.039$) but not in men with diabetes, while significant and borderline significant Group×Time interactions were observed for $\Delta[\text{HHb}]$ at 160 W and peak exercise, respectively.

Reference group had higher $\dot{V}O_{2\text{peak}}$ at baseline (54 ± 10 mL/min/kg FFM) than men with diabetes ($P = 0.024$). $\dot{V}O_{2\text{peak}}$ did not change (+3% ± 6%, $P = 0.245$), no within-group changes in peak work rates or pulmonary or cardiovascular responses were observed, and no evident changes in $\%\Delta[\text{HHb}]$ or $\Delta[\text{HHb}]$ were seen at any work rate in Reference group ($P > 0.05$). Based on the Reference group data, a typical
percentage error for \( \text{V}O_2\text{peak} \) (mL/min/kg FFM) was 2.9% ± 3.1% (451). Overall, both these CPET data as well as the earlier presented data on anthropometry, hematology, and spirometry reflect the repeatability of the methods employed in this study.

### Table 8  
**Exercise training performed per month by the training groups during the intervention in Study IV.**

<table>
<thead>
<tr>
<th></th>
<th>T1D (n = 8)</th>
<th>Controls (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total volume</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (training sessions/month)</td>
<td>16 ± 4</td>
<td>18 ± 4</td>
<td>0.454</td>
</tr>
<tr>
<td>Duration (h:min/month)</td>
<td>16:58 ± 6:07</td>
<td>16:52 ± 4:39</td>
<td>0.967</td>
</tr>
<tr>
<td>Energy expenditure (kcal/month)</td>
<td>7759 ± 4540</td>
<td>7762 ± 3812</td>
<td>0.462</td>
</tr>
<tr>
<td><strong>Mode</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endurance training frequency (sessions/month)</td>
<td>13 ± 4</td>
<td>15 ± 6 a</td>
<td>0.317</td>
</tr>
<tr>
<td>Resistance training frequency (sessions/month)</td>
<td>3 ± 1</td>
<td>3 ± 3 a</td>
<td>0.927</td>
</tr>
<tr>
<td><strong>Intensity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean heart rate (bpm)</td>
<td>122 ± 12</td>
<td>122 ± 6</td>
<td>0.994</td>
</tr>
<tr>
<td>Mean heart rate (% of HRR)</td>
<td>57 ± 5</td>
<td>55 ± 8</td>
<td>0.553</td>
</tr>
<tr>
<td><strong>Progression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The 1st third of the intervention:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure (kcal/month) b</td>
<td>7749 ± 4583</td>
<td>7206 ± 2179</td>
<td>0.529</td>
</tr>
<tr>
<td>The 2nd third of the intervention:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure (kcal/month) b</td>
<td>6975 ± 3935</td>
<td>8592 ± 5396</td>
<td>0.401</td>
</tr>
<tr>
<td>The 3rd third of the intervention:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy expenditure (kcal/month) b</td>
<td>8610 ± 5637</td>
<td>7342 ± 4408</td>
<td>0.753</td>
</tr>
</tbody>
</table>

Data are means ± SD. T1D, men with type 1 diabetes; HRR, heart rate reserve.

a n = 6 (two subjects in Controls did not report their exercise modes), b Nonnormally distributed data: Nonparametric Mann-Whitney U test is used to compare the groups.
### Table 9
**Effects of the training intervention on work rates and pulmonary and cardiovascular responses at peak exercise in Study IV.**

<table>
<thead>
<tr>
<th></th>
<th>T1D (n = 8)</th>
<th>Controls (n = 8)</th>
<th>P for Group×Time a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
</tr>
<tr>
<td>Work rate (W) b</td>
<td>237 ± 34</td>
<td>254 ± 27*</td>
<td>255 ± 17</td>
</tr>
<tr>
<td>Work rate (W/kg FFM)</td>
<td>3.5 ± 0.4</td>
<td>3.9 ± 0.5**</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>VO₂ (L/min)</td>
<td>3.04 ± 0.60</td>
<td>3.27 ± 0.53*</td>
<td>3.22 ± 0.24</td>
</tr>
<tr>
<td>VO₂ (mL/min/kg)</td>
<td>38 ± 4</td>
<td>41 ± 3**</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>VO₂ (mL/min/kg FFM)</td>
<td>45 ± 5</td>
<td>49 ± 6**</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>V̇E (L/min)</td>
<td>130 ± 33</td>
<td>131 ± 31</td>
<td>143 ± 26</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>96 ± 1 c</td>
<td>97 ± 2 c</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>CaO₂ (mL O₂/L blood)</td>
<td>186 ± 10 c</td>
<td>196 ± 13 c **</td>
<td>185 ± 4 c</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>175 ± 11</td>
<td>173 ± 9</td>
<td>184 ± 12</td>
</tr>
<tr>
<td>O₂ pulse (mL/beat)</td>
<td>18 ± 4</td>
<td>19 ± 3</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>O₂ pulse, (mL/beat/kg FFM)</td>
<td>0.26 ± 0.03</td>
<td>0.28 ± 0.03*</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>216 ± 16 d</td>
<td>217 ± 26 d</td>
<td>193 ± 15 c †</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>113 ± 36 d</td>
<td>101 ± 24 d</td>
<td>100 ± 15 c</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>147 ± 26 d</td>
<td>140 ± 10 d</td>
<td>131 ± 11 c</td>
</tr>
</tbody>
</table>

Data are means ± SD. T1D, men with type 1 diabetes; SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure. For other abbreviations, see the Abbreviations section.

a Between-group difference in change from baseline to post is evaluated with Group (T1D vs. Controls) as a between-subjects factor and Time (Baseline, Post) as a within-subject factor; b Nonnormally distributed data: Nonparametric tests are used to compare the groups (Mann-Whitney U) and Baseline vs. Post (Wilcoxon signed-rank), and use of repeated-measures ANOVA is inappropriate; c n = 7; d n = 6.

* Significantly (P < 0.05) different from Baseline, ** Significantly (P < 0.01) different from Baseline, † Significant (P < 0.05) difference between T1D and Controls.
Figure 18 NIRS recordings from leg muscle (m. vastus lateralis) during maximal incremental cycling (Study IV). Normalized relative (%Δ[HHb]) and relative (Δ[HHb]) concentration changes in deoxygenated hemoglobin as a function of work rate in men with type 1 diabetes (T1D) (A, C; squares; n = 8) and controls (B, D; circles; n = 8) at Baseline (white plots) and after the 1-year training intervention (= Post; black plots). Presented work rates include unloaded cycling, work rates accomplished by all subjects, and mean peak work rate. * Significant (P < 0.05) within-group difference between Baseline and Post, ** Significant (P < 0.01) within-group difference between Baseline and Post.

6.3 STATISTICAL POWER

In Study II, post hoc calculation provided statistical power of 84.5% (two-tailed test) or 90.8% (one-tailed test) (alpha <5%) to detect the between-group difference in $\dot{V}O_{2}\text{peak}$ (40 ± 6 vs. 46 ± 5 mL/min/kg FFM in PCOS women vs. controls) (466). Similarly, post hoc calculation in Study III provided statistical power of 87.1% (two-tailed test) or 92.6% (one-tailed test) (alpha <5%) to detect the difference in $\dot{V}O_{2}\text{peak}$ (47 ± 5 vs. 56 ± 7 mL/min/kg FFM in men with type 1 diabetes vs. controls) (466).
In Study IV, post hoc calculation demonstrated that at least six subjects per training group were needed to obtain statistical power of 80% for the observed training-induced change in $\dot{V}O_2\text{peak}$ ($\Delta\dot{V}O_2\text{peak} = +9\% \pm +8\%$ in a pooled population [i.e., all 16 trained men included in the eventual analyses], two-tailed alpha <5%) (467).
7 DISCUSSION

This thesis comprises four original studies examining integrated components of O2 delivery and utilization during acute dynamic exercise in both healthy individuals and individuals with PCOS or type 1 diabetes. The adaptations induced by long-term exercise training have also been evaluated in individuals with type 1 diabetes and compared with those in individuals without diabetes.

In conclusion, while integrated data on local and whole-body responses to maximal incremental treadmill exercise in healthy adults were provided by Study I, performing maximal incremental cycling exercise revealed early signs of cardiovascular dysfunction related to PCOS and type 1 diabetes in Studies II and III, respectively. Moreover, long-term adherence to exercise training was particularly found to induce similar improvements in cardiorespiratory fitness in adults with and without type 1 diabetes in Study IV. However, according to the results of Study IV, long-term exercise training as such resulted in deficient adaptations of active muscle microvasculature and was not able to improve glycemic control in individuals with type 1 diabetes.

The following Sections 7.1-7.4 explore the details and implications of the findings of Studies I-IV and particularly do so from a perspective of the integrated pathway for O2 from the atmosphere to the mitochondria. Section 7.5 provides a methodological discussion of the thesis, and Section 8 summarizes the main conclusions of the study overall. Section 9 eventually discusses future directions.

7.1 LOCAL TISSUE-SPECIFIC IMBALANCE BETWEEN OXYGEN DELIVERY AND UTILIZATION AS PART OF WHOLE-BODY RESPONSE TO ACUTE DYNAMIC EXERCISE (STUDY I)

Study I was the first study to simultaneously examine alveolar gas exchange and local O2 delivery and utilization within active muscle, less active muscle, and cerebral tissues during maximal incremental treadmill exercise. As tHb-mass of the study subjects was also determined, the setting of Study I overall provided a possibility to interpret integrated coupling of some key adjustments of whole-body systemic O2 delivery and local tissue-specific imbalance between O2 delivery and utilization during acute dynamic exercise. Regarding the NIRS data of Study I (as well as those of Studies III and IV), it is notable that while local tissue (de)oxygenation depends on SpO2, tissue blood volume and flow, and metabolic demand of a tissue of interest, SpO2 remained relatively constantly between 95% and 100% at submaximal intensities and averaged no less than 93% (i.e., reflecting mild-to-moderate EIAH) at peak exercise. This suggests that any changes observed in the NIRS data mainly resulted from alterations in tissue blood volume and flow and in local tissue-specific O2 extraction and utilization. The findings of Study I are mainly applicable to healthy, relatively fit adult men.

Leg muscle (de)oxygenation patterns observed in Study I are in accordance with previous studies that have investigated incremental treadmill exercise (468,469): At
the onset of baseline walking, ∆[HHb] decreased below and TSI increased above standing levels. These immediate onset responses reflect the expulsion of pooling venous blood towards the heart as a result of the activated muscle pump (238,460). Subsequently at speeds of ≥8 km/h, ∆[HHb] and TSI gradually increased and decreased, respectively, with the increases in locomotion speed, active muscle O₂ demand, and active muscle imbalance between O₂ delivery and utilization. A plateau of ∆[HHb] near peak exercise was observed in 11 of 22 subjects. Parallel to ∆[HHb] and TSI responses, after decreasing at the onset of exercise due to the muscle pump activation, ∆[tHb] increased at speeds of ≥8 km/h, reflecting increased leg muscle blood volume. However, ∆[tHb] decreased at high intensities (≥12 km/h) in agreement with previous findings (468), likely primarily reflecting sympathetically mediated vasoconstriction within the active muscle microvasculature. In this regard, it is of critical importance to realize that ∆[tHb] is not an estimate of blood flow but just local blood volume; hence, the observed decrease in leg ∆[tHb] at high intensities does not directly oppose the findings of earlier reports (175-177) or Study III, according to which active muscle microvascular blood flow actually seems to accelerate near peak cycling exercise.

When it comes to NIPs observed in active leg muscle, two main findings emerged: First, the observed NIPs coincided with AT and/or RC, and second, both NIP_{LegAT} and NIP_{LegRC} were manifested only in nine subjects. After the publication of Study I, several studies (182-184) have reported a consistent temporal coincidence of the onset of active muscle ∆[HHb] plateau and RC during maximal incremental cycling. This coincidence has been suggested to be evidence of a whole-body “metabolic boundary partitioning heavy from very heavy exercise domains”, the detection of which with NIRS technology might be of valuable practical importance (183,184). Simply put, these findings together with our results concerning leg NIPs would imply that metabolic changes within active muscle tissue are coupled with pulmonary function during incremental exercise. However, Boone et al. (470) have very recently concluded in their extensive review that despite a consistent correlation between active muscle ∆[HHb] plateau and RC, relatively large intra- and interindividual variability characterizes the findings in the field. As an example, our finding of NIP_{LegAT} and NIP_{LegRC} manifesting in only nine of the 22 subjects demonstrates this variability. Thus, it is currently unclear whether active muscle and whole-body responses to acute dynamic exercise are mechanistically linked and whether local (e.g., leg NIPs) and whole-body (e.g., RC) thresholds can be interpreted interchangeably (470).

Arm muscle (de)oxygenation profile was initiated similarly to the above-described leg muscle pattern: ∆[HHb] decreased below and TSI increased above standing levels at the onset of baseline walking likely due to arm movements and the concomitantly activated muscle pump. Thereafter, at speeds of ≥8 km/h, ∆[HHb] and TSI first increased and decreased, respectively, in a moderate manner, which was followed by accelerated changes during more strenuous exercise. These findings are consistent with previous findings made during incremental cycling (236). Interestingly, the extent of exercise-induced deoxygenation was much greater in arm muscle than in leg muscle or cerebral tissue, which likely highlights whole-body blood flow redistribution during acute dynamic exercise: at high exercise intensities, sympathetically mediated vasoconstriction diminishes blood flow in less active muscles (143,144) so that local O₂
extraction in such tissues needs to increase to guarantee their adequate O₂ utilization (41).

As regards NIPs observed in arm muscle, NIP_{ArmAT} coincided with AT but was evident only in six subjects, suggesting that arm muscle deoxygenation is hardly associated with pulmonary function at low exercise intensities. At higher intensities, NIP_{ArmRC} was observed later than RC. Moreover, a NIP reflecting accelerating arm deoxygenation was observed in 10 subjects during the last two minutes of exercise, and an accelerated rise of V̇ₚ occurred in nine of those 10 subjects—one minute before the deoxygenation acceleration. Ogata et al. (237) have accordingly observed that the magnitude of decrease in O₂ delivery to less active muscle is coupled to the magnitude of increase in hyperventilation during incremental cycling. Moreover, coupling between inactive arm muscle blood flow and respiratory muscle fatigue near peak cycling exercise has also been reported after the publication of Study I (144). Such associations have been suggested to be due to metabolite accumulation resulting from increased anaerobic metabolism and provoking both hyperventilation and decrease in O₂ delivery to less active muscle (237), likely as a result of increased sympathetic outflow (144). On a larger scale, these findings take us back to one of the two fundamental physiological challenges faced during acute dynamic exercise: as appropriate maintenance of MAP is always needed, sufficient blood flow to working locomotor but also respiratory muscles is guaranteed at the expense of blood flow and O₂ delivery to less active tissues (22,41,42).

Cerebral oxygenation response to maximal incremental treadmill exercise, reflected by cerebral TSI curve, was predictably rather similar to that observed previously during cycling (212): TSI increased from low intensities to the speed of 11 km/h and then started declining towards peak exercise. Meanwhile, Δ[HHb] increased exponentially along with running speed up to peak exercise after a short initial plateau, indicating an accelerating imbalance between prefrontal O₂ delivery and utilization. Cerebral deoxygenation, reflecting the above-mentioned cerebral O₂ imbalance, particularly accelerated after RC, demonstrated by NIP_{CerRC}, which followed RC. This finding has been repeated by Racinais et al. (471) after the publication of Study I. PETCO₂ (i.e., a surrogate for PaCO₂) began decreasing at RC due to hyperventilation as expected (60,64). While decreasing PaCO₂ is generally known to induce cerebral vasoconstriction (213), we observed no evidence of prefrontal vasoconstriction as Δ[tHb] just plateaued at high intensities. However, this does not indicate that cerebral blood flow would not have decreased in our subjects at high intensities: In fact, it has been shown that when cerebral blood flow and Δ[tHb] are measured simultaneously during incremental exercise, the former decreases while the latter remains elevated near peak exercise, which is likely due to a dominant contribution of increasing Δ[HHb] to the Δ[tHb] signal (472). Collectively, earlier studies have reported cerebral Δ[tHb] remaining elevated in trained individuals at peak exercise (212), whereas Bhambhani et al. (242) have shown a post-RC decrease in cerebral blood volume (i.e., Δ[tHb]) in less fit individuals. As discussed earlier in Section 2.1.2.3, this interindividual variability of Δ[tHb] levels at peak exercise may at least partly be explained by lower chemosensitivity and lower submaximal V̇ₚ of trained individuals, which attenuate PaCO₂ reduction at high intensities, thus resulting in less cerebral vasoconstriction (214).
Regarding tHb-mass and its links with the CPET data of Study I, we observed that a 1 g change in tHb-mass was associated with a 3.6 mL/min change in \( \dot{V}O_2^{\text{peak}} \), according with previous literature (129). tHb-mass also correlated with the extent of exercise-induced leg muscle deoxygenation (i.e., exercise-induced change in \( \Delta [HHb] \) from baseline walking to peak exercise). In other words, based on Equation 11, tHb-mass correlated with the extent of exercise-induced increase in optical density of leg muscle \( \Delta [HHb] \), as other variables of Equation 11 remain constant during any experiment period. Since an increase in tHb-mass is a well-known adaptation to endurance training (287), the described correlation is simply explained by associations between tHb-mass and local endurance training adaptations taking place in active muscles. First, structural vascular adaptations such as capillary proliferation (291) and luminal enlargement of arterioles (293) increase intramuscular surface for O\(_2\) diffusion and shorten diffusion distance. This leads to longer mean blood transit time (102), during which more O\(_2\) can be extracted (290) and diffused from the microvasculature to the active myocytes. Local active muscle O\(_2\) extraction may also be enhanced by training-induced decreases in blood flow heterogeneity (290). Second, endurance training also increases the oxidative capacity of the skeletal musculature via increases in mitochondrial content (296,473) and activities of mitochondrial oxidative enzymes (296,298). These vascular and mitochondrial adaptations together enable the attainment of higher exercise-induced increase in optical density of leg muscle \( \Delta [HHb] \). Herewith, rather than having any independent contribution to the extent of exercise-induced leg muscle deoxygenation, tHb-mass simply reflects overall training adaptations and is thus strongly associated also with the vascular and mitochondrial adaptations. This hypothesis is supported by the finding that no associations between tHb-mass and the extent of deoxygenation were observed in arm muscle or cerebral tissue; adaptations to endurance training in these tissues are not as obvious as they are in active skeletal musculature.

Overall, Study I describes in healthy adult men how local imbalance between O\(_2\) delivery and utilization in different tissues seems to be physiologically linked to alveolar gas exchange (i.e., whole-body) responses to acute treadmill exercise. Importantly, the findings closely resembled those observed previously in cycling protocols. However, the existing evidence of associations between local tissue-specific (de)oxyg enation and whole-body exercise responses is overall somewhat variable (470). It therefore remains to be confirmed whether local and whole-body adjustments are mechanistically linked and whether local (i.e., NIPs) and whole-body (e.g., AT and RC) thresholds can be interpreted interchangeably (470). However, the findings of Study I for their part form a base for the following discussions of Studies II-IV, which examine integrated adjustments to acute dynamic exercise from a viewpoint of disease.

7.2 ALTERATIONS IN PERIPHERAL RATHER THAN SYSTEMIC ADJUSTMENTS TO ACUTE DYNAMIC EXERCISE IN PCOS (STUDY II)

No study before Study II has examined cardiac pump function during acute dynamic exercise in women with PCOS. Besides monitoring cardiac pump function, we also
determined subjects' [Hb], measured SpO₂, and hence, estimated CaO₂ along with alveolar gas exchange measurements during our maximal incremental cycling protocol. This enabled us to draw integrated (albeit only whole-body data-based) conclusions regarding potential early defects in organ systems responsible for O₂ delivery and utilization in overweight and obese PCOS women. The findings of Study II can mainly be applied to adult women with PCOS and excess weight but without clinically overt cardiovascular disease. In addition, hyperandrogenism did not have a major effect on the findings since PCOS women overall had relatively low androgen levels, and only 27% (4/15) of them had diagnostic signs of hyperandrogenism.

We observed 13% lower V̇O₂peak in PCOS women than in controls, while HOMA-IR did not differ significantly between the groups. An Italian research group has previously reported even 48-63% lower V̇O₂peak in overweight and obese women with PCOS than in age- and BMI-matched healthy women (31-33). Notably, while being normoglycemic, PCOS women in those studies have had almost two times higher fasting insulin level, hence much higher HOMA-IR, and therefore, a markedly worse metabolic profile than PCOS women in our study (31). Two smaller studies (34,35) have observed that if exhibiting a similar IR profile, obese women with PCOS might also have similar V̇O₂peak to that of age- and BMI-matched women without PCOS. As the magnitude of IR has also been shown to have a strong inverse association with V̇O₂peak in PCOS women (31), IR has been suggested to be the leading pathophysiological feature affecting V̇O₂peak in this patient group. However, V̇O₂peak has been scaled to body weight in all of these five studies (31-35). This dismisses body composition and may introduce a bias against overweight and obese women (456), as discussed later in Section 7.5. A negative correlation between FFM-adjusted V̇O₂peak and HOMA-IR did not reach statistical significance in our study. Instead, when V̇O₂peak was scaled to body weight, the correlation between V̇O₂peak and HOMA-IR in all subjects turned out to be significant. Taken together, it seems that the magnitude of IR is likely linked to V̇O₂peak in overweight and obese women with PCOS but the link becomes weaker when the confounding influence of adipose tissue is taken into account. This suggests that some pathophysiological features in addition to IR also have a significant effect on V̇O₂peak in women with PCOS. It must be highlighted, however, that such a concluding suggestion carries the caveat of IR being interpreted by HOMA-IR in previous studies (31,34,35) as well as in our Study II; when Stepto et al. (314) using the “gold standard” hyperinsulinemic-euglycemic clamp method reported that PCOS involves intrinsic syndrome-specific IR compared with well-matched women without PCOS, they did not observe a significant between-group difference in HOMA-IR.

Behind the reduced V̇O₂peak, we observed both reduced C(a-v)O₂ and a steeper $\Delta Q/\Delta VO_2$ slope in PCOS women when compared to controls. C(a-v)O₂ also tended to be lower in PCOS women than in controls throughout the incremental exercise until reaching the significant difference at peak exercise. While SpO₂ and CaO₂ were similarly maintained in the groups throughout the exercise, PvO₂ and CvO₂ at peak exercise must have been higher in PCOS women than in controls after first tending to be higher at submaximal intensities. These findings together indicate that PCOS women exhibited a pronounced response of $\dot{Q}$ to increasing O₂ utilization from the very
beginning of incremental exercise to compensate for alterations in peripheral adjustments to exercise.

Why would \( P_{\text{vO}_2} \) and \( C_{\text{vO}_2} \) be higher in PCOS women than in controls during acute dynamic exercise, thereby leading to the reduced \( \dot{V}_O^2_{\text{peak}} \)? While this question cannot be answered by Study II, the answer(s) must lie in derangements of peripheral \( O_2 \) delivery and/or utilization. Herewith, the possible answers can be hypothesized in light of the integrated pathway for \( O_2 \). First, endothelial dysfunction, which is an intrinsic feature of PCOS (332), has been shown to correlate with reduced exercise capacity in women (474). In disease, such a correlation may be due to impaired active muscle blood flow during exercise, resulting from reduced bioavailability of NO (333). In fact, particularly NO-mediated microvascular vasodilator dysfunction has recently been observed in PCOS (35). In addition, exercise training has been shown to enhance NO-mediated endothelial function with a parallel improvement in \( \dot{V}_O^2_{\text{peak}} \) in obese women with PCOS (35). Whether endothelial dysfunction is associated with IR status in PCOS remains to be elucidated (332), but endothelial dysfunction and IR do have a well-acknowledged reciprocal relationship in several disease states (334). Second, exercise-induced blood flow redistribution may also be pathologically affected by the autonomic nervous system dysfunction that is characteristic of PCOS: as sympathetic nerve activity has been concluded to be increased in PCOS (336), this might theoretically lead to inappropriate distribution of blood flow during exercise so that less active regions such as splanchnic regions and less active muscles would receive unnecessarily high amounts of flowing blood at the expense of active muscle blood flow. To demonstrate pronounced sympathetic outflow, Tekin et al. (335) reported an exaggerated systolic blood pressure response to maximal exercise in PCOS women. Contrarily, no such exaggeration was evident in our study, but MAP and SVRi were similar between women with and without PCOS. This suggests that vascular conductance at the whole-body level was not decreased in PCOS women. However, we cannot draw conclusions concerning exercise-induced redistribution of blood flow between active muscles and less active tissues, because local active muscle blood flow differs considerably from systemic circulatory responses during incremental exercise (175). Third and last, but not least, in conditions in which there are defects in mitochondrial oxidative metabolism, such as in specific myopathies, the findings of reduced \( \dot{V}_O^2_{\text{peak}} \), reduced \( C(a-v)O_2 \), and a pronounced \( \Delta \dot{Q}/\Delta \dot{V}_O^2 \) slope resemble our findings in PCOS women (196, 197). As already mentioned in Section 2.2.4.1, defects in skeletal muscle insulin signaling pathways (317, 318) and expression of genes involved in mitochondrial oxidative metabolism (319) have been observed in women with PCOS and pronounced IR. When present, such mitochondrial dysfunction could potentially reduce responsiveness to substrate and \( O_2 \) utilization within the skeletal musculature, which is evident in diabetes-related insulin-resistant metabolic derangements (337). However, evidence of potential mitochondrial dysfunction in PCOS is highly controversial since intact gene expression (338) and function (339, 340) of skeletal muscle mitochondria have also been found in women with the syndrome. Therefore, it is unclear whether any mitochondrial defect could reduce \( O_2 \) utilization and thus increase \( C_{\text{vO}_2} \) during exercise in PCOS women in the present study.

In terms of systemic \( O_2 \) delivery, alveolar gas exchange hardly sets any limitation as \( \text{SpO}_2 \) and \( \text{CaO}_2 \) were similarly maintained in women with and without PCOS.
throughout the incremental exercise. Furthermore, after exhibiting the pronounced response of $\dot{Q}$ to increasing $O_2$ demand, PCOS women ended up at similar peak levels of $\dot{Q}$ and $\dot{Q_i}$ when compared with controls. We also calculated CPO (and CPO$_i$), which particularly conveys the hydraulic power of the heart by relating changes in systemic blood flow and afterload (454); no differences between PCOS women and controls were observed for CPO or CPO$_i$ at peak exercise. While cardiac pump function during exercise in women with PCOS has never before been estimated, let alone measured, previous case-control echocardiographic studies have reported both diastolic (324-326) and systolic (326,327) dysfunction as well as normal cardiac pump function (328-330) at rest in women with PCOS. Moreover, there may be an inverse association between cardiac function and the severity of IR in PCOS since associations between cardiac dysfunction and IR have been observed in women with PCOS (324-327). Accordingly, Rees et al. (329) recently reported that diastolic dysfunction is linked to IR in overweight and obese women, but is not further contributed by PCOS itself. Thus, our finding of similar cardiac function at peak exercise in PCOS women and controls might be due to rather similar (and relatively low) HOMA-IR in the groups. These data overall indicate that while cardiac pump function has its vital role in the integrated pathway for $O_2$ (23-30), the cardiovascular system’s total ability to generate appropriate convective blood flow during increasing $O_2$ demand was not further diminished in PCOS women. In conclusion, our findings suggest that PCOS per se does not contribute to limitations of systemic $O_2$ delivery during exercise.

Two previous paragraphs can be distilled into Figure 19, which illustrates four different possibilities of how the observed reduction of $\dot{V}O_2$peak in PCOS women can be explained in light of the integrated model of $\dot{V}O_2$peak (see Section 2.1.3.2). Higher peak $P_vO_2$ and $C_vO_2$ can lead to reduced $\dot{V}O_2$peak in PCOS women via reduced muscle $O_2$ diffusional capacity (Figure 19A) or reduced mitochondrial metabolic capacity to utilize $O_2$ (Figure 19B). In addition, it is possible that both the peripheral derangements are present in PCOS, but either reduced diffusional capacity (Figure 19C) or reduced metabolic capacity (Figure 19D) finally determines the level of the $\dot{V}O_2$peak reduction. It is of note that no limitation of systemic $O_2$ delivery during exercise was observed in PCOS women, as already discussed.

In summary, the findings of Study II revealed reduced $\dot{V}O_2$peak, reduced peak C(a–v)$O_2$, and a steeper $\Delta\dot{Q}/\Delta\dot{V}O_2$ slope in overweight and obese PCOS women relative to well-matched control women without PCOS. In parallel, similar cardiac function at peak exercise was observed in the two groups, while Ca$O_2$ was also similarly maintained in the groups. These observations indicate that PCOS per se is linked to alterations in peripheral adjustments to exercise rather than to limitations of systemic $O_2$ delivery in overweight and obese women. From a clinical point of view, these findings provide important information. First, reduced $\dot{V}O_2$peak can be regarded as an independent early sign of overall cardiovascular dysfunction, and hence, have a negative impact on the prognosis of women with PCOS in terms of their cardiovascular morbidity (268,269). Second, such a reduction of exercise capacity/tolerance and the suggested peripheral derangements may indirectly further worsen the cardiovascular prognosis as well as the overall health of women with PCOS; they may hypothetically decrease adherence to regular exercise, which is highly recommended to be an essential part of healthy lifestyle of PCOS women, particularly in overweight and obese.
individuals (308,309). Third, the pronounced $\Delta \dot{Q}/\Delta \dot{VO}_2$ slope in PCOS women reflects pronounced exercise-induced stress on the heart, which may theoretically predispose women with PCOS to an increased risk of adverse cardiac events during exercise in the long term.

Figure 19 Different possibilities of how the observed reduction of peak pulmonary $O_2$ uptake ($\dot{VO}_{2peak}$; black circles [●]) in PCOS women can be explained in light of the integrated plots of $\dot{VO}_2$ against partial pressure of muscle venous $O_2$ ($PvO_2$). Notably, no limitation of systemic $O_2$ delivery during exercise was observed in PCOS women. Instead, higher $PvO_2$, reflecting higher venous $O_2$ content, at peak exercise and hence lower $\dot{VO}_{2peak}$ in PCOS women can be due to reduced muscle $O_2$ diffusional capacity (A), reduced mitochondrial metabolic capacity to utilize $O_2$ (B), or their combinations so that either reduced diffusional capacity (C) or reduced metabolic capacity (D) eventually determines the level of the $\dot{VO}_{2peak}$ reduction in these women. White circles (○) reflect how reduced metabolic capacity (C) or reduced diffusional capacity (D) may be present in PCOS, without determining $\dot{VO}_{2peak}$. 
7.3 BOTH SYSTEMIC AND PERIPHERAL CARDIOVASCULAR IMPAIRMENTS ARE MANIFESTED DURING ACUTE DYNAMIC EXERCISE IN TYPE 1 DIABETES (STUDY III)

The novelty of Study III resided in the simultaneous examination of both systemic and peripheral mechanisms contributing to \( \dot{V}O_2 \) response to acute dynamic exercise in patients with type 1 diabetes. Not only were the PhysioFlow method and NIRS employed to estimate cardiac pump function and active muscle responses during maximal incremental cycling, respectively, but also BV was measured by the CO-rebreathing method to examine its associations with cardiac responses to incremental exercise. This integrated setting provided a possibility to identify and specify potential early signs of cardiovascular dysfunction induced by type 1 diabetes. The results of Study III are primarily applicable to physically active adult men with relatively well-controlled type 1 diabetes and no clinically overt micro- or macrovascular complications.

Our finding of 16% lower \( \dot{V}O_{2\text{peak}} \) in men with diabetes than in controls is rather consistent with the ~20% lower \( \dot{V}O_{2\text{peak}} \) reported previously in adults with type 1 diabetes (236,375-378). However, the finding disagrees with studies (379-386) that have observed similar \( \dot{V}O_{2\text{peak}} \) in adults with and without type 1 diabetes. As already extensively discussed in Section 2.3.4.1, such a discrepancy is likely due to the effect of glycemic control on the integrated entity of \( \dot{V}O_{2\text{peak}} \): adults with type 1 diabetes are likely capable of attaining \( \dot{V}O_{2\text{peak}} \) similar to that of healthy adults, but this most likely depends on them maintaining good glycemic control. To at least weakly support this conclusion originally presented by Baldi and Hofman (36), a relatively high albeit statistically nonsignificant negative correlation coefficient between \( \dot{V}O_{2\text{peak}} \) and HbA1c (i.e., \( r = -0.54 \)) was observed in men with diabetes in our study.

Systemic O\(_2\) delivery may decrease \( \dot{V}O_{2\text{peak}} \) in type 1 diabetes due to impairments of both pulmonary (370,378,382,389) and cardiac (371,372,385,389) function. In addition, reduced blood O\(_2\) carrying capacity may impair systemic O\(_2\) delivery and hence \( \dot{V}O_{2\text{peak}} \) in type 1 diabetes (376). In Study III, the levels of SpO\(_2\) and CaO\(_2\) were well maintained in men with and without diabetes throughout the incremental exercise, suggesting that pulmonary function had no limiting effect on \( \dot{V}O_{2\text{peak}} \). As regards cardiac function, earlier studies have demonstrated reduced SV and \( Q \) in type 1 diabetes patients during both submaximal (371,372) and maximal (385,389) exercise. Accordingly, we also observed lower SV\(_i\) and lower \( Q_i \) at peak exercise in men with diabetes, indicating decreased cardiac performance. In addition, both peak SV\(_i\) and peak \( Q_i \) correlated inversely with HbA1c. The latter supports previous studies (371,384,385) that have reported associations between glycemic control and cardiac performance during exercise in type 1 diabetes as well as large registry studies (18,368) reporting pronounced heart failure risk in type 1 diabetes patients with poorer glycemic control.

Reduced SV and subsequently diminished overall cardiac function in diabetes are primarily due to diastolic, but possibly also systolic (particularly at later stages of the disease), dysfunction (15,16). While the overall pathogenesis of this diabetic cardiomyopathy phenomenon is poorly understood, the mechanisms behind the diabetes-specific diastolic dysfunction include impairments of isovolumic relaxation,
ventricular filling/preload, and ventricular compliance (15,16). In Study III, mean EDVi in men with diabetes was 9% lower at rest and on average 10% lower during exercise than in controls, agreeing with previous findings in adolescents with type 1 diabetes (371). However, the between-group differences in EDVi failed to reach statistical significance, which is discussed later in this section. Reduced diastolic function, associated with reduced BV, has previously been observed in adults with type 2 diabetes (392). Regarding BV, before hypothesizing about the contribution of reduced BV to decreased cardiac performance in diabetes, it must be noted here that specific mechanisms of diabetes-related reduction of BV are not only beyond the scope of this thesis but also largely unknown, and, in fact, reduced BV in patients with diabetes remains to be confirmed by larger population studies. However, we did observe a 9% lower BV scaled to FFM in men with diabetes than in controls, which is congruent with a previous finding from our laboratory (376). Thus, because BV also correlated positively with peak values of EDV, SV, and $\dot{Q}$, it is physiologically justified (86,289) to suggest that lower BV reduces reserves of ventricular preload, SV, and $\dot{Q}$ in type 1 diabetes. This emphasis on the reduced preload is further supported by our finding of similar peak EDV/BV and peak SV/BV ratios in men with and without diabetes. However, no association between HbA1c and BV scaled to FFM was observed in men with diabetes. This accords with our previous finding (376) and indicates that other more hyperglycemia-related components of diastolic function and SV (e.g., impaired ventricular relaxation and/or compliance) were also present in men with diabetes and led to the strong inverse associations between HbA1c and cardiac performance. Strict interpretation of the data of Study III would suggest that the combination of nonsignificantly reduced peak EDVi but significantly reduced peak SVi implies impaired peak systolic function in men with diabetes. However, peak EF was rather similar between the study groups and no evidence of reduced systolic function in men with diabetes was provided.

As mentioned above, the between-group differences in EDVi failed to reach statistical significance both at rest and during exercise. This may partly be due to the “derived nature” of the PhysioFlow method, but likely results mainly from diastolic dysfunction being evident only in ~30% of otherwise healthy type 1 diabetes adults (366,367); these two issues, combined with the relatively small sample size of Study III, probably led to the relatively high SD of the observed EDVi values. To interpret the missed statistical significance further, it must be remembered that the amount of diastolic filling is affected by HR (83). Reduced peak HR has occasionally been reported in type 1 diabetes (370,372,391) and is one component of reduced cardiac reserve in individuals with diabetes (396,397). Reduced peak HR might be explained by hypoglycemia-related reduction of β-adrenoceptor sensitivity (394) and be pronounced in patients with diabetes-related autonomic neuropathy (391). In Study III, no patient with diabetes had previous evidence of neuropathy. However, it is of note that only two patients with diabetes, but six controls, attained age-predicted HR (475) at peak exercise, leading to a statistically nonsignificant 6 bpm lower peak HR in men with diabetes.

Reduced $Q_{VL}$, independent of $\dot{Q}$, was observed in men with diabetes at peak exercise. Previously, reduced leg blood flow, independent of $\dot{Q}$, has been observed in patients with type 2 diabetes during submaximal exercise (476). In Study III, men with diabetes
also had higher SVRi and tended to have higher systolic arterial blood pressure at peak exercise than controls. It is well known that type 1 diabetes is characterized by vascular dysfunction manifested as endothelial dysfunction (399), reduced arterial compliance (374,400,401), decreased capillary-to-muscle fiber ratio (402,403), and impaired microvascular muscle blood flow and O2 diffusional capacity (403-405). Based on the combination of these previously reported derangements and our present findings, it can be suggested that in men with type 1 diabetes, peripheral vascular dysfunction independently limited blood flow and O2 delivery to the active skeletal musculature at peak exercise. Importantly, peak $\dot{Q}_{\text{VL}}$ tended to correlate inversely with HbA1c in men with diabetes and may thus depend on glycemic control.

Although peak blood flow, and thus, peripheral O2 delivery to active muscles were limited in men with diabetes, the C(a-v)O2 response was similar in men with and without diabetes throughout the exercise. This is congruent with previous findings in adolescents (372) and adult women (384) with type 1 diabetes and indicates that O2 extraction was not limited at the whole-body level for men with diabetes. The combination of reduced peak $\dot{Q}_{\text{VL}}$ but preserved peak C(a-v)O2 in men with diabetes might be explained by exaggerated fractional O2 extraction from microvascular blood flow; reductions of microvascular muscle blood flow (404) and microvascular O2 partial pressure (477) have been observed in type 1 diabetes rats at rest and suggest that fractional O2 extraction from the adjacent blood flow could be higher in active muscles of men with diabetes. In addition, as a sign of slower $\dot{V}O2$ kinetics, before attaining a similar steady-state level, microvascular O2 partial pressure has been observed to fall more rapidly and to far lower levels during 3-min electrical muscle stimulation in rats with type 1 diabetes than in healthy rats (477). These findings suggest that if fractional O2 extraction from the adjacent blood flow was higher in men with diabetes in our present study, it conceivably was so at rest and immediately after each transition from the previous work rate to the next one but not after attaining steady state. As we used a step incremental CPET protocol, which enables attaining steady state (up to AT) or at least getting close to steady state (after AT) at each submaximal work rate (452), it can be concluded that fractional O2 extraction was likely similar in the study groups during the last 30 s of each work rate. Thus, diabetes hardly affected the method used to quantify $\dot{Q}_{\text{VL}}$ at submaximal work rates. If diabetes affected the method at peak exercise (i.e., if fractional O2 extraction was higher in men with diabetes because of not attaining steady state before volitional exhaustion), it only led to underestimating of the between-group difference in peak $\dot{Q}_{\text{VL}}$, not to incorrect conclusions.

As was the case with Study II, all of the above-discussed findings of Study III can eventually be interpreted and summarized in light of the integrated model of $\dot{V}O2_{\text{peak}}$ (see Section 2.1.3.2). Such an interpretation is illustrated by Figure 20 and relies on the assumption that peak PvO2 in men with and without diabetes was rather similar at peak exercise. In this context, Figure 20 shows how lower $\dot{V}O2_{\text{peak}}$ in men with type 1 diabetes is due to both reduced O2 delivery to active muscles and reduced muscle O2 diffusional capacity. The latter may at least partly be reflected by the observed reduction of peak $\dot{Q}_{\text{VL}}$ in men with diabetes, but may also include some other unidentified factors such as lower subsarcolemmal mitochondrial content (303).
In conclusion, Study III showed that both systemic and peripheral cardiovascular impairments were manifested in physically active adult men with type 1 diabetes during maximal incremental cycling and led to lower \( \dot{V}O_{2\text{peak}} \) than in well-matched healthy men without any diabetes. Systemically, lower BV has potential to reduce capacities of ventricular preload, SV, and \( \dot{Q} \), thereby limiting \( O_2 \) delivery in individuals with diabetes. Peripherally, peak microvascular blood flow within active skeletal muscles was observed to be independently reduced in men with diabetes, thus further contributing to their reduced \( \dot{V}O_{2\text{peak}} \). Importantly, systemic and probably also peripheral limitations seem to be associated with HbA\(_{1c} \) in type 1 diabetes patients. While the early development of cardiac dysfunction in type 1 diabetes has already been shown to have significant clinical importance (18), the findings of Study III also have clinical importance in many ways and share many similarities with the implications of the findings of Study II. First, reduced \( \dot{V}O_{2\text{peak}} \) independently weakens the prognosis of patients with type 1 diabetes in terms of their cardiovascular health (19). Second, this reduction of exercise capacity/tolerance may indirectly further worsen the cardiovascular prognosis as well as the overall health of type 1 diabetes patients since it may hypothetically decrease adherence to regular exercise, which is recommended for all patients with the disease (39). In this context of promoting overall health, any additional cardiovascular limitation to exercise tolerance is problematic since type 1 diabetes itself already creates a barrier to physical activity via potential glycemic imbalance (478). Third, the observed associations between \( O_2 \) delivery and HbA\(_{1c} \) highlight the importance of maintaining good glycemic control.

![Figure 20](image)

**Figure 20** Reduced peak pulmonary \( O_2 \) uptake (\( \dot{V}O_{2\text{peak}} \); black circles [●]) in men with type 1 diabetes (T1D) explained in light of the integrated plots of \( \dot{V}O_{2} \) against partial pressure of muscle venous \( O_2 \) (PvO\(_2\)). It is first of note that peak PvO\(_2\) in the groups is rather similar reflecting the postulate according to which the study groups attained rather similar level of venous \( O_2 \) content at peak exercise. In this context, lower \( \dot{V}O_{2\text{peak}} \) in men with type 1 diabetes is due to both reduced \( O_2 \) delivery to active muscles (arrow 1) and reduced muscle \( O_2 \) diffusional capacity (arrow 2). Apart from the observed reduction of active muscle microvascular blood flow at peak exercise, other derangements contributing to reduced diffusional capacity in men with type 1 diabetes could not be specified. The white circle (○) reflects how reduced \( O_2 \) delivery to active muscles for its part decreases \( \dot{V}O_{2\text{peak}} \) in men with type 1 diabetes.
7.4 EFFECTS OF LONG-TERM EXERCISE TRAINING ON RESPONSES TO ACUTE DYNAMIC EXERCISE AND GLYCEMIC CONTROL IN TYPE 1 DIABETES (STUDY IV)

Performing laboratory experiments before and after one year of individualized exercise training intervention made Study IV the first study to examine training-induced adaptations after such a long training period in patients with type 1 diabetes. Previously, different cardiovascular and respiratory adaptations as well as glycemic control adaptations induced by training interventions of only six weeks to five months have been studied in this patient group (see Table 2). Simultaneous monitoring of alveolar gas exchange, SpO2, HR (and thus also peak O2 pulse), as well as continuous wave NIRS data during CPET enabled examination of training adaptations from an integrated viewpoint with a focus again particularly on different components of O2 delivery and utilization but also glycemic control. Chiefly, the observations of Study IV can be applied to nonathlete adult men with rather well-controlled type 1 diabetes and without signs of clinically overt micro- or macrovascular disease.

The existing evidence on the effects of exercise training on $\dot{V}O_2$peak, glycemic control, and thus cardiovascular morbidity in type 1 diabetes has been presented in depth in Section 2.3.4.2 and Table 2. Aerobic training interventions of six weeks to five months have consistently resulted in 5-27% increases in $\dot{V}O_2$peak in type 1 diabetes patients (407-414,432-439). Moreover, three (411,435,436) of those intervention studies have compared the extent of training-induced increases in $\dot{V}O_2$peak between individuals with and without type 1 diabetes and observed no differences in $\dot{V}O_2$peak improvements between the groups. Accordingly, Study IV also showed that $\dot{V}O_2$peak increased similarly in men with (+10%) and without (+8%) type 1 diabetes after long-term adherence to regular exercise training. In light of vast epidemiological evidence (19,275), this similar improvement of $\dot{V}O_2$peak in the groups for its part suggests that exercise training reduces a risk of cardiovascular complications to a similar extent whether one has type 1 diabetes or not. Regarding glycemic control, it has remained uncertain whether physical activity has any beneficial influence on HbA1c in type 1 diabetes (416-421). The findings of Study IV support the data of several earlier short-term intervention studies (407-410,412-414,433,436-439) and a meta-analysis (421) according to which such a glycemic benefit does not exist; HbA1c did not decrease in men with diabetes, although the duration of the training intervention was as long as one year. This finding further questions whether physical activity has potential benefits regarding the occurrence of micro- and macrovascular complications in type 1 diabetes since the magnitude of hyperglycemia is known to be a major risk factor for cardiovascular disease in type 1 diabetes (18,354,365,368). Regarding the unchanged HbA1c, it has been suggested that training-induced reduction of daily insulin requirements could partly contribute to the finding (415). This might also be the case in Study IV, as a daily dose of basal insulin decreased in men with diabetes as a result of the intervention. However, Study IV is a small study and certainly does not provide any “end-point data” regarding cardiovascular morbidity. Its findings still accord with previous literature and simply indicate that, in adults with type 1 diabetes, recreational-like training seems to have a similar influence on $\dot{V}O_2$peak to that in
individuals without any diabetes, and a reduction of HbA1c is not a prerequisite for or a consequence of an increase in \( \dot{V}O_{2\text{peak}} \).

Regarding different components of \( \dot{V}O_{2\text{peak}} \), endurance-type exercise training has been shown to improve cardiac pump function in animal models of type 1 diabetes (440,441). However, human studies examining training effects on cardiac pump function or, on the whole, systemic \( O_2 \) delivery in type 1 diabetes are sparse. In this context, only a single finding of a 10% increase in peak \( O_2 \) pulse (i.e., a surrogate for peak \( SV \) (20,123)) has been observed after three months of aerobic training in type 1 diabetes adults (412). Meanwhile, data on type 2 diabetes patients have shown both training-induced improvements (442,443) and no improvements (444,445) in myocardial function when assessed by echocardiography parameters. Peak \( O_2 \) pulse\( \i\)rose in a similar manner in men with (+10%) and without (+11%) type 1 diabetes in Study IV. This indirectly suggests a similar improvement in \( SV \), and thus, cardiac pump function in the groups (20,123). However, it must be noted that changes in different components of peak \( C(a-v)O_2 \) also affect changes in peak \( O_2 \) pulse (see Equation 7); in this regard, no statistically significant differences in adaptations of peak \( \text{SpO}_2 \), [Hb], and therefore, peak \( \text{Ca}_O_2 \) were observed between the groups in Study IV, whereas \( \text{CvO}_2 \) was not measured. Thus, acknowledging both the highly surrogate nature of peak \( O_2 \) pulse and also its inability to separate diastolic and systolic components of cardiac function, the data of Study IV suggest an equivalent net effect of the 1-year training intervention on peak systemic \( O_2 \) delivery in men with and without type 1 diabetes.

While the integrated nature of \( \dot{V}O_{2\text{peak}} \) makes \( \dot{V}O_{2\text{peak}} \) depend also on peripheral \( O_2 \) delivery and utilization, two findings of Study IV reflect peripheral vascular defects characteristic of type 1 diabetes (374,399-405). First, at peak exercise, MAP was pronounced in men with diabetes (i.e., significant Group effect), whereas the components of \( \dot{Q} \) (HR and \( SV \) estimated roughly by \( O_2 \) pulse) were similar in men with diabetes and healthy men. This suggests higher peak SVR in men with diabetes, hence consistent with the findings of Study III. Second, we observed that leg muscle \( \%\Delta[Hb] \) at submaximal work rates decreased in controls but not in men with diabetes, whereas \( \Delta[Hb] \) at peak exercise increased in controls but not in men with diabetes. These NIRS findings agree well with each other and can be interpreted as follows: \( \Delta[Hhb] \) at peak exercise increased in controls due to enhanced local microvascular \( O_2 \) extraction capacity, which resulted from previously summarized skeletal muscle (i.e., vascular and mitochondrial) adaptations to endurance training (see Section 2.1.4.2). This rise in peak \( \Delta[Hhb] \) was for its part reflected by decreases in submaximal \( \%\Delta[Hhb] \), which simply describes local \( O_2 \) extraction in relation to maximal extraction capacity. The training-induced increase in peak \( \Delta[Hhb] \) has previously been reported by both cross-sectional (182) and longitudinal (302) studies. However, it is a novel finding that such enhancement of local microvascular \( O_2 \) extraction capacity was totally absent in men with diabetes. While we have no data to explain this in more detail, it can be postulated that the finding must result from unidentified diabetes-related maladaptations regarding active muscle \( O_2 \) delivery and utilization. Previously, animal (402) and short-term training intervention (436) studies have showed that training-induced capillary proliferation is deficient in type 1 diabetes patients relative to individuals without diabetes. Thus, it is plausible that the longer 1-year training intervention of Study IV also led to such a deficient angiogenic
adaptation in men with diabetes, and thereby, to their unchanged muscle microvascular O₂ extraction. Theoretically, both deficient muscle fiber type transformation (from type IIb to IIa or even from IIa to I) and/or unaltered enzymatic mitochondrial capacity for oxidative metabolism could also explain the retained level of O₂ extraction (299). However, while neither of these two possible maladaptations can be interpreted by Study IV, at least the latter is highly unlikely since even poorly controlled type 1 diabetes patients have displayed a normal training response in mitochondrial enzyme activities (435,436). In any case, however, any maladaptations at the muscle level in men with diabetes were not sufficiently severe to prevent further improvements in exercise capacity (i.e., in peak work rate and VO₂peak).

Individually collected training data enabled the examination of associations between the characteristics (i.e., dose) and the outcomes (i.e., response) of training: While training dose was consistently associated with observed training responses in healthy men (e.g., the higher the training volume, the greater the increase in peak work rate), no such dose-response associations were evident in men with diabetes. This may highlight the need for greater individualization of exercise prescription in type 1 diabetes (39), although it is noteworthy that the intervention as such was beneficial also for men with diabetes despite the lack of obvious dose-response associations.

To sum up, one-year adherence to exercise training, on average consisting of 3-4 weekly endurance training sessions and one resistance training session performed at moderate intensity and for approximately one hour at a time, led to similar increases in VO₂peak in well-matched adult men with and without type 1 diabetes. This similarity was accompanied by equivalent improvements in peak O₂ pulse. On the contrary, training enhanced NIRS-derived active muscle microvascular O₂ extraction at peak exercise in controls but not at all in men with diabetes. Meanwhile, training led to no reduction of HbA1c in men with diabetes. These findings provide novel evidence of clinical importance. First, the similar increases in VO₂peak and peak O₂ pulse suggest that an equivalent long-term dose of exercise training reduces an overall risk for cardiovascular complications (19,275) and improves cardiac pump function (20,123), respectively, to a similar extent in adult men with and without type 1 diabetes. Second, however, the lack of improvements in active muscle microvascular O₂ extraction in men with type 1 diabetes suggests that even long-term training, to this extent, does not induce significant adaptations regarding active muscle microvascular O₂ delivery and utilization in men with diabetes. This maladaptation might be due to deficient capillary proliferation and may also be linked to contradictory evidence according to which physical activity either is (423) or is not (424) protective against microvascular complications in type 1 diabetes. Particularly in this context, it is important to note that while improved glycemic control has strongly been proved to decrease the risk of microvascular complications (354), even long-term training does not seem to reduce HbA1c in type 1 diabetes. Importantly, the lack of associations between training dose and responses in men with diabetes may highlight the need for better individualization of exercise regimens in type 1 diabetes (39).
7.5 METHODOLOGICAL STRENGTHS AND LIMITATIONS

Methodological strengths of this thesis include the use of the various setting of noninvasive methods, enabling the examination of O$_2$ delivery and utilization during acute dynamic exercise from an integrated viewpoint. Furthermore, matching the subject groups (i.e., PCOS women vs. controls [Study II] and men with type 1 diabetes vs. controls [Studies III and IV]) carefully for age, anthropometry, LTPA (Studies II and III), and baseline V$\dot{O}_{2peak}$ (Study IV) is a strength, minimizing the potential confounding effect of these factors on between-group comparisons. Hence, any observed between-group differences in Studies II-IV likely reflect disease-related differences between healthy individuals and those with PCOS or type 1 diabetes.

The main methodological limitation of all four original studies is the small number of participants, which means that the main results of this thesis overall must be verified by further studies with larger populations. This should be done, although the post hoc statistical power calculations presented in Section 6.3 naturally indicate that the magnitudes of the attained statistical power were adequate. The use of a single question to obtain a measure of LTPA is also a methodological limitation. In this regard, it is notable that the criterion validity of a single-item measure against more quantitative accelerometry has suggested that a single question is a valid method to screen physical activity in both men and women (479,480). However, it must be acknowledged that more detailed physical activity questionnaires and/or objective measuring of physical activity (e.g., accelerometries) are more accurate ways to quantify LTPA. While the in-depth discourse of Sections 7.1-7.4 also include some methodological discussion, the following paragraphs discuss a few more methodological considerations study by study.

In Study I, the most important methodological detail is perhaps that all of the results were interpreted in light of the strict criteria used for NIP determination (see Section 5.1.5.3); for instance, seven NIPs in leg muscle were concluded to reflect oxygenation (i.e., $\Delta[O_2Hb] \uparrow$, $\Delta[HHb] \downarrow$, and TSI $\uparrow$), although more detailed inspection of the data does show that leg muscle $\Delta[HHb]$ actually reached “some kind of” plateau around RC in 11 subjects. However, the results of Study I can overall be physiologically reasonably interpreted and are in accordance with literature published both before and after Study I (see Section 7.1). Thus, the criteria used for NIP determination can be regarded as appropriate for the aims of Study I. Another methodological note concerning this study is that during the treadmill exercise interindividual variability in anthropometry as well as different locomotion strategies (e.g., stride frequency, stride length) certainly affect muscle recruitment patterns, levels of muscle work, and hence, local O$_2$ delivery and utilization responses. Furthermore, not only the NIRS data of Study I but also those of Studies III and IV carry the caveat that only the biceps brachii (Study I) and the vastus lateralis (Studies I, III, and IV) muscles were under examination, while spatial heterogeneity of local O$_2$ delivery and utilization within and between muscle groups is well acknowledged (185,186,194).

In Study II, $\dot{V}O_{2peak}$ scaled to FFM was lower in PCOS women than in controls, whereas $\dot{V}O_{2peak}$ expressed as L/min did not differ between the groups, which may raise a question as to whether or not $\dot{V}O_{2peak}$ was truly different between the two well-matched groups. The contribution of adipose tissue to O$_2$ utilization has been shown
to be negligible and independent of $\dot{V}O_{2\text{peak}}$ (481). Instead, $\dot{V}O_{2\text{peak}}$ expressed as L/min follows a linear function of FFM (455,456). In addition, Krachler et al. (456) recently demonstrated how scaling $\dot{V}O_{2\text{peak}}$ data collected during cycling to FFM introduces a much smaller bias against overweight and obese women than quantifying $\dot{V}O_{2\text{peak}}$ in a more traditional manner (i.e., L/min, mL/min/kg body weight), although cycling is not a weight-bearing exercise mode. Thus, in Study II involving overweight and obese women, VO$_2$ was appropriately scaled to FFM to avoid ignoring any differences in body size and composition between PCOS women and controls. The superiority of using this FFM approach is due to the apparently good ability of FFM to reflect the amount of active skeletal muscle mass, which is mainly responsible for O$_2$ utilization during acute dynamic exercise; a magnetic resonance imaging study by Abe et al. (482) demonstrated strong positive associations both between FFM and total skeletal muscle mass and also between FFM and lower leg skeletal muscle mass in normal-weight women. Although it is not known whether such studies have been conducted in overweight or obese individuals, the above-mentioned findings of Krachler et al. (456) strongly suggest that FFM likely reflects the amount of active muscle mass well also in individuals with excess weight. One limitation of Study II is the use of feasible HOMA-IR as a method to assess IR; using an intravenous frequently sampled glucose tolerance test or a euglycemic-hyperinsulinemic clamp technique would have provided a more sensitive and in-depth assessment of insulin sensitivity.

The primary methodological limitation of Study III is the use of the assumed values of muscle (a-v)O$_2$ (55,461) when estimating $\dot{Q}_{VL}$. Accepting this limitation, the actual patterns of $\dot{Q}_{VL}$ should remain similar to those presented here in Figure 17B unless large deviations existed between the actual muscle (a-v)O$_2$ and the published values (55,461) used.

As already mentioned, the comparatively long 1-year duration of the training intervention is the main strength and novelty of Study IV. In addition, Study IV is also the first longitudinal study to examine the effect of exercise training on active muscle deoxygenation profiles in individuals with diabetes. As a shortcoming of Study IV, interpreting its findings in light of the integrated model of $\dot{V}O_{2\text{peak}}$ was hindered by SV and $\dot{Q}$ not being measured or even estimated by PhysioFlow. Moreover, the allocation of the subjects to the training groups was performed in a nonrandom fashion, which can also be regarded as a limitation. Such a nonrandom allocation was due to the substantial burden on subjects imposed by the relatively long-term intervention, including intensive collecting of individual exercise data. While it is acknowledged that a randomized design would have been necessary to preclude selection bias, the nonrandom allocation hardly had any effect on comparisons between the training groups. Lack of consistent progression in training of the training groups may also be regarded as a limitation. However, more often than not, such lack of progression is certainly characteristic of recreational-like training. Hence, the completed training intervention likely reflected real-world circumstances, which was one purpose of Study IV. Moreover, the completed training can truly be regarded as “exercise training” rather than “habitual exercise” since the subjects changed their physical activity habits, and thereby, improved their exercise capacity and cardiorespiratory fitness by ~10%, which would hardly have happened if they had just kept on exercising in a similar fashion as before the intervention.
8 SUMMARY AND CONCLUSIONS

This study entity examined O₂ delivery and utilization during acute dynamic exercise in both healthy individuals and individuals with PCOS or type 1 diabetes. Acute dynamic exercise was thus used as a tool to identify early signs of cardiovascular dysfunction in PCOS and type 1 diabetes. Whether long-term exercise training alleviates such early signs related to type 1 diabetes was also assessed. Importantly, all of the findings were approached from the perspective of the integrated pathway for O₂ from the atmosphere to the mitochondria. In summary, individuals with PCOS or type 1 diabetes but without clinically overt cardiovascular disease were characterized by early signs of cardiovascular dysfunction, eventually reflected as reduced $\dot{V}O_2^{\text{peak}}$. Furthermore, long-term adherence to exercise training was partially but not fully able to alleviate the early signs of cardiovascular dysfunction associated with type 1 diabetes.

The main conclusions of the four original studies are specifically as follows:

I Local imbalance between O₂ delivery and utilization within active leg muscle, less active arm muscle, and cerebral tissues was integrated with alveolar gas exchange (i.e., whole-body) responses to maximal incremental treadmill exercise in healthy adult men similarly to what has previously been reported during maximal incremental cycling.

II In comparison with overweight and obese women without PCOS, reduced $\dot{V}O_2^{\text{peak}}$, reduced peak C(a-v)O₂, and a pronounced $\Delta Q/\Delta VO_2$ slope but otherwise intact systemic O₂ delivery were observed in overweight and obese PCOS women during maximal incremental cycling. These findings indicate that PCOS per se is linked to alterations in peripheral adjustments to acute dynamic exercise rather than to limitations of systemic O₂ delivery in overweight and obese women.

III In comparison with healthy men, reduced peak cardiac pump function, being associated with reduced BV, and independently deteriorated peak microvascular blood flow within active muscle were observed during maximal incremental cycling in adult men with type 1 diabetes. Thus, O₂ delivery is limited both systemically and peripherally during acute dynamic exercise in type 1 diabetes, which is further reflected as reduced $\dot{V}O_2^{\text{peak}}$ in this patient group. Importantly, the findings of Study III also suggest that both systemic and peripheral limitations are associated with glycemic control.

IV The long-term (1-year) individualized exercise training intervention improved $\dot{V}O_2^{\text{peak}}$ and peak O₂ pulse similarly in adult men with and without type 1 diabetes. By contrast, training had no effect on NIRS-
derived local active muscle O$_2$ extraction or HbA$_{1c}$ in men with type 1 diabetes. These findings suggest that adherence to regular exercise improves cardiorespiratory fitness, probably cardiac pump function, and hence, the overall prognosis of cardiovascular morbidity in type 1 diabetes to the same extent as it does in individuals without diabetes. Instead, regular exercise, at least to the extent in Study IV, is unable to alleviate disease-related defects within active muscle microvasculature or to improve glycemic control in this patient group. Importantly, consistent associations between training dose and responses were observed in healthy men, but not in men with diabetes, highlighting the need for greater individualization of exercise prescription in type 1 diabetes.
9 FUTURE PERSPECTIVES

The conclusions summarized in Section 8 provide a basis for future multi-disciplinary research to identify more detailed mechanisms underlying the observed early disease-specific signs of cardiovascular dysfunction. Moreover, while $\dot{V}O_2\text{peak}$ itself is known to be a strong predictor of cardiovascular health (19), detailing the clinical significance of the present findings further is also warranted. Examples of large and intriguing questions that remain to be answered include the following: What are the defects reducing peripheral $O_2$ delivery and/or utilization within active muscle tissue of women with PCOS and excess weight? How does the magnitude of IR relate to such unidentified PCOS-specific peripheral defects? Can these unidentified PCOS-related defects in the periphery be alleviated by chronic exercise training? What are the mechanisms that make chronic hyperglycemia lead to such an early development of cardiac dysfunction in type 1 diabetes? Can chronic adherence to exercise training really reverse or even delay the early development of diabetes-related cardiac dysfunction that has recently been shown to have significant clinical importance in type 1 diabetes (18)? What features in exercise training could improve microvascular dysfunction and/or HbA$_1c$ in type 1 diabetes?

In addition to answering the open scientific questions exemplified above, all exercise scientists and clinicians are encouraged to approach any observed responses to acute dynamic exercise from the integrated perspective highlighted here instead of attempting to answer overly simplified questions like “what single factor limits exercise capacity or $\dot{V}O_2\text{peak}$?”. Such an integrated approach enables identification of specific mechanisms underlying pulmonary and/or cardiovascular dysfunction as well as exercise intolerance in terms of a single disease or a single person. This is vital when prescribing effective, health-promoting, and safe exercise training to any population or individual.
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After some long days,

Antti-Pekka Rissanen
Helsinki, April 2017
APPENDICES

APPENDIX 1 SCALING OF THE FIGURES PLOTTED FOR NIP DETERMINATION (SEE SECTION 5.1.5.3)

- Figure plotting performed by Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA).
- Worksheet zoom level: 100%.
- Figure titling: Title (e.g., Leg $\Delta$[HHb]) above each figure with bolded Calibri font size 18.
- Figure sizes:
  - Width: 15 intact columns
  - Height: 20 intact rows
- Figure axes:
  - $\text{Leg } \Delta [\text{HHb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Leg $\Delta$[HHb]): height 30 $\mu$mol/L
  - $\text{Leg } \Delta [\text{O}_2\text{Hb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Leg $\Delta$[O$_2$Hb]): height 35 $\mu$mol/L
  - Leg TSI vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Leg TSI): height 70% (from 20% to 90%)
  - $\text{Arm } \Delta [\text{HHb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Arm $\Delta$[HHb]): height 50 $\mu$mol/L
  - $\text{Arm } \Delta [\text{O}_2\text{Hb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Arm $\Delta$[O$_2$Hb]): height 55 $\mu$mol/L
  - Arm TSI vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Arm TSI): height 90% (from 0% to 90%)
  - Cerebral $\Delta [\text{HHb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Cerebral $\Delta$[HHb]): height 12 $\mu$mol/L
  - Cerebral $\Delta [\text{O}_2\text{Hb}]$ vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Cerebral $\Delta$[O$_2$Hb]): height 30 $\mu$mol/L
  - Cerebral TSI vs. Time
    - x-axis (Time): width 2500 s
    - y-axis (Cerebral TSI): height 55% (from 40% to 95%)
REFERENCES


(22) Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. Physiol Rev 2015;95(2):549-601.


(102) Saltin B. Hemodynamic adaptations to exercise. Am J Cardiol 1985;55(10):42D-47D.


(116) Sramek BB, Rose DM, Miyamoto A. Stroke volume equation with a linear base impedance model and its accuracy, as compared to thermodilution and magnetic flowmeter techniques in humans and animals. Proceedings of the Sixth International Conference on Electrical Bioimpedance, Zadar, Yugoslavia 1983:38-41.


(190) Poole DC, Copp SW, Hirai DM, Musch TI. Dynamics of muscle microcirculatory and blood-myocyte O(2) flux during contractions. Acta Physiol (Oxf) 2011;202(3):293-310.

(191) Krogh A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J Physiol 1919;52(6):409-415.


(345) Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. Diabetologia 2001;44(7):914-922.

(346) Groop L, Pociot F. Genetics of diabetes--are we missing the genes or the disease? Mol Cell Endocrinol 2014;382(1):726-739.


(369) Austin A, Warty V, Janosky J, Arslanian S. The relationship of physical fitness to lipid and lipoprotein(a) levels in adolescents with IDDM. Diabetes Care 1993;16(2):421-425.


