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Muscle oxygen availability in response to exercise: influence of intensity and duration.

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Equations

List of Abbrevations

A	Amplitude
AIT	Aerobic interval training
A.U.	Arbitrary units
AT	Anaerobic threshold
ATT	Adipose tissue thickness
(a-v)O_2	Arteriovenous oxygen difference
ANOVA	Analysis of variance
ATT	Adipose tissue thickness

BASE	Baseline
BF	Blood Flow
C	Concentration
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CO_2	Carbon dioxide
COX	Cyclooxygenase
CP	Critical power
DPF	Differential pathlength factor
CWS	Continuous-wave spectroscopy
EE	End-exercise
eNOS	Endothelial nitric oxide synthase
ε	Extinction coefficient
GET	Gas exchange threshold
GET60–	Subjects with GET below 60% VO_2 peak
GET60+	Subjects with GET above 60% VO_2 peak
Hb	Hemoglobin
HCO_3^-	Bicarbonate
HHb	Deoxygenated hemoglobin
HIIT	High intensity interval training
HIT	High intensity training
INT	Experimental session “exercise intensity”
λ	Wavelength
L	Photon pathlength
LT	Lactate threshold
Mb	Myoglobin
MCT	Moderate continuous traininig
MLSS	Maximal lactate steady state
MRT	Mean response time
mVO_2	Muscular oxygen uptake
NIRS	Near-infrared spectroscopy
NO	Nitric oxide

OD	Optical density
OD_S	Light attenuation by scattering
O_2Hb	Oxygenated hemoglobin
OS	Postexercise overshoot
O_2	Oxygen
PT	Preliminary testing
Q	Blood flow
Q_{cap}	Capillary blood flow
RCP	Respiratory compensation point
REC	Recovery period
Reoxy	Reoxygenation rate
RER	Respiratory exchange ratio
RST	Repeated sprint training
SIT	Sprint interval training
τ	Time constant
TIME	Experimental session “exercise duration”
TD	Time delay
THb	Total hemoglobin
VCO_2	Carbon dioxide emission
V_E	Ventilation
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
V-Slope	Method to detect the anaerobic threshold

1. Summary

Interval training evidentially triggers aerobic adaptions like improved fatty acid and carbohydrate oxidation rates. Increased local blood supply is supposed to be one important mechanism that underlies these effects. Two important determinants of interval training are intensity and duration of work intervals. However, knowledge is scarce on the detailed effect of exercise intensity (Study 1) and exercise duration (Study 2) on the other hand on post-exercise blood supply and oxygen availability. In order to study those issues, the effects of six different, interval training associated exercise intensities and durations on post-exercise musclular oxygen availability and relative changes in hemoglobin concentration have been examined.

For both (1) and (2), relative changes in oxygenated and deoxygenated hemoglobin and total hemoglobin (ΔO_2Hb, ΔHHb, ΔTHb) were monitored with near-infrared spectroscopy of the vastus lateralis muscle and pulmonary oxygen uptake (VO_2) was assessed. In Study 1, 17 male subjects performed an experimental protocol consisting of 180 s cycling bouts at six exercise intensities (40–90% peak oxygen uptake, VO_{2peak}) in randomized order, separated by 5 min rests. In order to estimate local muscle blood supply and oxygen provision, $\Delta HHb/\Delta VO_2$ ratio and estimated capillary blood flow (Q_{cap}) were calculated during recovery using a bi-exponential model. In Study 2, 18 healthy male subjects performed an experimental protocol of five exercise bouts (30 s, 60 s, 90 s, 120 s and 240 s) at 80% VO_{2peak} in randomized order, separated by 5 min rests. To examine the influence of submaximal aerobic performance, subjects with gas exchange thresholds (GET) above 60% VO_{2peak} (GET60+) were compared with subjects reaching GET below 60% VO_{2peak} (GET60-).

The results of Study 1 revealed a progressively increased $\Delta HHb/\Delta VO_2$ ratio from 40% to 60% VO_{2peak}. Above 60% VO_{2peak}, it decreased progressively. Post-exercise ΔTHb and ΔO_2Hb showed an overshoot in relation to pre-exercise values, which was equal following exercise at 40–60% VO_{2peak} and rose significantly following higher exercise intensities. A plateau was reached following exercise at ≥80% VO_{2peak}. Mean response time (MRT) of Q_{cap} recovery increased significantly with increasing exercise intensity. Study 2 showed significantly increased post-exercise oxygen

availability and local blood supply following 90 s exercise duration without a further increase following longer exercise bouts. Considering submaximal aerobic performance, the GET60+ group reached maximum post-exercise oxygen availability also with shorter exercise (60 s) than the GET60- group (90 s).

Based on the results, Study 1 shows a progressively increasing mismatch of local O_2 delivery and utilization with increasing exercise intensity up to 60% VO_{2peak} after 3 min exercise bouts as suggested by increasing end-exercise $\Delta HHb/\Delta VO_2$ ratio. This suggests that microvascular perfusion does not adequately meet the increased metabolic demand up to this point. Interestingly, $\Delta HHb/\Delta VO_2$ decreased above 60% VO_{2peak}. Consequently, the matching of HHb and VO_2 gets progressively impaired from 40% VO_{2peak} to 60% VO_{2peak} but is progressively improved at exercise intensities above 60% VO_{2peak} up to 90% VO_{2peak}. Postexercise oxygen availability also was improved above above 60% VO_{2peak}, which was according to the transition from moderate to heavy intensity exercise (gas exchange threshold 59 ± 13% VO_{2peak}). Consequently, beginning acidosis could have promoted local vasodilation. Study 2 results give evidence for a slower adjustment of local vasodilation in subjects with GET60- than GET60+. The key mechanism behind those effects presumably is an enhanced endothelium and flow-mediated vasodilation superimposing sympathetic vasoconstriction.

These results suggest that cycling exercise is most efficient for enhancing local postexercise oxygen availability and blood supply when it is conducted (A) at least at 80% VO_{2peak} and (B) with a minimum duration of 90 s for subjects wit GET60- while such with GET60+ have same effects following 60 s of exercise. Hence, interval training should be prescribed accordingly in order to promote aerobic effects.

2. Introduction: Local muscle blood supply and its relevance for exercise performance and exercise induced adaptions

Every physical exercise has its specific demands on the human physiological system. These demands include metabolic, cardiovascular, endocrinological and neurological issues, which are closely linked among each other. Thus, it is not possible to quantify the impact of one of those systems e.g. on exercise performance without considering concomitant processes. Despite of this, it is possible to define them by their functional role within the entire physiological system.

The cardiovascular system is mainly responsible to supply the body with metabolites, hormones and oxygen (O_2). It includes the heart and all kind of vessels – arteries, veins and capillaries - and hence enables the perfusion of all organs including brain and muscles. Perfusion is usually expressed as blood flow (Q), which describes how many liters of blood are perfusing a certain region in the body per minute.

During exercise, Q closely matches the current metabolic demand (60). It has particular significance for exercise performance, because it directly influences both the supply of working muscles with energy substrates and the clearance of metabolites such as lactate (74). In addition, there is also a dependency of certain types of metabolism on Q (56, 66, 88, 91), which are discussed below.

Q is most importantly responsible for the transport and demand-orientated distribution of O_2 as well as clearance of carbon dioxide (CO_2). An athlete's capability to quickly start aerobic processes in response to exercise reduces the requirement of energy, which has to be synthesized by less economical pathways like lactate metabolism (21).

Summed up, local blood supply to active muscles and the associated O_2 provision are crucial for exercise performance.

Aerobic interval training or high intensity (interval) training (AIT/HIIT/HIT) have become increasingly popular as endurance training and their positive effects on the anaerobic (17, 110) and aerobic (38, 46, 55, 95) system are proven. While especially HIIT has been mostly applied to healthy, trained subjects, intensive training is now getting more and more popular in clinical applications, e.g. in patients with heart

disease (43, 51). Recent studies could show that HIIT and AIT improve microvascular functions (9, 22, 73) and hence facilitate local blood supply.

Although there is knowledge about the importance of local blood supply and O_2 provision during and following exercise, the relationship of local blood supply and O_2 provision to exercise intensity and duration has not been examined in detail. Because HIIT and AIT have been reported to enhance local hemodynamics effectively, this project aims to examine those issues, which are mentioned above, in relationship to exercise intensity and duration, which are associated to HIIT and AIT. Because of the substantial lack of knowledge in this field, the project focuses on isolated exercise bouts and not with repeated bouts, as they are usually applied for HIIT. This second step will be addressed in future studies. With the results of this study, interval training prescription shall be improved in order to maximize aerobic effects that are supposed to be supported by enhanced muscle perfusion.

Local blood supply and O_2 provision can be noninvasively monitored using near-infrared spectroscopy (NIRS) (25, 33, 39, 58, 80). This technique takes advantage of wavelength-dependent specific near-infrared absorptions properties of hemo- and myoglobin (Hb, Mb) and uses the modified Lambert-Beer law to calculate Hb+Mb concentrations (105). NIRS enables measurements during exercise and can be attached to various parts of the human body. In this study, cycling exercise was focused, so the NIRS probe was attached to the vastus lateralis muscle, which is commonly used for NIRS measurements of lower body exercise (15, 33, 79).

Because muscle perfusion is significantly affected by movement during exercise (muscle pump, see chapter 2.2.2) (100), this work does not solely focus on parameters during exercise, but in particular examines the early post-exercise phase. The reasons are that (1) work intervals within HIIT regimens had been suggested to be “trigger intervals” mediating effects during the low interval (16) and that (2) it had been shown that some metabolic pathways, i.e., fatty acid metabolism, are dependent on local perfusion (66). Furthermore, our approach is based on prior research, which could show intensity-dependent post-exercise hyperemia (6, 23, 27).

3. Background

3.1. Intensity and thresholds – what is HIIT/HIT?

Although HIT and HIIT definitions are varying among different authors or purposes of application (16), there is consensus about general characteristics of these training methods. By their intermittent character, they are clearly distinguished from training methods with continuous exercise intensity. Billat describes HIT as a training method, that "involves repeated short-to-long bouts of rather high-intensity exercise interspersed with recovery periods" (7) while Laursen et al. defined HIT as repeated bouts of short- to moderate-duration work intervals which are completed at an intensity above the anaerobic threshold with low-intensity resting intervals in-between (69).

The difference between HIT and HIIT is that HIIT usually refers to lower intensities during the work interval compared to HIT (38, 69, 95) and is sometimes also termed "HIT with long intervals" (16). Another aspect is that HIIT is often used with longer work intervals in relation to rest intervals and/or rest intervals at higher intensities compared to HIT. Some exemplary HIT and HIIT will be presented in this section.

3.1.1. Exercise intensity domains

Because definitions of HIT and HIIT in turn are based on distinct exercise intensities, it is necessary to define a framework for different exercise intensity domains covering the range between rest an maximal exercise capacity. Several frameworks have been suggested to characterize different levels of subjects' strain, perceived exertion or metabolic demand. Among those suggestions, one framework that is based on three different exercise domains has evolved as the most accepted approach (21, 75, 90). This so-called "three-zone-model" (Fig. 1) describes three different levels of energy supply and utilization. The boundaries of those intensity domains are defined by distinct metabolic turning points, often designated as "thresholds", although this term used in a metabolic context suggests a transition from one kind of metabolism to another, which is not true.

The turning points or thresholds are usually either assessed using spiroergometry or ergometry with lactate diagnostics. Both ways have their advantages and limitations, which are discussed in depth elsewhere (10). In spiroergometry, there exist two important turning points. These are derived from O_2 uptake (VO_2), carbon dioxide expiration (VCO_2) and ventilation (V_E) during an incremental ergometry. The first submaximal threshold is called “anaerobic threshold” (AT) and describes the point when non-metabolic CO_2 is released remarkably for the first time during an incremental exercise test (3, 21). This non-metabolic CO_2 has its origin in the bicarbonate buffer system (HCO_3^-) and is released in response to beginning acidosis by rising blood lactate concentration. In brief, glycolysis is promoted as a response to increased energy demand during heavy exercise. Because pyruvate uptake rate of the citrate cycle is limited, surplus pyruvate is metabolized to lactate. Up to a distinct level, local muscle cells themselves can remove lactate. This hypothesis is called “lactate shuttle” theory (37, 108). Hence, blood lactate concentration will not change remarkably compared to resting values. However, when the capability for lactate removal by local muscle cells is exceeded, lactate diffuses into blood and is delivered to other body regions (i.e. liver). This can be detected by plotting VO_2 vs. VCO_2 (V-Slope) (3) and appears as increase of the slope caused by additional CO_2 expiration from HCO_3^- (Fig. 2).

Commonly used synonyms are “gas exchange threshold” (GET) (42) or “ventilatory threshold 1” (VT1) (70). Confusingly, the term “aerobic threshold” had been introduced for AT in the German-speaking area. However, the international terms are used in this thesis for clarity.

The second submaximal threshold is termed “respiratory compensation point” (RCP) (112). It is associated with the exceedance of the maximal lactate steady state (MLSS). Once local and global lactate removal capacities have reached their limit, blood lactate accumulates when exercise is increased further. This leads to forced release of CO_2 from bicarbonate and forced ventilation while O_2 uptake rises with exercise intensity in an unaltered way. This threshold is located between the point when VCO_2 equals VO_2 for the first time, i.e. a respiratory exchange ratio of 1

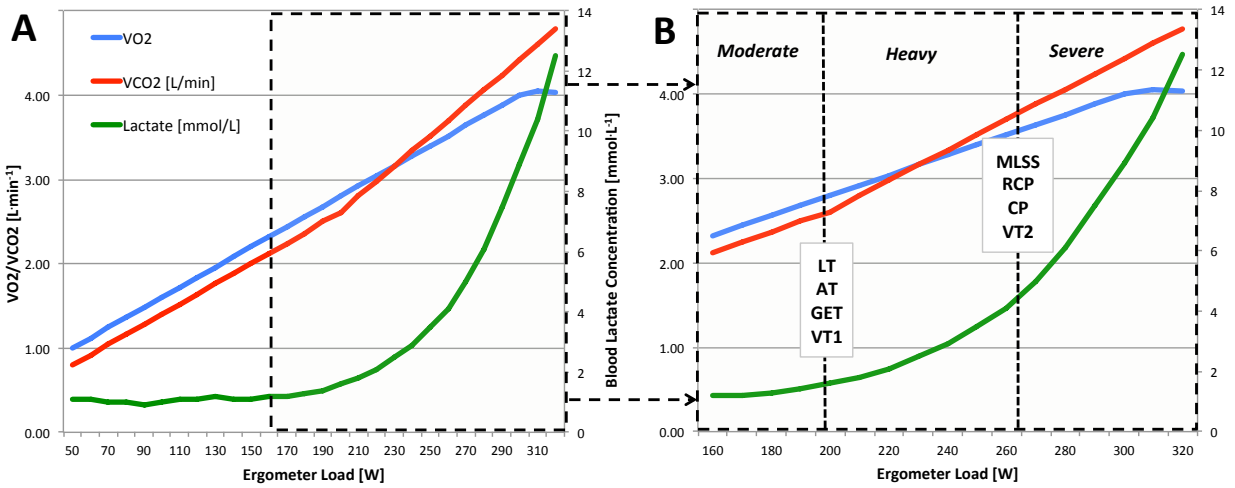

Figure 1 Lactate, VO_2 and VCO_2 kinetics in relation to cycling exercise intensity of a trained subject; (B) enlarged view on the same parameters beginning short before the first increase in blood lactate concentration with assigned thresholds and exercise intensity domains.

(RER=1) and VO_{2max}. Reaching RCP is indicated by a further increase in VCO_2 due to the increased HCO_3^- breakdown above MLSS and forced V_E to compensate CO_2 release from HCO_3^-. Commonly used synonyms are “critical power” (CP) (21) or “ventilatory threshold 2” (VT2) (70).

AT and RCP determine the framework for the three-zone-model of exercise intensity domains. AT therefore represents the upper bound of the first intensity domain, which is usually called “moderate intensity domain” (21). Exercise in this intensity domain is characterized by high to highest rates (occurring shortly before AT) of fatty acid oxidation (1, 88). Hence, oxidation of carbohydrates is supported by fatty acid oxidation most efficiently in this intensity domain. Since the demand on the anaerobic system is little in this domain, blood lactate values do not differ remarkably from resting values. Again, it has to be noted that anaerobic energy provision rises with increasing exercise intensity, but up to AT this can be equaled by local muscles lactate removal capacity (36, 74, 108). Moderate intensity exercise therefore mainly

stimulates aerobic carbohydrate and fatty acid oxidation with minor anaerobic demand. However, long exercise is required to gain training effects, because total energy demand is relatively low compared to more intense exercise.

Intensities between AT and RCP represent the “heavy intensity domain” (21). Exercise in this domain is characterized by higher rates of carbohydrate oxidation compared to moderate intensity exercise but a beginning decline in fatty acid oxidation. Anaerobic energy provision is enhanced and already induces rising blood lactate values causing non-metabolic CO_2 release from bicarbonate but will not accumulate during constant load exercise. Accordingly, the stimuli to the different energy systems are shifted.

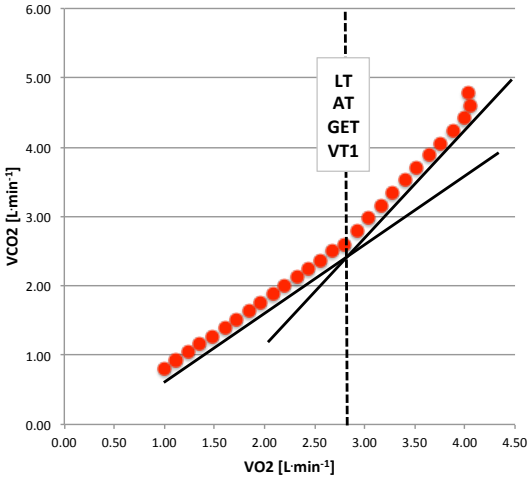

Figure 2 V-Slope (xy-plot of VO_2 and VCO_2); the first change in the relationship of VO_2 and VCO_2 indicates LT/AT/GET/VT1.

All Intensities above RCP up to VO_{2max} are summed up in the “severe intensity domain” (21), which characterizes exercise above MLSS. Beside high anaerobic demand, aerobic carbohydrate oxidation reaches its top level within this domain. Fatty acid oxidation has declined to near-resting values (88).

The exercise intensity corresponding to VO_{2max} assessed during an incremental exercise test does not represent the maximum intensity for short-duration exercise (e.g. sprinting). Hence there is a supramaximal intensity domain, which has its lower boundary at VO_{2max}. However, this intensity domain remains somehow uncertain, because the exercise intensity that elicits VO_{2max} is dependent on various factors such as the incremental exercise test protocol (111, 115).

HIT sessions are usually characterized by the repeated transition from severe or supramaximal exercise to moderate intensity exercise and back.

3.1.2. HIT regimens

There exist a lot of varying approaches for HIT/HIIT prescription. In general, one can manipulate interval training by adjusting intensity and duration of the work and relief interval as well as the number of repetitions. Furthermore, the modality of the relief interval, i.e. either applied passively or actively, can be adjusted. Buchheit et al. (16, 17) proposed the attempt of a classification for HIT/HIIT. They included all intensities corresponding to 90% VO_{2max} or higher, which are separated into submaximal to maximal intensities (90%-100% VO_{2max}) and the so-called “anaerobic speed reserve”, i.e. all supramaximal exercise intensities above the intensity corresponding to VO_{2max}. In brief, work interval durations of 2-4 min are described as “long intervals” (≈90% VO_{2max}) and interval durations of 10-60 s are described as “short intervals” (≈100-120% VO_{2max}). More intensive interval exercise is described as “repeated sprint training” (RST,≈4 s All-Out work intervals) and “sprint interval training” (SIT, ≈20-30 s All-Out work intervals). The distinction between the different sub-divisions of HIT/HIIT varies slightly among different authors. For example, some authors described lower exercise intensities like 80% VO_{2max} as a minimum exercise intensity for HIT/HIIT with long intervals (43, 84, 95). One of the major goals of HIT/HIIT is to increase exercise time at intensities near-to or at VO_{2max} (16). Continuous training does not engage such long times at high exercise intensities within one training session as it is possible within HIT/HIIT sessions for reasons of fatigue.

The relief interval can be applied either actively or passively. Within SIT or RST sessions, a passive recovery is recommended in order to enable a sufficient recovery and performance for the next work interval. But especially in HIT/HIIT with long intervals, there is the possibility to conduct both active or passive recovery. Active recovery is applied within the moderate intensity domain and logically has to last longer than passive recovery. Nevertheless, there are valuable reasons for the application of active recovery. In order to improve aerobic capacity, interval training is most effective when exercise time is maximized. This refers to the work interval on the one hand, for which it could be shown that HIT with long intervals extends time

near-to or at VO_{2max} more effectively compared to HIT with short intervals. On the other hand, active recovery increases the rate of aerobic metabolism (8).

In this thesis, HIT/HIIT refers to long interval training regimens. For clarity, HIT is used representative.

3.2. Determinants of local muscle blood supply

The increased O_2 demand of exercising muscles requires a well-balanced and adaptive regulation of local blood supply (Fig. 3). After ejection from the left ventricle, blood spreads in different regions of a complex structure of vessels. Blood flow regulation has to be closely linked to acute demands, which can differ among various body regions. Therefore, it appears logical that mechanisms of blood flow regulation do not only work globally across the whole body, but also work locally in different body regions and sometimes even antagonistically. Joyner et al. (60) describe two potentially competing physiological needs during exercise. On the one hand, there is the metabolic demand of active tissue while blood pressure also has to be regulated to ensure appropriate perfusion to all organs on the other hand. Both functions are described as “competing mechanisms” (60) because their regulation occurs antagonistically.

3.2.1. Central blood flow regulation

Central blood flow regulation mechanisms do have a “global” impact on body perfusion, i.e. they affect all body regions rather equally. These central mechanisms are most importantly heart rate and stroke volume (57). An increase in heart rate and stroke volume will induce an undirected increase in perfusion. Cardiac output can increase four- to eightfold compared to resting values (60). Another “global” mechanisms is sympathetic mediated vasoconstriction (67). It is regulated by the release of norepinephrine and aims to augment vascular tone in order to maintain a sufficient blood pressure to ensure adequate perfusion of important organs like the brain. Hence, sympathetic mediated vasoconstriction does impede blood flow. However, this mechanism is of high importance during exercise, when distinct body regions require very high perfusion in order to meet the metabolic demand. To avoid a drop in blood pressure in the other body regions, sympathetic mediated

vasoconstriction occurs simultaneously. For some body regions like kidneys and the splanchnic bed, this vasoconstriction induces a reduction in blood flow compared to resting condition (60), while other non-exercise regions, e.g. the brain, are perfused consistently across different levels of activity. As a central mechanism, sympathetic vasoconstriction works equally in all body regions and hence must be counteracted by other, local mechanisms when a rise in local blood flow is required. These "competing mechanisms" (60) provide a precise regulation of body perfusion.

3.2.2. Local blood flow

Local muscle blood flow can rise dramatically during exercise. In humans, 300 ml per min per 100g muscle mass could be observed (60). This implicates that there must be mechanisms which counteract or superimpose sympathetic vasoconstriction. Interestingly, the first response to a muscle's contraction is an immediate impairment or sometimes even obstruction of microvascular flow (60) due to the microvascular

Figure 3 Idealized distribution of blood flow during rest (left) and exercise in a untrained (middle) and a trained subject (right); dark blue=brain, red=heart, yellow=organs (kidney, liver and others), green=skin, light blue=muscle; adapted from Joyner et al. (60).

compression. If a contraction leads to microvascular occlusion or not depends on the contraction's intensity – de Ruiter et al. (30) found this threshold to be at 35% maximum torque capacity in the m. rectus femoris while it evolved to be 25% in the m. vastus lateralis and medialis. A sustained contraction above that threshold restricts arterial inflow and venous outflow simultaneously, similar to the effect of a passive

arterial occlusion using a blood pressure cuff (105). Arterial inflow is occluded when intramuscular pressure exceeds arterial pressure. If the intramuscular pressure appears to be between arterial pressure (approximately systolic blood pressure during resting conditions) and systolic blood pressure, arterial inflow to the certain tissue will be enabled while venous outflow is restricted. The effect will be the same as during venous occlusion by inflating a blood pressure cuff just above diastolic blood pressure (105). An intramuscular pressure below systolic blood pressure will engage full perfusion of the tissue.

Given the situation that microvascular flow has been restricted in response to a single muscular contraction, flow increases rapidly after relaxation of the muscle with a response time <1 s and returns to baseline thereafter (60). Since muscle perfusion pressure does not change markedly during the phase of reperfusion, local mechanisms must be responsible for this phenomenon. On the one hand, there is local vasodilation facilitating perfusion by the relaxation of local vessels. Considering that rapid vasodilation takes approximately 5 s to occur (48, 71), vasodilation fails to be the explanation for this first response. This rapid hyperemia is due to the so-called “muscle pump” (68, 89), which increases local blood flow by mechanical effects of rhythmic muscle compression and relaxation. Local vasodilation instead greatly influences local muscle perfusion after this rapid response. Both dimensions of “local blood flow enhancers” are discussed below.

Local Vasodilation

Vasodilation is one key mechanism that regulates local muscle perfusion. During exercise, vasodilation occurs at every part of the arteriolar network. Recent research has identified smallest muscle vessels to be most resistant to sympathetically mediated vasoconstriction and hence being responsible for local muscle vasodilation (106, 107) rather than large conduit artery vasodilation, which does not account for local muscle hyperemia in response to exercise in a major role (96).

Substances that are released from the endothelium of local vessels are intensively discussed in current literature. This kind of vasodilation has been shown to be dependent on various factors whereas two central pathways play a central role: the nitric oxide (NO) mediated pathway via endothelial NO synthase (eNOS) and the

prostacyclin mediated pathway via cyclooxygenase (COX) (54). Both eNOS and COX can be activated by at least partially the same stimuli, e.g. adenosine triphosphate, adenosine, acetylcholine, bradykinin, histamine and shear stress (54). Of the mentioned stimuli, especially adenosine triphosphate, adenosine, acetylcholine and shear stress presumably are associated with exercise induced effects on vasodilation (52, 67, 78, 80, 97, 100). In smooth muscle cells surrounding the endothelium, endothelial release of NO activates cyclic guanosine monophosphate (cGMP) while endothelial prostacyclin activates cyclic adenosine monophosphate (cAMP). Both enzymes effect Ca^{2+} release in smooth muscle cells and thus a relaxation of muscle fibers (54).

It is still not entirely evaluated to what extent and in which temporal pattern the several factors act that activate NO and prostacyclin during exercise. Acetylcholine has been pointed out to play a central role in rapid vasodilation within the first seconds of exercise (100). In contrast, Haram et al. (52) found a decreased responsiveness to acetylcholine immediately after exercise in rats, presumably due to local vascular stress caused by oxidative radicals. Post-exercise, this depression of acetylcholine-mediated vasodilation turns into a rapid increase which peaks 12-24 h after the cessation of exercise. Vasodilation mediated by extracellular adenosine triphosphate is also supposed to influence vasodilation during exercise (64). This mechanism seems to be tightly linked to adenosine mediated vasodilation within a redundant system (67, 86). Tinken et al. (97) studied the influence of shear stress on acute and long term vasoregulatory effects in humans. They found a significant dependency of vasodilation on shear stress. Due to the contribution of multiple substances to local muscle vasodilation, it is hard to differentiate the detailed relationship of the single mediators and exercise intensity. In case of shear stress mediated vasodilation, it seems logical that this stimulus is linked to blood flow, which is linearly related to exercise intensity (89).

Beside endothelium-mediated vasoregulation, smooth muscle cells can be directly influenced, e.g. by metabolites. There is evidence for an activation of cGMP within smooth muscle cells by lactate in rats (24). This is supported by Gerbino et al. (45), who proposed local vasodilation mediated by lactate induced residual acidosis as a key factor for speeded VO_2 kinetics following prior exercise in humans. It has to be

mentioned that Chen et al. (24) used neutralized lactate in their experiments. Consequently, this group suggested other, cGMP independent vasoregulative mechanisms being sensitive to pH-level. Hence, lactate induced acidosis could cause vasodilation via two different pathways, i.e. by lactate itself and by the concomitant acidosis. However, the role of lactate is discussed controversy (59).

The muscle Pump

The quickest response of local muscle perfusion to non-static exercise is the so-called “muscle pump” (89). During a muscle contraction, blood flow through the muscle is impeded (100). The blood is either pumped into the venous system or backwards, into the arterial system. In the phase of relaxation, blood can travel back to the muscle, whereby venous valves prohibit reperfusion from the venous system. Consequently, reperfusion from the arterial system is forced because of the pressure gradient. However, there exist several explanation for how the muscle pump accelerates blood flow (100), but the contribution of the muscle pump to exercise induced hyperemia is very likely (68).

3.3. Local muscle blood supply and exercise

Local muscle blood supply is enhanced during (22, 43, 79, 100) and following exercise (6, 27). In literature, one can find hints that a prolonged enhanced local blood supply is dependent on the modality of exercise, i.e. exercise duration, exercise intensity and the possible presence of a priming exercise. Priming exercise means an exercise bout that is conducted prior to the main exercise in order to effect a metabolic preconditioning of metabolic pathways and cardiovascular mechanisms (59). For example, aerobic metabolism is enhanced by intensive prior exercise. In fact, interval training somehow is a repeated set of low intensity exercise bouts, each with an intensive prior exercise bout. So it is not surprising that interval training can enhance aerobic metabolism. There is evidence that interval training improves local microvascular blood supply very effectively (5, 43), whereas this effect is mainly triggered by heavy intensity work (79). Murias et al. could show a faster adjustment of microvascular O_2 delivery during moderate intensity exercise following a prior heavy intensity exercise bout. Heavy intensity bouts followed by moderate intensity bouts

represent the approach of HIT. A faster adjustment of local blood supply to exercise on-transitions reduces the O_2-deficit and hence reduces anaerobic energy contribution (21). The concept of O_2 (Fig. 4) debt describes the depletion of phosphocreatine stores and lactic acid accumulation as a compensation of lagged aerobic metabolic pathways during exercise on-transitions (21, 59). For moderate exercise in example, this means that the difference of currently aerobically synthesized adenosine triphosphate (ATP) during exercise on-transition to that amount of aerobic energy, which would be gained during steady-state conditions with the same exercise intensity. Because glucose provides 19 times more energy when it is metabolized aerobically, lactic metabolism very inefficient. Consequently, a faster response of aerobic metabolism during exercise on-transition preserves valuable energy sources. Furthermore, aerobic metabolism is enhanced during interval

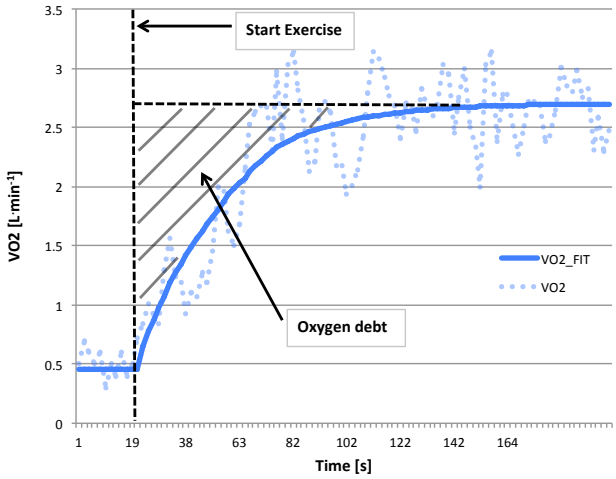

Figure 4 Schematic graphic oft the concept of O_2 debt.
VO_2_FIT is the mono-exponentially fitted raw signal (VO_2).

training and therefore provides a different stimulus to an isocaloric constant load exercise. In combination with anaerobic adaptations caused by high intensities within HIT (17), this kind of training is favorable in games like soccer or basketball.

The key mechanisms underlying improved blood supply and O_2 delivery to active muscles following priming exercise are not entirely determined. However, many determinants triggering local blood supply have been identified (see chapter 2.2). The activity of vasoactive substances does not stop immediately with cessation of exercise, so it is likely that a residual vasodilation enables a prolonged hyperperfusion (45) to recently active tissue and, hence, a better O_2 availability

during the next exercise on-transition. Beside the mechanisms that are mentioned above, metabolic acidosis could be presumed for playing a major role in regulation of O_2 availability. On the one hand, it has been shown in rats that lactate can act as a local vasodilator. Hence, exercise that is accompanied by lactic acid accumulation may promote local vasodilation. In general, blood lactate level increases at exercise intensities above GET. However, this approach is discussed controversy in literature (59).

Metabolic acidosis also triggers O_2 availability by altering the chemical affinity of oxygen to hemoglobin. The Bohr-effect describes the pH-dependence of the O_2 dissociation curve and therefore the diffusional gradient for O_2 between capillary blood and mitochondria of exercising muscles (12, 45), which is improved at lower pH-levels.

However, the relative contribution of the described factors on end-exercise and post-exercise O_2 availability has not been quantified in detail.

3.4. Advantages of increased local blood supply during exercise

It had been generally accepted that O_2 availability does not appear as the bottleneck in delayed O_2 uptake kinetics in response to exercise on-transitions (2). Instead, a limitation of O_2 utilization, i.e. the capacity of cellular oxidative pathways, had been suggested to be responsible for delayed O_2 uptake kinetics. However, those studies usually measured thigh blood flow and O_2 extraction when estimating local O_2 delivery and utilization. (2, 49). Although showing clearly that O_2 availability is not limiting O_2 uptake kinetics on that level, researchers already considered a mismatched blood flow distribution to the exercising muscles. This hypothesis has been supported by later studies using NIRS to monitor local muscle oxygenation (32, 79, 114). Those studies could show a dependency of local muscle O_2 utilization on O_2 delivery whereas high delivery could enhance O_2 utilization during exercise on-transition (78, 79). A better blood supply to working muscles has several advantages; on the one hand, a better O_2 availability, as mentioned above, enhances oxidative metabolism during exercise on-transitions and therefore preserves energy sources by avoiding a greater contribution of anaerobic metabolism. On the other hand, it has been shown that local perfusion has implications on metabolic pathways by

regulating substrate availability (88) and substrate uptake rates of muscle cells (66). In general, Romijn et al. could show a restricted fatty acid availability during severe exercise but this restriction is turned in an increased availability after exercise (88). This was attributed to reduced perfusion of adipose tissue during severe exercise and a “wash out” of fatty acid following exercise secondary to reperfusion. Hence, a better local muscle blood supply will improve fatty acid availability in working muscles. Laaksonen et al. could show a significant dependency of free fatty acid uptake in muscle cells on local blood flow. Taken together, both mechanisms could contribute positively to fatty acid oxidation, most importantly post-exercise, or, referred to HIT, during the low intervals. Adaptations in succession to exercise are triggered by perturbation of homeostasis, e.g. by very high-energy demand. Positive adaptations aim to prepare the organism for those situations by improving all systems accordingly to their specific demands during exercise (53, 98). In case of fatty acid oxidation, those adaptations include increased mitochondrial mass and enzyme activity triggered by high rates of fatty acid oxidation during low interval within HIT regimens. In conclusion, it is conceivable that improvements in fatty acid oxidation by HIT (95) could be facilitated by enhanced local blood supply.

3.5. NIRS as a functional tool to monitor local muscle blood supply

Near infrared spectroscopy (NIRS) is a technique with growing popularity over the last two decades. Its advantages are its noninvasiveness and flexibility, which enables various applications in clinical, basic and applied sciences. In the last year, mobile devices with integrated data-logging option came up. This development provided new options, especially for measurements during exercise in field and laboratory. In humans, NIRS is applied to measure tissue oxygenation in organs, brain and connective tissue (11).

In brief, NIRS is based on specific NIR-light absorption properties of hemo- and myoglobin (Hb, Mb) (39). Generally, the propagation of light through a certain tissue is influenced by reflection, scattering and absorption (61) Distinct NIR-wavelength travel through skin and subcutaneous tissue and are solely partially absorbed by Hb

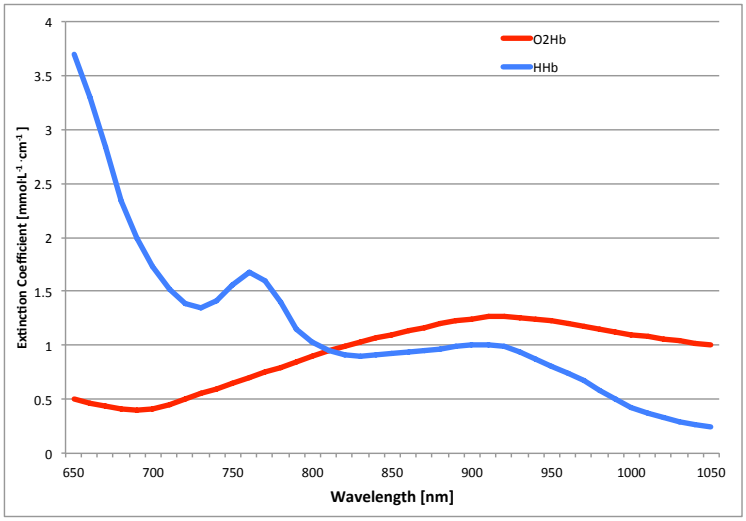

Figure 6 Extinction coefficients of O_2Hb and HHb for wavelengths from 650 nm to 1050 nm; modified from Wray et al. (113)

and Mb. To gain information, it is important that the specific wavelength is not absorbed completely. This principle is called “relative transparency” (102). Wavelengths from 700-1300nm comply with those requirements while wavelengths below are completely absorbed by hemoglobin and the transmission of wavelengths

Figure 5 Left panel: NIRS device „portamon“, Artinis (Zetten, NL); middle panel: NIRS device upside down (three transmitter optodes and one receiver optode), covered in plastic foil for protection against humidity and placed on adhesive tape; right panel: subject sitting on a bicycle ergometer (Lode excalibur, Groningen, NL), wearing the NIRS device (covered to avoid influences of ambient light) on the right thigh and a spirometry mask.

above is prevented by water (61) (Fig. 5). Extinction of NIR-light of a distinct wavelength by Hb and Mb is not constant throughout the NIR-spectrum. Extinction coefficients have therefore been examined in laboratory experiments (113) (Fig. 5).

A NIRS device consists of one or more transmitter optodes and one receiver optode (Fig. 6). To associate a NIRS measurement to a specific tissue, it is important that receiver and transmitter are located on the same site of a body segment.

Otherwise, photons would have to pass bones, tendons or other muscles, which are not under investigation in the particular measurement. Within a measurement, photons are scattered to different directions. Those photons which are received by the receiver optode do not penetrate the tissue linearly, but describe a curved trajectory (26) (Fig. 6). Consequently, this trajectory is longer, than the receiver-transmitter-distance. To determine exact photon pathlength, time-resolved spectroscopy is needed. This technique measures the time-of-flight of photons and therefore provides information about the pathlength. Because time-resolved spectrometers are of high-cost and unfeasible for many applications, continuous-wave spectrometers (CWS) are commonly used in applied sciences. Unfortunately, the pathlengths of photons are uncertain in CWS, but there exist recommendations for pathlength-corrections factors (differential pathlength factor, DPF) for various human tissues including muscle and brain (34) that can be used for CWS measurements. These DPFs have been derived from laboratory measurements using time-resolved spectroscopy. The penetration depth of NIR-light is supposed to be approximately half of the receiver-transmitter distance (102).

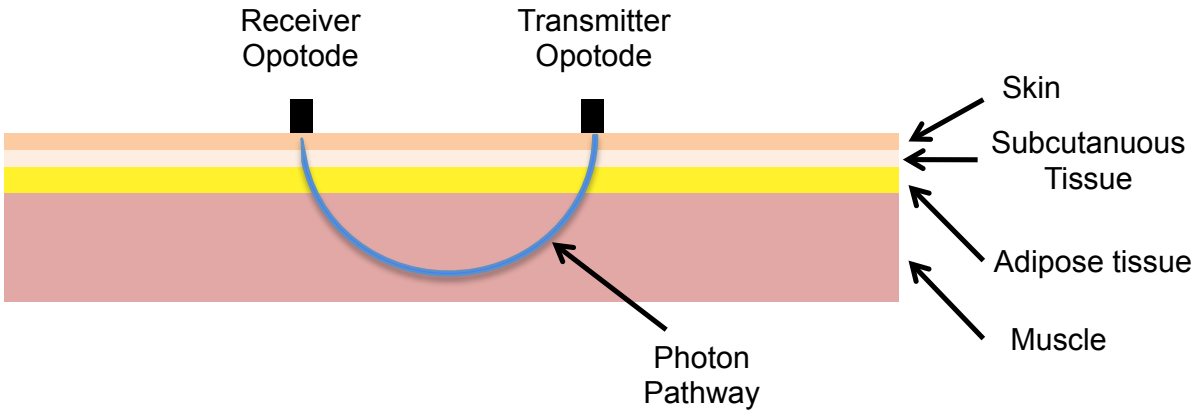

Figure 7 Schematic photon trajectory and passed-through tissue.

Beside Hb and Mb, NIR-light between 700 and 1300nm is absorbed by cytochrome C oxidase, a mitochondrial protein (104). But because of the small content of

cytochrome C oxidase compared to Hb and Mb, the contribution to NIRS signal is minimal and usually is hidden by the noise of the signal.

Considering the parameters mentioned above, one can apply the Lambert-Beer-law (Eq. 1 & 2), which describes the dependency of light attenuation (optical density, OD) from the concentration of a chromophor (c), the wavelength-specific (λ) extinction coefficient (ε) and the distance from the transmitter optode to the corresponding receiver optode (L). By solving this equation, the concentration of a chromophor can be determined. Because the distance of transmitter and receiver is not known, the Lambert-Beer-Law has been modified (34, 35) by including the DPF. Furthermore, light attenuation by scattering (OD_S) is considered. However, the latter cannot be quantified and is assumed to be constant throughout a measurement.

$$OD_\lambda = \varepsilon_\lambda \cdot c \cdot L$$

Equation 1 Lambert-Beer law; OD_λ = optical density of a distinct wavelength λ; ε_λ = extinction coefficient of the chromophor for a distinct wavelength λ; c = concentration of the chromophor; L = pathlength of the photons between transmitter and receiver optode.

$$OD_\lambda = \varepsilon_\lambda \cdot c \cdot L \cdot DPF \cdot OD_{S\,\gamma}$$

Equation 2 Modified Lambert-Beer law (34, 35); L represents the linear distance between transmitter and receiver optode and is corrected with the DPF; $OD_{S\,\lambda}$ is assumed to be constant throughout a measurement.

Because of their different absorption properties, it is possible to distinguish between oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin (39). Accordingly, this results in two sets of equations, which differ in the use of the different extinction coefficients for a specific NIR-wavelength. Each equation contains one unknown, which can be solved by substitution.

The estimates for scattering (OD_S) and the DPF are varying slightly among different authors. Therefore, several algorithms have been published for NIRS (72). This individuality of tissue absorption properties is the reason why CWS NIRS is used in qualitative approaches rather than as a quantitative method. Concentrations of O_2Hb, HHb and total hemoglobin (THb = O_2Hb + HHb) are monitored as relative changes

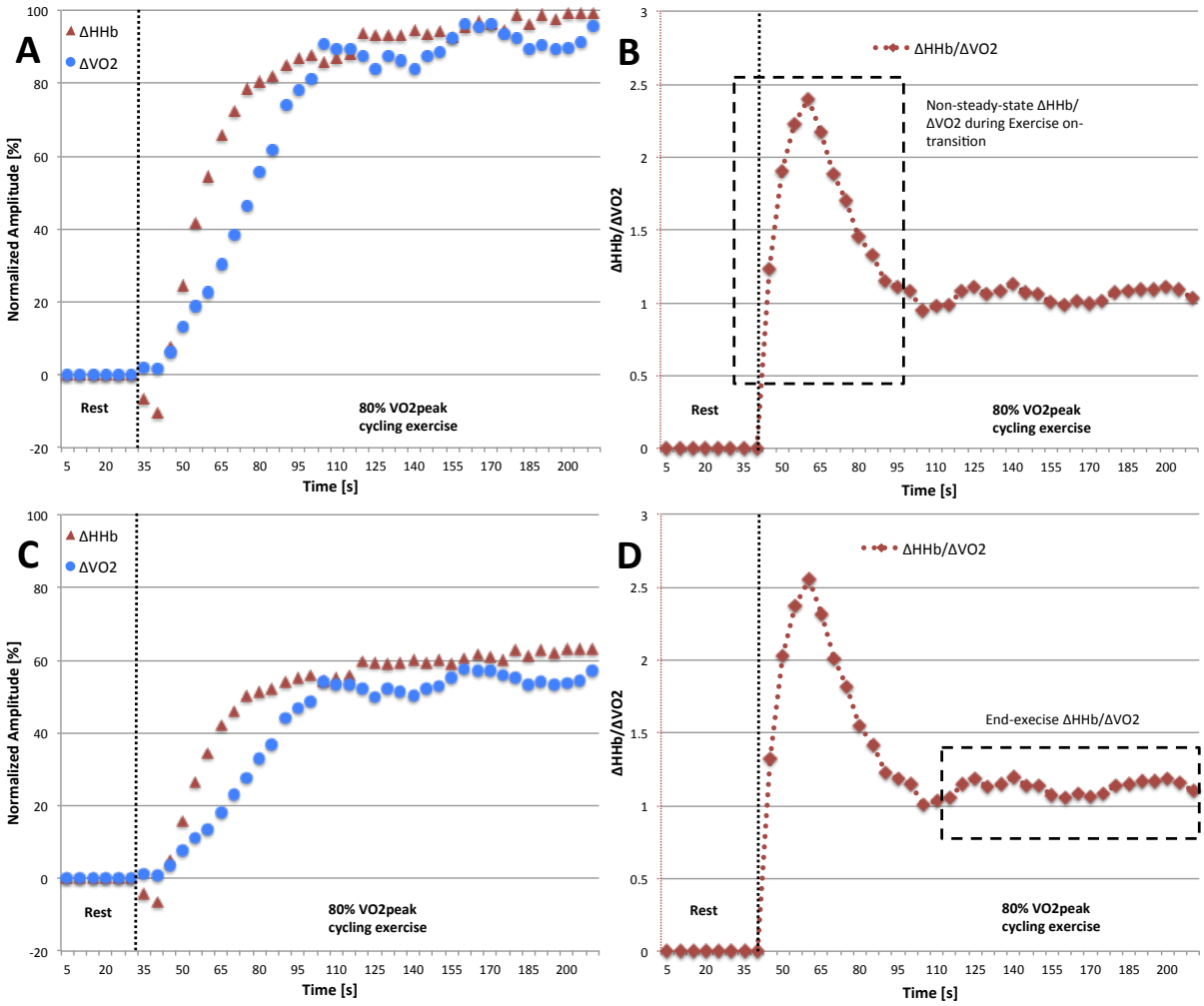

Figure 8 (A) and (C) show time courses of VO_2 and ΔHHb during cycling exercise at 60% VO_{2peak}; VO_2 and ΔHHb in (A) are normallized to the corresponding end-exercise values while data shown in (C) were normalized to maximum values derived from an prior incremental exercise test to exhaustion; (B) shows the corresponding ΔHHb/ ΔVO_2 ratio to (A) with focus on the exercise on-transition, like it has been applied by Murias et al. (80); (D) shows the corresponding ΔHHb/ ΔVO_2 ratio to (C) with focus on the end-exercise ratio, like it has been applied in the present project.

(ΔO_2Hb, ΔHHb, ΔTHb) in relation to a prior measured baseline, described either in µmol/L or in arbitrary units (A.U.). Quantitative measurements address muscular O_2 uptake (mVO_2) and muscle capillary blood flow (Q_{cap}). Those parameters can only be determined by the use of interventions in order to apply a "physiological calibration". These interventions are the arterial and venous occlusion method (105) and have been applied in several studies (14, 18, 30, 31, 58, 101). During arterial occlusion, a

blood pressure cuff is inflated rapidly well above the systolic pressure and will restrict both, in- and outflow. By the rate of muscle deoxygenation, mVO_2 can be estimated and the maximum deoxygenation of the specific tissue location can be assessed. When the cuff is only inflated above the diastolic pressure, only the venous outflow is restricted. This intervention enables the assessment of Q_{cap} by monitoring the increase in ΔTHb. The disadvantage of both interventions is that they cannot be applied continuously.

Using the qualitative approach, ΔO_2Hb and ΔHHb are influenced by O_2 utilization (20, 31) and by muscle perfusion (30). For example, when perfusion is limited while local O_2 utilization is unchanged, ΔO_2Hb will decrease. If local O_2 utilization rises and local perfusion cannot compensate the higher O_2 demand completely, ΔHHb will rise. If local perfusion had been sufficient to compensate the higher O_2 demand, ΔHHb would have stayed unchanged.

The sum of ΔO_2Hb and ΔHHb, ΔTHb, has been reported to reflect blood volume changes (25, 39, 58, 109).

Additionally to the dynamic balance of muscle perfusion and O_2 extraction, which is indicated by ΔO_2Hb, ΔHHb and ΔTHb, NIRS parameters have been combined with other physiological measures for further investigations. The ratio of pulmonary O_2 uptake (VO_2) and ΔHHb has been proposed as an indicator for an microvascular matching of O_2 delivery and utilization (78). Originally, this parameter has been introduced by DeLorey in 2004 (33), but has become more and more popular within the last six years. Murias et al. used this method in several studies (78-81). In these experiments, exercise on-transitions were investigated. Therefore, the amplitude of VO_2 and ΔHHb responses of an exercise bout were normalized to end-exercise values. Then, $\Delta HHb/\Delta VO_2$ ratio was calculated. In older subjects and untrained subjects, this ratio reached values well above 1 in the early phase of the exercise on transition (Fig. 8B). This effect was minor in trained subjects, after a training period or in younger subjects. The theorem behind is that with increasing local O_2 demand, VO_2 increases because local muscle O_2 extraction is enhanced. Accordingly, O_2Hb is unloaded, which should be visible by the measured ΔHHb. As mentioned above, the characteristic of the change in ΔHHb is dependent of change in microvascular perfusion. Consequently, VO_2 and ΔHHb can evolve slightly different, which provides

information about microvascular perfusion. In example, when VO_2 and ΔHHb are rising but the increase in ΔHHb is minor compared to that of VO_2, then microvascular perfusion adapts better or maybe quicker to exercise as if ΔHHb would have been rising equally or even stronger than VO_2. In the present project, $\Delta HHb/\Delta VO_2$ ratio will be calculated with data normalized to maximum values of a prior incremental test in order to enable comparisons of the end-exercise matching of O_2 delivery and utilization of different exercise bouts (Fig. 8C/D).

Summed up, qualitative CWS NIRS provides valuable information about microvascular perfusion, blood supply and O_2 availability despite of the inability to quantify blood flow in l/min as the original perfusion parameter. This issue has been addressed by Ferreira and colleagues (40), who modeled capillary blood flow (Q_{cap}) by re-arranging the Fick principle. Recently, this approach has been doubted and $\Delta HHb/\Delta VO_2$ ratio has been suggested as a more accurate estimate for microvascular perfusion (81). Details to Q_{cap} are provided in chapter 5.2.

4. Aim of the project

This project aimed to examine the relationship between post-exercise microvascular blood supply and O_2 availability in relationship to exercise intensity and exercise duration.

Acute effects of exercise on local muscle perfusion have been focused in several investigations (4, 22, 57, 89, 100). Surprisingly, post-exercise effects have been addressed only by few studies and the relationship to exercise duration and intensity has been focused rather undifferentiated, i.e. only few different exercise durations or intensities had been studied.

In 1992, Chance et al. showed a marked overcompensation of muscle oxygenation (percentage of O_2Hb to THb) following rowing exercise. Later, Bhambhani et al. examined four different exercise intensities (40%, 80% LT and 25%, 50% LT-VO_{2max}) on muscle oxygenation (6). They found a proportional decrease of muscle oxygenation with increasing exercise intensity. During early recovery, they observed a post-exercise overcompensation in muscle oxygenation, which also happened in

proportion to work rate. This “physiological payback” to exercise (27) could also been observed in further studies.

Although those studies could show an increased overcompensation in post-exercise muscle oxygenation with increasing exercise intensity, the significance of those findings is limited, because muscle saturation solely describes O_2Hb as a percentage of THb. Thus, only limited information is provided about changes in concentrations of oxygenated Hb (O_2Hb), deoxygenated Hb (HHb), or THb, and consequently neither can indicate a hyperemia nor are appropriate estimates for microvascular perfusion.

Ferreira et al. (40) investigated Q_{cap} in response to two different exercise intensities (below and above LT). Q_{cap} recovery showed to be slowed following heavy intensity exercise indicating a higher post-exercise perfusion.

Even less studies are available on post-exercise muscle perfusion and exercise duration. Zafeiridis et al. compared three different training regimens (continuous exercise, HIT with long intervals and short intervals). No differences could be observed regarding the matching of O_2 delivery and utilization among all protocols. However, because the authors applied different exercise intensities for each training regimen, these results cannot be assigned causally to exercise duration (detailed information is provided in chapter 6.3, section “Discussion”).

Beside the limitations of muscle oxygenation as an estimate for microvascular perfusion, the number of different exercise intensities or durations is crucial. Most studies compared two or three different exercise intensities or durations, which may suggest linear relationships but might mask the real dependency on exercise intensity or duration. Moving ahead, this project aims to examine the detailed relationship of post-exercise blood supply and O_2 availability in relation to HIT-associated exercise intensities and durations during cycling exercise.

5. Study design

The detailed methodology of all parts of this study is contained in the “Methods” section in chapters 6.1, 6.2 & 6.3.

The general protocol of this cross-sectional study was separated in three different sessions (Fig. 9). After inclusion to the study (inclusion criteria are displayed "Methods" section in chapters 6.1 & 6.3), the participants reported to the laboratory for a graded exercise on a cycle ergometer test to exhaustion. This session aimed to determine VO_{2peak} and GET. In the second and third session, a customized protocol was used to determine post-exercise blood supply and O_2 availability in following different exercise intensities and durations. The order of these sessions was assigned using a cross-over design.

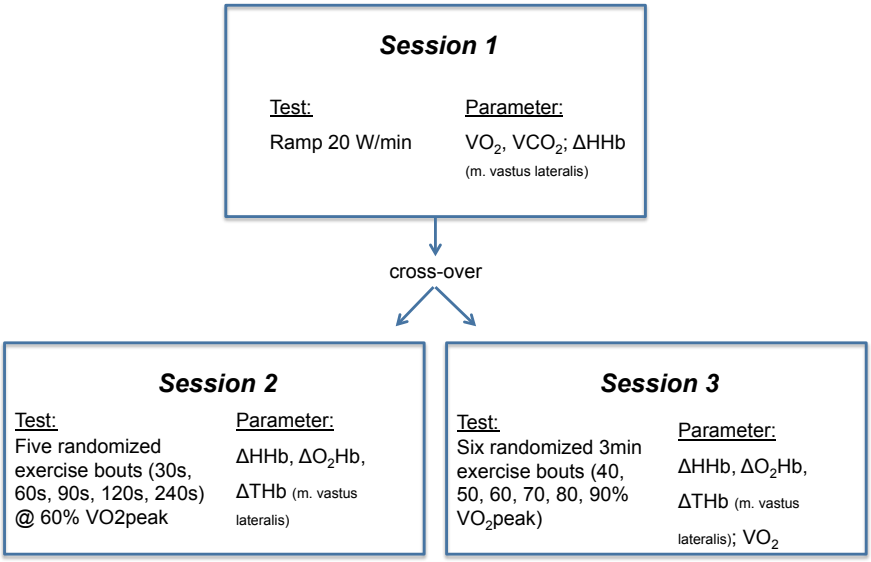

Figure 9 Schematic order of the study design; detailed descriptions of each session can be derived from the chapters 5.1 & 5.3.

6. Project Studies: Introductions, Methods, Results and Discussions

6.1. Study 1: End-exercise ΔHHb/ΔVO2 and post-exercise local oxygen availability in relation to exercise intensity

Stöcker F, **Oldershausen Von C**, **Paternoster FK**, **Schulz T**, **Oberhoffer R**. End-exercise ΔHHb/ΔVO2 and post-exercise local oxygen availability in relation to exercise intensity. *Clin Physiol Funct Imaging* [epub ahead of print], 2015. doi: 10.1111/cpf.12314.(92)

End-exercise $\Delta HHb/\Delta VO_2$ and post-exercise local oxygen availability in relation to exercise intensity

Short title: End-exercise $\Delta HHb/\Delta VO_2$ and post-exercise oxygen availability

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Abstract

Increased local blood supply is thought to be one of the mechanisms underlying oxidative adaptations to interval training regimes. The relationship of exercise intensity with local blood supply and oxygen availability has not been sufficiently evaluated yet. The aim of this study was to examine the effect of six different intensities (40–90% peak oxygen uptake, VO_{2peak}) on relative changes in oxygenated, deoxygenated and total hemoglobin (ΔO_2Hb, ΔHHb, ΔTHb) concentration after exercise as well as end-exercise $\Delta HHb/\Delta VO_2$ as a marker for microvascular O_2 distribution. Seventeen male subjects performed an experimental protocol consisting of 3 min cycling bouts at each exercise intensity in randomized order, separated by 5 min rests. ΔO_2Hb and ΔHHb were monitored with near-infrared spectroscopy of the vastus lateralis muscle and VO_2 was assessed. $\Delta HHb/\Delta VO_2$ increased significantly from 40% to 60% VO_2peak and decreased from 60% to 90% VO_2peak. Post-exercise ΔTHb and ΔO_2Hb showed an overshoot in relation to pre-exercise values, which was equal after 40–60% VO_{2peak} and rose significantly thereafter. A plateau was reached following exercise at ≥80% VO_{2peak}. The results suggest that there is an increasing mismatch of local O_2 delivery and utilization during exercise up to 60% VO_2peak. This insufficient local O_2 distribution is progressively improved above that intensity. Further, exercise intensities of ≥80% VO_{2peak} induce highest local post-exercise O_2 availability. These effects are likely due to improved microvascular perfusion by enhanced vasodilation, which could be mediated by higher lactate production and the accompanying acidosis.

Keywords

muscle oxygenation; hyperemia; near-infrared spectroscopy; exercise intensity; post-exercise muscle perfusion; prior exercise

Introduction

Interval training regimes have become increasingly popular as a result of well-documented beneficial effects on different aspects of physical performance (16, 69).

Especially high intensity interval training (HIIT) has been successfully applied with highly trained (69), recreationally active subjects (38, 95) as well as sedentary, older subjects and such with

cardiac disease (43, 46, 55). In recent years, many positive effects have been demonstrated not only anaerobically, as a result of the high anaerobic energy contribution (17), but also aerobically. Studies have shown that interval training is an effective training method to improve (micro) vascular (9, 22, 73) and oxidative (38, 55, 95) functions.

Possible mechanisms underlying oxidative adaptations are enhanced blood flow and local blood supply of small vessels in the active tissue (22, 43). Recently, the effect of priming exercise, i.e. exercise that is meant to activate metabolic processes prior to the real exercise, has been attributed to an enhanced microvascular oxygen (O_2) distribution (79) by improved capillary perfusion (33). Capillary perfusion has been outlined as a determinant for fatty acid metabolism (66, 91). Because fatty acid oxidation is restricted during exercise at high intensities (88), it is likely that mechanisms causing oxidative adaptations in terms of fatty acid oxidation take effect in the resting interval of an interval training session and profit from the elevated microvascular perfusion. Thus, in order to study the effect of work intervals in interval training regimes, the recovery from the work interval has to be considered in addition to acute effects during the work interval itself.

Interval training regimes have become more and more popular for training with sedentary, older subjects and such with cardiac disease (43, 46, 55). This leads to the question which minimum exercise intensity is needed to "trigger" elevated microvascular perfusion.

In order to examine the relationship between exercise intensity and O_2 availability during and after exercise, two issues were addressed with this study: The first aim was to determine the minimum exercise intensity, which is sufficient to elicit higher post-exercise local blood supply and O_2 availability. The second aim was to assess the detailed relationship between exercise intensity and microvascular O_2 distribution. To examine those issues, relative changes in hemoglobin (ΔO_2Hb, ΔHHb, ΔTHb) concentrations of small muscle vessels in the vastus lateralis muscle and pulmonary oxygen uptake (VO_2) were assessed during and following six levels of exercise intensity.

Methods

Subjects

Seventeen healthy, physically active males volunteered to participate in the study (see Tab 1). The participants exercised at least two times per week in various sports whereas aerobic exercise contributed on average with 4 ± 3 h per week. Only subjects aged 18-35 yrs with physically active background were recruited while subjects with acute or chronic diseases such as chronic heart disease, diabetes type II, epilepsy, relevant diseases of liver and kidney as well as smokers and subjects with excessive alcohol consumption were excluded from the study. All subjects were familiar with cycling exercise and exhaustive exercise testing. They were provided detailed information about the purpose and procedures of the study and their written informed consent was obtained. The local Ethics Committee approved the study protocol (#67/14).

Table 1. Subject data

Parameter	
n	17
Age (years)	28 ± 5
Weight (kg)	77.4 ± 4.9
Height (cm)	180.1 ± 5.2
Skinfold thickness (mm)	7.5 ± 3.1
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	50.1 ± 5.8
GET (%VO_2peak)	59 ± 13

values are expressed as mean ± SD

Test design and procedures

Subjects reported to the laboratory on two sessions. The first session consisted of preliminary testings, and the following experimental session aimed to examine local blood supply and oxygen availability in relation to exercise intensity. Both sessions had to be completed within one week, and sessions had to be separated by at least 48 h. The subjects were instructed not to perform exhaustive activity 24 h before each session and not to eat and to drink caffeinated beverages or such with high content of carbohydrates 2 h before each session. Special bicycle shoes and pedals were not allowed during the ergometer tests in order to reduce the influence of cycling technique.

Preliminary testing

In the first session, all subjects performed an incremental test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur; Lode, Groningen, The Netherlands) to determine VO_{2peak} and the gas exchange threshold (GET) according to the v-slope method (Beaver et al., 1986). The load was increased from an initial 60 W by 10 W every 30 s. The cadence could be freely chosen by the subjects but had to be maintained >70 revolutions/min. The test was terminated when the cadence could not be maintained any longer.

Experimental testing

The subjects were asked to complete a randomized protocol consisting of one 3 min interval at each of the six different exercise intensities (40, 50, 60, 70, 80, and 90% VO_{2peak}) separated by a 5 min passive recovery period. The order of the bouts was assigned by lot. Previously, recovery periods of 3 min have been used for similar measurements (Buchheit et al., 2011) but based on prior observations, a 5 min recovery period was chosen. Prior to the first interval, a 3 min baseline measurement ($BASE_0$) was conducted with the subject sitting passively on the ergometer, followed by a warm-up, consisting of 1 min at 60% VO_{2peak} and 3 min at 80% VO_{2peak}. This priming exercise effected a preconditioning of local muscle blood flow and metabolism in order to avoid order effects for the first experimental condition. During $BASE_0$ and during the rest periods in between exercise bouts, the subjects had to maintain a standardized position keeping the right foot on a bar in between the pedals to minimize movement artifacts by unwanted muscular activity on the NIRS signal.

Fig 1 shows a scheme of an exemplary experimental protocol.

Gas exchange and heart rate measurement

During all tests, heart rate was monitored (Polar Team[2,] Polar Electro, Kempele, Finland) and respiratory parameters were measured continuously with a breath-by-breath spirometer (ZAN 600USB; Nspire Health, Oberthulba, Germany). Following the incremental exercise test and the experimental test, oxygen uptake (VO_2) curve was smoothed with a 30 s moving average while heart rate data were smoothed with a 5 s moving average. Peak heart rate was assessed for each exercise bout of the experimental test. VO_2 data obtained during the incremental exercise test was used to determine VO_{2peak} and GET. VO_{2peak} was determined as the highest value reached during exercise and was expressed relatively to body weight ($ml \cdot kg^{-1} \cdot min^{-1}$). GET was determined using the v-slope method (3) and confirmed with the ventilatory equivalents for O_2 and CO_2.

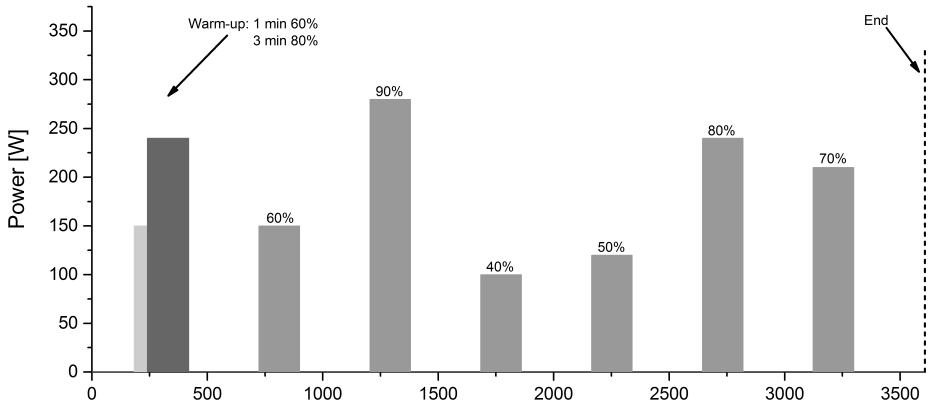

Fig. 1 Scheme of an exemplary experimental protocol. Subjects had to maintain in a standardized recovery position sitting on the ergometer during the baseline measurement prior to the warm-up and the recovery periods in-between the exercise bouts.

Near-infrared spectroscopy

Local changes in hemoglobin concentration of the right vastus lateralis muscle were measured continuously with a wireless continuous-wave NIRS device (PortaMon, Artinis Medical Systems, Zetten, The Netherlands) during all sessions. The PortaMon probe consists of three light sources (wavelengths: 760 nm and 850 nm) separated by 3, 3.5, and 4 cm from the receiving optode. The probe was firmly attached to the shaved skin above the distal part of the right vastus lateralis muscle with adhesive tape. The probe was covered with a lightproof cloth to prevent any influences of

ambient light. The position of the probe was marked with a surgical marker to detect possible shifts of the probe during the test.

PortaMon simultaneously uses the modified Lambert–Beer law and spatially resolved spectroscopy (39), which enables the observation of relative changes in O_2Hb, HHb, and THb. Because the exact pathlength of the photons, which penetrate the tissue is unknown when using continuous-wave spectroscopy, a differential pathlength factor has to be used (39). In this study, a differential pathlength factor of 4 was chosen. It is commonly accepted that THb reflects blood volume (39, 58, 99, 105). Because hemo- and myoglobin have equal absorption properties, it is not possible to distinguish between the contributions of hemo- and myoglobin on the NIRS signal. However, the contribution of myoglobin to the NIRS signal is assumed to be minimal (39).

Table 2. OS_THb/O_2Hb/HHb, Hemoglobin recovery times, Time-to-peak

$\%VO_{2peak}$	40%	50%	60%	70%	80%	90%
OS_THb ($\Delta\mu mol \cdot l^{-1}$)	4.32 ± 1.42	4.89 ± 2.19	5.17 ± 6.33	6.33 ± 2.04	7.52 ± 2.26	7.54 ± 3.14
OS_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	2.78 ± 2.40	2.86 ± 2.02	3.20 ± 2.78	5.41 ± 2.93	6.54 ± 2.70	6.65 ± 3.53
Recovery of ΔTHb (s)	193 ± 83	155 ± 64	190 ± 76	191 ± 68	201 ± 72	216 ± 54
Recovery of ΔO_2Hb (s)	208 ± 70	206 ± 76	222 ± 49	244 ± 43	220 ± 66	221 ± 50
Recovery of ΔHHb (s)	217 ± 50	210 ± 84	231 ± 55	236 ± 43	213 ± 79	196 ± 80
SD_REC_ ΔTHb ($\Delta\mu mol \cdot l^{-1}$)	0.33 ± 0.17	0.33 ± 0.15	0.32 ± 0.22	0.28 ± 0.12	0.59 ± 1.19	0.29 ± 1.83
SD_REC_ ΔO_2Hb ($\Delta\mu mol \cdot l^{-1}$)	0.26 ± 0.11	0.30 ± 0.14	0.30 ± 0.19	0.22 ± 0.11	0.48 ± 0.99	0.24 ± 0.11
SD_REC_ ΔHHb ($\Delta\mu mol \cdot l^{-1}$)	0.16 ± 0.10	0.20 ± 0.18	0.19 ± 0.12	0.16 ± 0.07	0.22 ± 0.21	0.15 ± 0.11
Time-to-peak ΔTHb (s)	35 ± 35	52 ± 64	52 ± 69	50 ± 33	60 ± 38	78 ± 48
Time-to-peak ΔO_2Hb (s)	234 ± 82**	185 ± 102**	125 ± 93	78 ± 40	91 ± 56	106 ± 45

n = 17; values are expressed as mean ± SD; Significant results for OS_THb/O_2Hb are presented in Fig 4; SD_REC_ΔTHb/O_2Hb/HHb represent the average standard deviation of the last 30 s of recovery; Time-to-peak values indicate the time from cessation of exercise to the highest value reached during the subsequent recovery period (asterisks indicate significant higher values compared to the other values, ** = $P < 0.01$)

NIRS data were collected with the software Oxysoft (Artinis Medical Systems) and were sampled with a frequency of 10 Hz and expressed relative to baseline values as $\Delta\mu mol \cdot L^{-1}$. Only data measured with the 3.5 cm optode distance were taken into account, corresponding to a penetration depth of approximately 1.75 cm (23, 102). After collection, the data were smoothed with a 1 s moving average. The data of the last 30 s of $BASE_0$ were averaged. Baseline values were measured again for each exercise bout in the last 30 s of the previous recovery period ($BASE_{40–90}$). Furthermore, the recovery time of ΔTHb/O_2Hb/HHb was determined. Therefore, the time elapsed from cessation of

exercise and the first value which was between the average of the last 30 s of recovery ± five standard deviations was calculated (Tab 2). End-exercise values (averaged over the last 10 s of exercise) were assessed and expressed in relation to the respective baseline values for each exercise bout (EE_ΔO2Hb/ΔHHb/ΔTHb). The early post-exercise overcompensation appears as an overshoot (OS) of ΔO_2Hb and ΔTHb after exercise and was also expressed in relation to baseline values (Fig 2) as well as the time-to-peak from cessation of exercise (Tab 2). To control possible changes of BASE, the difference between the baseline before and after each exercise bout was calculated ($\Delta BASE_{40–90}$).

Microvascular O_2 distribution

End-exercise values of ΔHHb were expressed per unit ΔVO_2. First, ΔHHb and VO_2 data from every exercise bout was physiologically calibrated for each subject. In detail, ΔVO_2 and ΔHHb from each BASE to corresponding end-exercise values were calculated for every exercise bout and normalized to the maximum ΔVO_2 and ΔHHb obtained during the preliminary incremental test. The averaged normalized data of ΔHHb and ΔVO_2 obtained during the last 10 s of each exercise bout was used to calculate $\Delta HHb/\Delta VO_2$. A similar procedure has been used previously (33).

Statistical analysis

Sample size was determined *apriori* using G*Power (Faul et al., 2009) and designated six subjects to find statistical significance at a value of $P < 0.05$ with a statistical power of 0.8. Nevertheless, 17 subjects were recruited to increase power. Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). A repeated measures analysis of variance (ANOVA) was used to detect possible differences of the effect of the various exercise intensities on OS_O_2Hb/THb, end-exercise concentrations of $\Delta O_2Hb/\Delta HHb/\Delta THb$, peak heart rate, end-exercise $\Delta HHb/\Delta VO_2$, and ΔBASE for $\Delta O_2Hb/\Delta HHb/\Delta THb$. Data were tested for sphericity with Mauchly's test and were corrected using the Greenhouse–Geisser method (Greenhouse and Geisser, 1959) when necessary. If ANOVA showed a significant main effect, pairwise comparisons were performed with Bonferroni–Holm corrected post-hoc tests. Baseline values of ΔO_2Hb and ΔTHb were individually compared with the corresponding OS values using *t*-tests. The same procedure was conducted to

compare baseline values before and after the particular exercise bouts to test for possible baseline drifts. Statistical significance was set to $P \leq 0.05$.

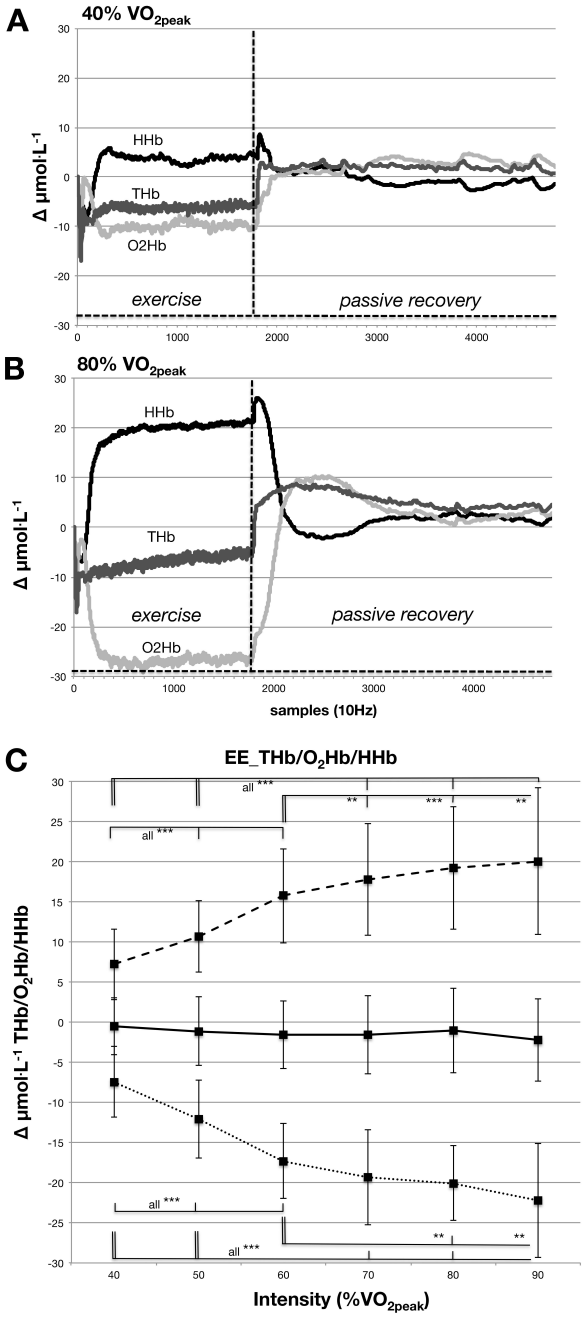

Fig. 2 (A, B) Typical kinetics of ΔO_2Hb, ΔHHb, and ΔTHb during and after 3-min cycling at 40% VO_{2peak} and 80% VO_{2peak} in relation to baseline values (set to zero). Note the marked overshoot in ΔO_2Hb following exercise at 80% VO_{2peak} compared with exercise at 40% VO_{2peak}, where highest post-exercise values are reached at the end of the 5-min recovery period. Despite an undershoot in ΔHHb after 80% VO_{2peak}, there is a ΔTHb overcompensation. Data was recorded with10-Hz and filtered with a 1-s moving average afterwards. (C) End-exercise (EE) values (mean ± SD) of ΔO_2Hb (dotted line), ΔHHb (broken line), and ΔTHb (solid line). Asterisks represent results from post-hoc tests in relation to values marked with double lines; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Results

Parameters during the exercise bouts

A representative time-course of ΔHHb, ΔO_2Hb and ΔTHb kinetics of one subject following exercise at 40% and 80% VO_{2peak} is displayed in Fig 2A/B. Exercise at 80% VO_{2peak} elicited a significantly higher deoxygenation, i.e. an increase in ΔHHb and thus a decrease in ΔO_2Hb. End-exercise values and corresponding results of the post-hoc tests are displayed in Fig 2C.

ANOVA reported a significant influence of *intensity* on end-exercise $\Delta HHb/\Delta VO_2$ ($F_{2,31} = 8.0$; $P = 0.002$). In detail, $\Delta HHb/\Delta VO_2$ increased progressively with exercise intensity from 40% to 60% VO_{2peak} ($P = 0.003$ for 40% vs. 60%, Fig 3). Above 60% VO_{2peak}, $\Delta HHb/\Delta VO_2$ decreased as shown by significantly lower values at 70%, 80% and 90% VO_{2peak} compared to 60% VO_{2peak} ($P \leq 0.007$) as well as significantly lower values at 90% vs. 70% and 80% VO_{2peak} ($P \leq 0.016$).

As expected peak heart rate increased significantly with exercise intensity ($F_{5,80} = 253.0$; $P < 0.001$) indicating significant differences between all intensity stages (all $P < 0.001$) (Fig. 4B).

Post-exercise Parameters

Immediately after cessation of exercise, ΔO_2Hb, ΔHHb and, as a consequence, ΔTHb increased rapidly. Subsequently, ΔHHb decreased and ΔO_2Hb increased persistently. ΔHHb decreased in an exponential manner and then reached a stable level within 236 s (for details, see Tab 3). ΔO_2Hb increased invertedly to ΔHHb after 40% and 50% VO_{2peak}. After 60%, 70%, 80%, and 90% VO_{2peak}, ΔO_2Hb increased rapidly and reached a peak value (OS_O_2Hb) within 125 s whereas peak values were reached significantly later following exercise at 40% and 50% VO_{2peak} (Time-to-peak, for details and post hoc results, see Tab 2). This peak value was higher than baseline, and O_2Hb subsequently decreased to baseline level (Fig 2 & 4A) within 244 s (Tab 3). $OS\_O_2Hb_{60–90}$ were significantly higher than the corresponding baseline values ($P < 0.001$). This post-exercise overcompensation also occurred for ΔTHb at all intensities ($P < 0.001$), whereas peak values where reached within 78 s. In contrast to ΔO_2Hb, Time-to-peak in ΔTHb was not significantly influenced by exercise intensity. Although there was no overcompensation with a subsequent decrease in O_2Hb after 40% and 50%

VO_{2peak} (Fig. 2A), the highest post-exercise value (which actually occurred in the end of the recovery period) was determined as the OS_O_2Hb value for 40% and 50% VO_{2peak}.

ANOVA identified a significant effect of the factor *intensity* on OS_O_2Hb ($F_{3,56}$ = 5.8; P < 0.001) and OS_THb ($F_{2,43}$ =4.7; P < 0.001). Based on post-hoc tests, OS_O_2Hb$_{70}$, OS_O_2Hb$_{80}$ and OS_O_2Hb$_{90}$ were significantly higher than OS values obtained after the exercise bouts of 40%, 50% and 60% VO_{2peak} ($P \leq 0.015$; Fig 4A).

For OS_THb, post-hoc tests revealed similar results (see Fig 4B): OS_THb$_{80}$ and OS_THb$_{90}$ were significantly higher than OS values after exercise at 40%, 50% and 60% VO_{2peak} ($P \leq 0.007$) whereas OS_THb$_{80}$ was significantly higher than OS_THb$_{70}$ (P = 0.020) and OS_THb$_{70}$ was significantly higher than OS_THb$_{40}$ (P = 0.003). Overshoot data can also been seen in Tab 2.

As mentioned above, all NIRS parameters (ΔO_2Hb, ΔHHb, and ΔTHb) reached stable values at the end of each recovery period (which was defined as the new baseline for the subsequent exercise bout). These new baselines (Tab 3) did not show any significant intensity-related differences, neither when they were sorted by exercise intensity, nor by chronological order of the exercise bouts.

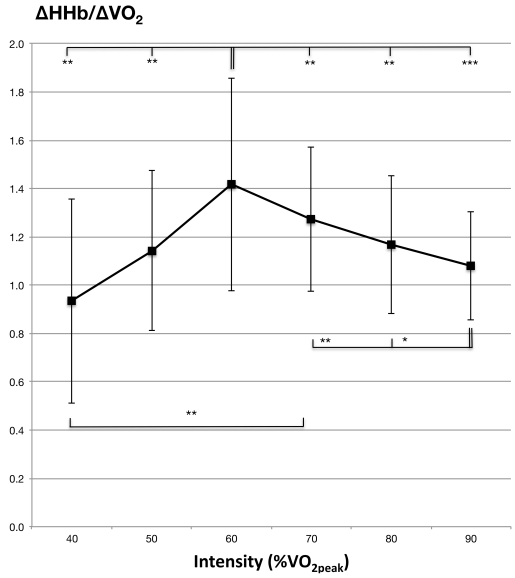

Fig. 3 End-exercise ΔHHb/ΔVO_2 values (mean ± SD) after the six exercise bouts. Asterisks represent results from post-hoc tests in relation to values marked with double lines; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

Discussion

The major finding of this study is that end-exercise $\Delta HHb/\Delta VO_2$ increased progressively with exercise intensity up to 60% VO_{2peak} and decreased at intensities above 60% VO_{2peak} (Fig 3). Further, we have shown that 60% VO_{2peak} also has evolved as a crucial point for post-exercise ΔO_2Hb and ΔTHb kinetics as is shown by rising post-exercise overshoot values for ΔO_2Hb and ΔTHb following exercise above that intensity (Fig 4).

End-exercise $\Delta HHb/\Delta VO_2$

$\Delta HHb/\Delta VO_2$ describes the microvascular fractional O_2 extraction per unit VO_2 (33). A similar approach has been used to study the matching of O_2 delivery and O_2 utilization during the adjustment phase of exercise on-transitions whereas low values indicate a better microvascular O_2 distribution (78, 81). In our study, we used end-exercise values, which were steady state values for exercise intensities below GET and non-steady state values for exercise intensities above GET due to the intensity-dependent presence of the VO_2 slow component (21).

Our results show a progressively increasing mismatch of local O_2 delivery and utilization with increasing exercise intensity up to 60% VO_{2peak} after 3 min exercise bouts as suggested by increasing end-exercise $\Delta HHb/\Delta VO_2$. Hence, end-exercise ΔHHb increased stronger in proportion to VO_2 values. This suggests that microvascular perfusion does not adequately meet the increased metabolic demand up to this point. Interestingly, $\Delta HHb/\Delta VO_2$ decreased above 60% VO_{2peak}. Consequently, the matching of HHb and VO_2 gets progressively impaired from 40% VO_{2peak} to 60% VO_{2peak} but is progressively improved at exercise intensities above 60% VO_{2peak} up to 90% VO_{2peak}. This is an interesting finding because the local O_2 extraction is supposed to increase stronger with increasing exercise intensity due to an enhanced motor unit recruitment (21). Obviously, this assumption is only valid up to a certain point, which evolved on average as 60% VO_{2peak} in the population tested within this study. However, our data provide only information about microvascular O_2 distribution following 3 min of cycling exercise. It is known that microvascular blood flow reaches a steady state within 90 s with a little further increase during very intense exercise (Saltin et al., 1998). Based on our measurements, we cannot speculate about the development of microvascular O_2 distribution during prolonged exercise.

Post-exercise hemoglobin overcompensation

THb has been reported to be a valid measure for microvascular blood volume changes (25, 39, 58, 109), so our results suggest that exercise intensities of 80% VO_{2peak} and above elicit a higher local post-exercise blood volume in the area of interrogation and hence a pronounced filling of local capillaries. This effect can be attributed solely to small vessels because NIRS is only sensitive for signals from vessels with a diameter < 1 mm (39). Smallest vessels are more relevant to study exercise induced hyperemia because vasodilation in conduit arteries does not play a functional role in regulation of blood flow in exercising muscles (96) but is most pronounced in smallest arterioles (106 & 107).

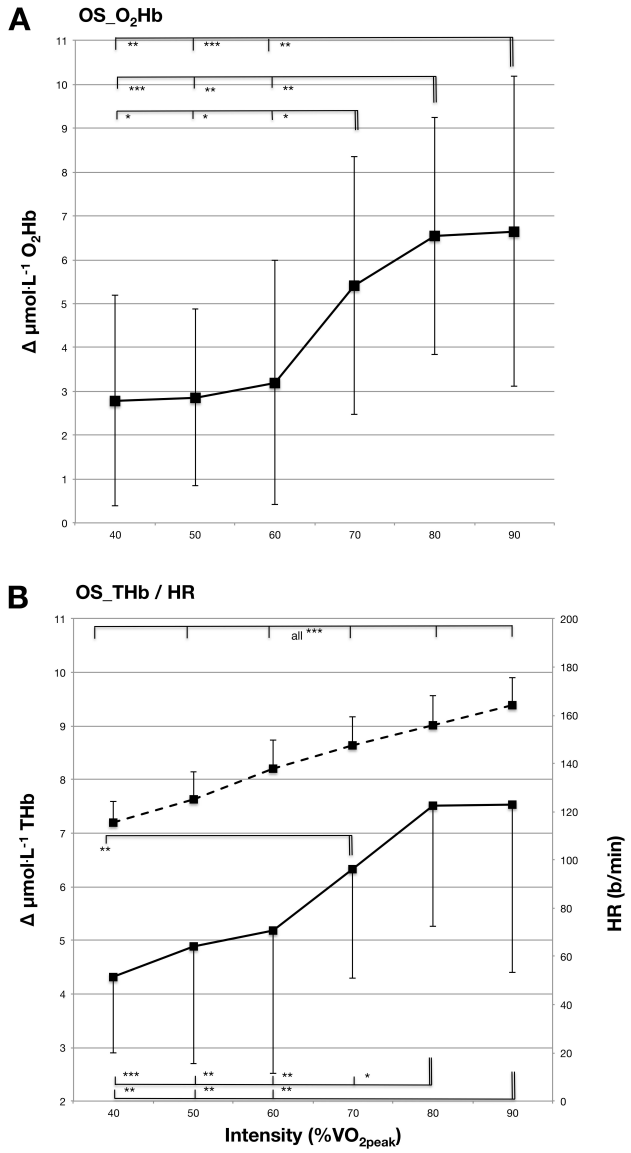

Fig. 4 (A) Overshoot values of ΔO_2Hb (mean ± SD) after the six exercise bouts. (B) Overshoot values of ΔTHb (solid line) and end-exercise (EE) heart rate values (broken line) (mean ± SD) after the six exercise bouts. Asterisks represent results from post-hoc tests in relation to values marked with double lines; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Heart rate values all differed significantly from each other at $P < 0.001$

OS_THb is a superposition of a rapid decrease in ΔHHb and a pronounced post-exercise overshoot of O_2Hb. Fig 4A shows that the initial significant increase in the OS_O_2Hb-intensity relationship starts above 60% VO_{2peak} and reached a plateau above 80% VO_{2peak}. Due to the nearly identical relation to exercise intensity, the overshoot in ΔTHb signal can be explained by ΔO_2Hb recovery kinetics and thus by arterial blood supply leading to an intensity dependent early post-exercise "blood pooling". Thus, post-exercise O_2 availability is elevated following exercise >60% VO_{2peak} and reaches a plateau following exercise ≥80% VO_{2peak}.

Possible mechanisms causing better microvascular O_2 distribution and post-exercise O_2Hb availability by exercise >60% VO_{2peak}

We have shown that exercise at 60% VO_{2peak} evolved as a crucial point for both microvascular O_2 distribution and local post-exercise O_2Hb availability. During dynamic exercise, the increased oxygen demand induces an increased cardiac output and conduit artery flow as well as sympathetic mediated vasoconstriction. Simultaneously, acute local vasodilation is effected by vasoactive substances and enables an augmented perfusion of capillaries (57, 67, 78). This results in an increased blood flow to active tissue and is accompanied by the so-called muscle pump (89), especially during upright exercise (60) as it was performed in this study. As mentioned above, there are several factors that affect local muscle perfusion. Focused on microvascular effects, local vasoactive substances likely are the major determinants. Global mechanisms do not seem to be the major determinants for the non-linear shape of $\Delta HHb/\Delta VO_2$ and OS_O_2Hb and OS_THb relationship with exercise intensity: The supportive action of the muscle pump was not active any longer when OS_O_2Hb and OS_THb occurred but stopped before with the cessation of exercise because the subjects were instructed to return to a standardized recovery position. Furthermore, end-exercise heart rate is linearly related to exercise intensity (Fig 4B) and hence would be expected to influence perfusion in a linear pattern.

So the improved microvascular O_2 distribution above 60% VO_{2peak} is apparently due to a enhanced endothelium and flow-mediated vasodilation (78) superimposing sympathetic vasoconstriction. This is also supposed to be the reason for the post-exercise overshoot of ΔTHb and ΔO_2Hb.

Vasodilation has been shown to be dependent on various factors. It can be evoked neurally and mechanically (muscle pump) but most importantly chemically and flow-mediated, either directly and or indirectly via stimulation of nitric oxide (NO) released from the vascular endothelium or both (60). In endothelium mediated vasodilation, two pathways play a central role: the NO mediated pathway via endothelial NO synthase (eNOS), which activates cyclic guanosine monophosphate (cGMP) and the prostacyclin mediated pathway via cyclooxygenase (COX), which activates cyclic adenosine monophosphate (cAMP) (54). Direct and endothelium mediated vasodilation can be activated at least partially by the same stimuli, e.g. adenosine triphospate, adenosine, acetylcholine, bradykinin,

histamine and shear stress (54). Of the mentioned stimuli, especially adenosine triphospate, adenosine, acetylcholine, and shear stress presumably are associated with exercise induced effects on vasodilation.

Table 3. Baseline drifts

A %VO_{2peak}	ANOVA	40%	50%	60%	70%	80%	90%
ΔBASE_THb ($\Delta\mu mol \cdot l^{-1}$)	P=0.099	1.39 ± 1.66	1.07 ± 1.97	0.78 ± 1.32	1.49 ± 1.83	2.30 ± 1.35	2.06 ± 1.50
ΔBASE_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	P=0.103	1.69 ± 2.18	1.16 ± 2.48	−0.13 ± 2.21	1.30 ± 2.33	1.99 ± 1.64	2.10 ± 2.65
ΔBASE_HHb ($\Delta\mu mol \cdot l^{-1}$)	P=0.205	−0.31 ± 1.34	−0.10 ± 1.48	0.91 ± 1.33	0.10 ± 0.86	0.31 ± 1.03	−0.04 ± 1.83
B No. of exercise bout		1	2	3	4	5	6
ΔBASE_THb ($\Delta\mu mol \cdot l^{-1}$)	P=0.223	1.70 ± 2.22	1.57 ± 2.36	1.82 ± 1.68	2.21 ± 1.60	0.92 ± 1.41	0.81 ± 1.90
ΔBASE_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	P=0.082	1.31 ± 2.71	2.03 ± 3.07	1.59 ± 1.77	2.32 ± 1.75	0.74 ± 1.76	0.20 ± 2.49
ΔBASE_HHb ($\Delta\mu mol \cdot l^{-1}$)	P=0.293	0.39 ± 1.07	-0.46 ± 1.72	0.22 ± 1.63	-0.11 ± 0.98	0.18 ± 1.23	0.59 ± 1.48

n = 17; values are expressed as mean ± SD; ΔBASE values are the differences between the baseline values before and after the exercise bouts; Panel A shows baseline drifts sorted by exercise intensity while panel B shows baseline drifts sorted by chronological order of the exercise bouts.

It is still not entirely evaluated to what extent and in which temporal pattern the several factors act that evoke vasodilation during and following exercise as they are organized within a redundant system (60). However, those vasodilators seem not to explain the progressively improving O_2 distribution and rising post-exercise O_2Hb availability at exercise intensities above 60% VO_{2peak}. In example, acetylcholine has been pointed out to play a central role in rapid vasodilation only within the first seconds of exercise (100) and 12-24 h after the cessation of exercise (52). In case of shear stress mediated vasodilation, it seems logical that this stimulus is linked to blood flow, which is linearly related to exercise intensity (89).

Our results show that there is a progressively increasing mismatch of local O_2 delivery and utilization with increasing exercise intensity up to 60% VO_{2peak}. This suggests that sympathetic vasoconstriction is not effectively counteracted by vasoactive substances in the vastus lateralis muscle up to that intensity. In turn, the matching of local O_2 delivery und utilization gets progressively improved above 60% VO_{2peak}. That intensity represents approximately the average intensity corresponding to GET (59 ± 13% VO_{2peak}). Beside endothelium-mediated vasoregulation, smooth muscle cells can be directly influenced, e.g. by metabolites. There is evidence for an activation of cGMP within smooth muscle cells by lactate in rats (24). Independently to the lactate molecule itself,

the concomitant lower pH-level also induces a relaxation of local arterioles (24). Hence, although the role of lactate is discussed controversy (59), lactate and the associated acidosis caused by heavy and severe intensity exercise above GET (21) might account for the improved O_2 distribution and higher post-exercise O_2Hb availability by superimposing sympathetic vasodilation above GET. Moreover, the elevated post-exercise O_2Hb availability following exercise at >60% VO_{2peak} suggests a prolonged vasodilation and thus an improved local perfusion immediately after exercise.

However, because we did not normalize exercise intensity to GET in our study, this hypothesis cannot be confirmed. Additionally, the physiological determinants for GET are not necessarily the determinants for lactate or acidosis mediated vasodilation. GET solely describes the point where VCO_2 kinetics and ventilation increases stronger in relation to VO_2 kinetics because blood lactate levels exceed resting levels. This is caused by an increased lactate production in relation to lactate elimination. Lactate production itself already increases before reaching GET (74). Consequently, locally increased lactate production could account for local lactate or acidosis mediated vasodilation even before GET is reached.

Advantages of improved post-exercise O_2 availability

We have shown that exercise above 60% VO_{2peak} elicits an improved local blood supply and microvascular O_2 distribution, presumably by enhancing microvascular perfusion. There are possible advantages of an improved O_2 availability. The increased filling of small vessels, as indicated by ΔTHb in the area of interrogation, leads to an increased functional cross-sectional area (22). This increases the capillary surface area (85), which facilitates local gas exchange. Further, recent studies reported that a higher local muscle perfusion is associated with an enhanced oxidative metabolism like fatty acid oxidation (56, 66, 88, 91). Consequently, especially fatty acid oxidation could be enhanced by improved post-exercise O_2 availability during the early recovery phase or, with regard to interval training, during the low interval. We studied post-exercise hemoglobin kinetics during passive recovery. In most HIT regimes, the recovery or relief interval consists of low intensity exercise. Low intensity exercise might enhance post-exercise muscle perfusion, e.g. by enhanced heart rate or the

muscle pump (89) and simultaneously engages adipose tissue perfusion and hence lipolysis (88). Consequently, it should promote aerobic effects better than passive recovery.

Study Limitations

Because we did not normalize exercise intensity to GET in our study, we can not entirely confirm the hypothesis that the improved matching of local O_2 delivery and utilization and enhanced post-exercise local O_2 availability during and following exercise above 60% VO_{2peak} is triggered by increasing lactic acidosis above GET. Nevertheless, this study aimed to obtain an overall view on the relationship of local O_2 availability and matching of O_2 delivery and utilization and normalizing exercise intensity to GET is a further step.

The NIRS signal is affected by subcutaneous adipose tissue thickness (ATT) (102). The penetration depth is roughly half of the optode distance (39, 102). In this study, an optode distance of 3.5 cm was used, so the penetration depth was approximately 1.75 cm. Skinfold thickness (measured using a caliper) at the measuring point was 7.5 ± 3.1 mm. Half of this value represents the skin and ATT. Therefore, it can be concluded that NIR light was able to penetrate the skin and ATT and reached muscle tissue.

ΔO_2Hb and ΔTHb did not recover completely during the 5 min recovery period, while ΔHHb reached baseline values, which might be a result of an insufficient recovery period. However, this baseline drift in ΔO_2Hb and ΔTHb was not related to exercise intensity. Prior exercise affects acute physiological adaptions to subsequent exercise bouts (59). This bias should be adequately compensated by the randomized order of the experimental exercise bouts and the prior intensive warm up. Further, we did not analyze early exercise on-transition kinetics and the overshoots in ΔTHb and ΔO_2Hb occurred within the first 3 min of the recovery periods. In order to enable a complete recovery, all exercise bouts could have been performed on separate days or recovery periods could have been extended to 8 – 10 min (40). However, it is unlikely that this would have led to different results but possibly would have reduced standard deviation and would have not changed the results substantially.

We are aware that moderate intensity exercise is not used for work intervals within HIIT regimes. However, interval training regimes have been increasingly applied to elderly or diseased people. In this regard, training should be applied in a gentle but effective form. Thus it is important to know the minimum intensity that evokes beneficial effects.

Conclusion

The finding that microvascular O_2 distribution is increasingly impaired by increasing exercise intensity up to a certain intensity, but improves progressively with higher exercise intensities is novel. This intensity appeared on average at 60% VO_{2peak} in this study. We have also shown that post-exercise O_2 availability is elevated following exercise above the same intensity. Those effects are presumably due to an enhanced local vasodilation, possibly effected by lactate induced acidosis resulting from exercise at intensities above GET, which occurred on average at 59% VO_{2peak}.

Hence, we suggest that interval training is most effective to enhance local perfusion and thus oxidative metabolism in active muscles immediately after cycling exercise when work intervals are performed at a minimum exercise intensity of 60% VO_{2peak}. But considering the plateau in the relationship of post-exercise O_2 availability and exercise intensity, a minimum exercise intensity of 80% VO_{2peak} is recommended.

Because interval training is not only described by the intensity of the work interval, further research is needed to examine the particular relevance of the relief interval intensity, as well as the influence of the interval duration on local hemodynamics. Moreover, the possible role of GET on local vasoregulation needs to be researched.

Acknowledgements

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6.2. Supplement to Study 1: Postexercise muscle perfusion and Hb kinetics in relation to exercise intensity

6.2.1. Comment on the Supplement 1

Originally, the modeling of capillary blood flow (Q_{cap}) from ΔHHb and VO_2 (15, 40, 41) should be applied in order to estimate post-exercise perfusion. However, during the development of study 1, we became aware of novel findings (81) suggesting to favor $\Delta HHb/\Delta VO_2$ as estimate for microvascular perfusion rather than Q_{cap} modeling, which consequently was removed from the published version of the manuscript (chapter 5.1). For sake of completeness, the results of Q_{cap} analysis is attached as supporting material to this thesis. A justification is given in chapter 5.2.2.

6.2.2. Introduction

Ferreira et al. (41) introduced a method to estimate capillary blood flow (Q_{cap}) by re-arranging the Fick principle using the pulmonary oxygen uptake (VO_2) kinetics and the HHb signal as surrogates for muscular VO_2 (mVO_2) and arteriovenous oxygen difference [$(a\text{-}v)O_2$]. This method has been used to analyze Q_{cap} during (15, 41) and after exercise (40). Q_{cap} recovery kinetics have been found to be slowed after heavy intensity exercise in comparison to moderate intensity exercise (40).

6.2.3. Methods

Muscle Capillary Blood Flow

Q_{cap} was estimated by rearranging the Fick principle [*Eq. 1*] using VO_2 kinetics and raw HHb signal as surrogates for mVO_2 and $(a\text{-}v)O_2$. Assumptions and pitfalls of this method have been discussed extensively before (15, 41), but it is important to highlight that the amplitude of Q_{cap} is quantitatively uncertain due to the unknown percentage of contribution of arterial and venous blood to the HHb signal in skeletal muscle. Assuming this percentage remains constant throughout the measurement, the kinetic of HHb and thus Q_{cap} will be preserved. Consequently, results for Q_{cap} are presented in arbitrary units (A.U.).

$$Q_{cap} = \frac{mVO_2}{(a-v)O_2} \propto \frac{VO_2}{[HHb]} \quad [Eq.1]$$

According to *Eq.1*, Q_{cap} kinetic depends on the relative amplitude of VO_2 and HHb recovery. Ferreira et al (40, 41) used frequency domain multidistance NIRS, which provides absolute values. In this study, a CWS device was used, which only provides relative changes in hemoglobin concentration. Hence, values were set to zero at the beginning of the measurement. This leads to problems using the Fick equation due to very high relative amplitudes in HHb recovery. Thus a bias of 40 µmol/L was added to the HHb values in order to calculate Q_{cap}. After adding the bias, mean relative amplitude (40-90% VO_{2peak}) in HHb recovery was 28% which complies the mean relative amplitude obtained by Ferreira et al. (40) (32%, averaged over all tested intensities). Because we added the same bias to all subjects and did not test for inter-individual effects, we assume this procedure did not affect the results.

To establish time-alignment of HHb (recorded at 10 Hz) and raw VO_2 data (recorded breath-by-breath), VO_2 kinetic was determined using nonlinear regression by applying a least squares technique using Excel Solver (Microsoft Inc., Redmond, WA, USA). A mono-exponential function [*Eq. 2*] (40) was used to calculate time delay (TD), time constant (τ) and amplitude (A) of the VO_2 recovery. VO_{2EE} represents the endexercise VO_2 values averaged over 10 s. U = 0 for $t \leq TD_{VO2}$ and U = 1 for $t > TD_{VO2}$.

$$VO_2(t) = VO_{2EE} - A_{VO2} \times \left[1 - e^{-(t - TD_{VO2})/\tau_{VO2}}\right] \times U \; [Eq.\ 2]$$

Q_{cap} was modeled using a bi-exponential function [*Eq. 3*] (40) to calculate TD, τ and A for the primary component ($_{BFp}$) and for the secondary component ($_{BFs}$) respectively. $U_1 = 0$ for $t \leq TD_{BFp}$ and $U_1 = 1$ for $t > TD_{BFp}$ while $U_2 = 0$ for $t \leq TD_{BFs}$ and $U_2 = 1$ for $t > TD_{BFs}$.

$$Q_{cap}(t) = Q_{capEE} - A_{BFp} \times \left[1 - e^{-(t - TD_{BFp})/\tau_{BFp}}\right] \times U_1 \; [Eq.3]$$

$$- A_{BFs} \times \left[1 - e^{-(t - TD_{BFs})/\tau_{BFs}}\right] \times U_2$$

The mean response time (MRT) was calculated using *Eq.5* where A'_{BFp} is the amplitude of the primary component at TD_{BFs} [*Eq.4*] and $A'_{BFs} = A'_{BFp} + A_{BFs}$.

$$A'_{BFp} = A_{BFp}\left[1 - e^{-(TD_{BFs})/\tau_{BFp}}\right] \; [Eq.4]$$

$$MRT = \frac{A'_{BFp}}{A'_{BFs}} \times [TD_{BFp} + \tau_{BFp}] + \frac{A_{BFs}}{A'_{BFs}} \times [TD_{BFs} + \tau_{BFs}] \; [Eq.5]$$

Furthermore, MRT/τ_{VO2} ratio was calculated.

Statistical Analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). A repeated measures analysis of variance (ANOVA) was used to detect possible differences of the effect of the various exercise intensities on Q_{cap} (EE, MRT, τ_{BFp}, τ_{BFs}). Data were tested for sphericity with Mauchly's test and were corrected using the Greenhouse–Geisser method when necessary. If ANOVA showed a significant main effect, pairwise comparisons were performed with Bonferroni–Holm corrected post-hoc tests. Statistical significance was set at $P \leq 0.05$.

6.2.4. Results

There was a significant influence of exercise intensity on estimated capillary blood flow ($F_{2,31}$ = 120.4; $P < 0.001$). In detail, Q_{capEE} enhanced with increasing exercise intensity from 40% to 90% VO_{2peak} (all $P < 0.001$, Fig. 1C). The mean response time of Q_{cap} recovery (MRT) showed also a dependency on exercise intensity ($F_{3,50}$ = 35.0; $P < 0.001$). Post hoc tests reported significant faster recovery kinetics after 40%, 50% and 60% in comparison to 80% ($P \leq 0.001$) and 90% VO_{2peak} ($P \leq 0.002$). Additionally, MRT after exercise at 90% VO_{2peak} was significantly slower in comparison to 70% VO_{2peak} (P = 0.007). From 40% to 70% VO_{2peak}, MRT increased progressively (P = 0.001 for 40% vs. 50%; P = 0.014 for 50% vs. 60%; P = 0.013 for 60% vs. 70%).

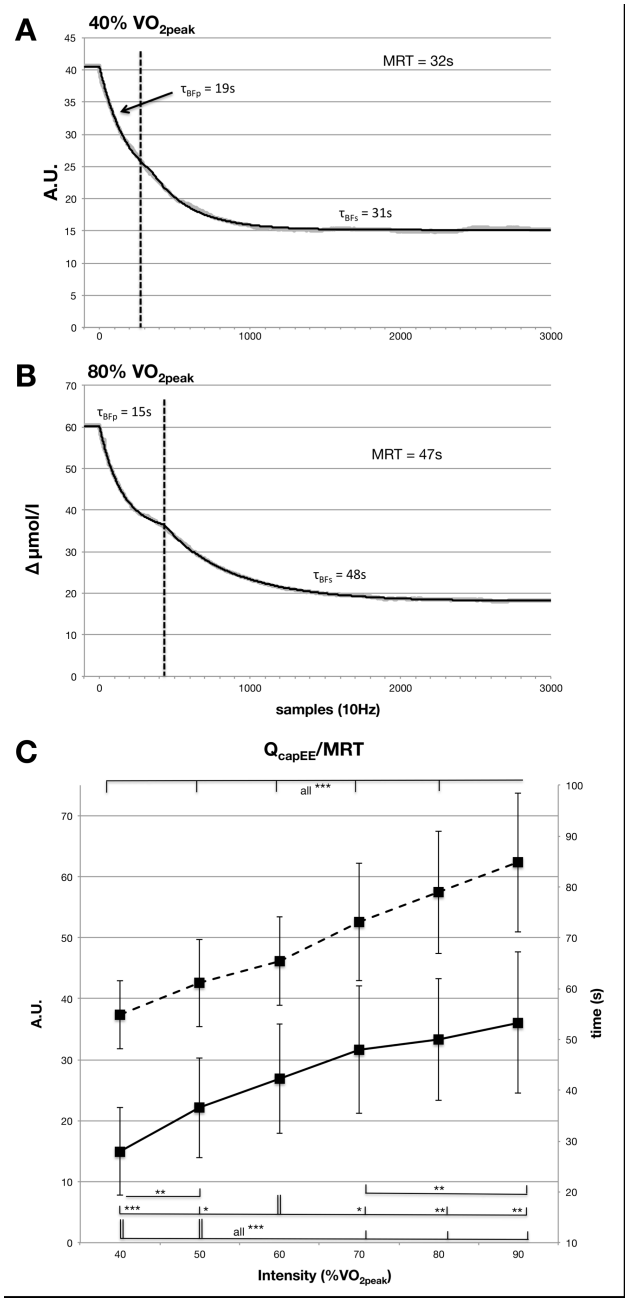

Fig. 1 (A,B) Exemplary time course of Q_{cap} recovery kinetics (grey: raw data, black: modeled data) following exercise at 40% and 80% VO_{2peak}. The vertical dashed line shows the transition from Q_{cap} primary to secondary component and hence corresponds to the time delay of the secondary component; (C) Endexercise estimated capillary blood flow (Q_{cap}, broken line) and mean response time (MRT, solid line) the six exercise bouts (mean ± SD). Asterisks represent results from post-hoc tests in relation to values marked with double lines; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

No significant effects of exercise intensity could be observed for the time constant of the Q_{cap} primary component. In contrast, the time constant of the secondary component (τ_{BFs}) was significantly influenced by exercise intensity ($F_{2,38}$ = 24.2; $P < 0.001$). τ_{BFs} increased with increasing exercise intensity from 50% to 80% VO_{2peak} (P = 0.014 for 50% vs. 60%; P = 0.025 for 60% vs. 70% and P = 0.003 for 70% vs. 80%).

MRT/τ_{VO2} ratio was significantly influenced by exercise intensity ($F_{2,32}$ = 10.2; $P < 0.001$) and was significantly greater after 60-90% VO_{2peak} than after 40% and 50% VO_{2peak} ($P \leq 0.007$ vs. 40%; $P \leq 0.004$ vs. 50%; Fig. 2).

6.2.5. Discussion

Endexercise Q_{cap} increased significantly with increasing exercise intensity (+67% up to 90% VO_{2peak}), which is in accordance with recent literature (15, 41, 57, 67). MRT of Q_{cap} recovery kinetics also increased (+20s) with exercise intensity up to 70% VO_{2peak}, but increased attenuated thereafter (+6s up to 90% VO_{2peak}). This is indicated by the significantly shorter MRT after exercise at 70% versus 90% VO_{2peak} whereas no significant differences could be seen after exercise at 80% VO_{2peak} in comparison to 70% and 90% respectively (Suppl. 1 Fig. 1).

MRT of Q_{cap} recovery represents the overall response of both, the primary and the secondary component of Q_{cap} recovery kinetics. The time constant of Q_{cap} primary component (τ_{BFp}) evolved independently from exercise intensity. τ_{BFp} is supposed to represent the cessation of the muscle pump as well as the onset of postexercise vasoconstriction (40). Values of τ_{BFp} are presented in table 1. It is remarkable that τ_{BFp} was quite unstable over the six exercise intensities. This is due to some cases, where time delay of the secondary component was shorter than the onset of primary components curvature and therefore, the definition of the primary component kinetics is more uncertain. τ_{BFp} appeared to be considerably longer in those cases. After removing all cases with $TD_s < \tau_{BFp}$ (all cases = 6 intensities x 17 subjects = 102 cases; 22 cases showed $TD_s < \tau_{BFp}$), values became more stable (see table 1) and approximate those reported by Ferreira et al. (15) with the exception of τ_{BFp} after exercise at 40%. This suggests a delayed vasoconstriction following exercise at very low exercise intensity.

Postexercise τ_{BFs} instead is related to exercise intensity due to vasoactive substances of neuronal, muscular and vascular origin (40). This could also be observed in our study. Because of the different postexercise kinetics of Q_{cap} and Hb overcompensation (which is presumably affected by a prolonged local vasodilation, see section "postexercise hemoglobin kinetics"), there seem to be more global influences on blood flow redistribution affecting τ_{BFs} like cardiac output and global vascular regulation. It can be speculated that the relationship of τ_{BFs} and exercise intensity shows a plateau above 80% VO_{2peak}, because there was no significant difference between τ_{BFs} after 80% and 90% VO_{2peak}. But this "missing" difference is likely due to the high standard deviation.

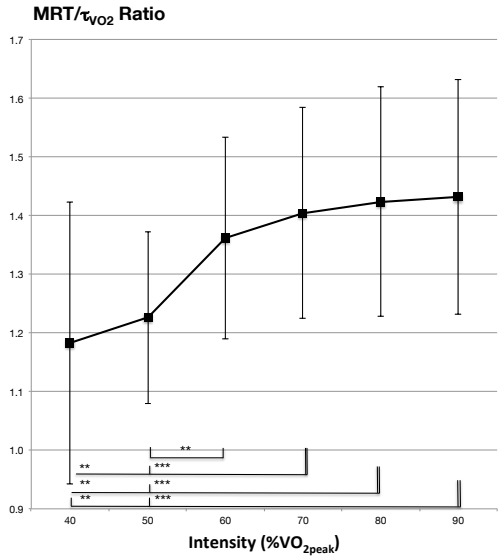

Fig. 2 MRT/τ_{VO2} Ratio after the six exercise bouts; ** = $P < 0.01$; *** = $P < 0.001$.

The temporal dissociation between MRT and the time constant of VO_2 recovery kinetics provides information about the balance between postexercise sympathetic vasoconstriction an the release of vasodilators (40, 103). In our study, the MRT/τ_{VO2} ratio was significantly greater after 60-90% VO_{2peak} than after 40% and 50% VO_{2peak} (Fig. 4A). This is a further indicator for a dominant local vasodilation superimposing sympathetic vasoconstriction reaching following exercise above 60% VO_{2peak}.

Table 1. Baseline Changes and Q_{cap} Parameters

$\%VO_{2peak}$	40%	50%	60%	70%	80%	90%
τ_{BFp} (s)	17 ± 8 (16)	21 ± 12 (13)	13 ± 9 (10)	16 ± 10 (13)	12 ± 6 (12)	16 ± 10 (13)
τ_{BFs} (s)	24 ± 8	28 ± 5	33 ± 9*	37 ± 7*	41 ± 8*	43 ± 7
TD_{BFs} (s)	21 ± 9	22 ± 5	27 ± 6*	32 ± 8*	35 ± 9*	37 ± 13
A'_{BFp} (A.U.)	11 ± 5	12 ± 4	13 ± 5	15 ± 8	18 ± 9*	19 ± 12
A'_{BFs} (A.U.)	18 ± 4	25 ± 8*	30 ± 7*	34 ± 5*	40 ± 7*	42 ± 10
τ_{VO2} (s)	24 ± 7	29 ± 6*	31 ± 6	34 ± 5*	35 ± 6*	37 ± 6

n = 17; values are expressed as mean ± SD; asterisks stand for significantly different values compared to the next lower exercise intensity; ΔBase values are the differences between the baseline values before and after the exercise bouts; values in brackets for τ_{BFp} show averages after removing all cases with $TD_s < \tau_{BFp}$ (see discussion „Q_{cap} recovery from exercise").

6.2.6. Point-Counterpoint discussion on Q_{cap} modeling

Upon modeling of Q_{cap}, the Fick principle can be re-arranged and $(a\text{-}v)O_2$ may be replaced by ΔHHb. This is supported, for example, by Grassi et al. (49), who observed substantial similarities between $(a\text{-}v)O_2$ and ΔHHb kinetics during exercise. As pointed out by Ferreira et al. (40), the Fick principle can provide direct information of blood flow, albeit modeling of Q_{cap} may allow the evaluation of relative changes in Q_{cap} only. However, Murias et al. (81) have doubted the potency of this approach to reflect microvascular O_2 availability. They rather argued that the $\Delta HHb/\Delta VO_2$ ratio cannot provide direct information about local capillary blood flow (78), because ΔHHb might be an inacceptable approximation for $(a\text{-}v)O_2$. In fact, they failed to model Q_{cap} reliably for the complete cohort of subjects of their study (78). In some subjects, the suggested mono- or bi-exponential model could be applied, while data of other subjects could not be fitted with acceptable quality. This problem was attributed to varying proportional increases in ΔHHb during exercise (81). Furthermore, the proportional increase in ΔHHb substantially exceeded that of VO_2 in many subjects, which led to a distorted shape of Q_{cap} kinetics. This problem was even more evident when using CWS NIRS, which requires a reset of the device before each measurement, thus precluding to solve the Fick equation properly. In order to compensate for this, a bias of 40 was added in our project, which generated data comparable to those reported by Ferreira et al. (40) and Buchheit et al. (15). The latter group followed a similar strategy and used the same NIRS device as we did in the current project. In agreement with the observations of Murias et al. (81), Q_{cap} kinetics could be reliably fitted in a subset of the cohort only, but not for all subjects.

After removing those cases with a shorter time delay of the secondary component compared to the onset of primary components curvature, the results support those that have been derived from $\Delta HHb/\Delta VO_2$ and post-exercise O_2Hb overshoot assessment. Summed up, it is likely that Q_{cap} modeling reflects microvascular perfusion when the model can be applied properly. However, considering all assumptions and data fitting, the method remains questionable. In this regard, the outcomes of Q_{cap} modeling have not been considered for any conclusions.

6.3. Study 2: Relationship of post-exercise muscle oxygenation and duration of cycling exercise

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Original Article

Relationship of post-exercise muscle oxygenation and duration of cycling exercise

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ABSTRACT

BACKGROUND: Aerobic adaptations following interval training are supposed to be mediated by increased local blood supply. However, knowledge is scarce on the detailed relationship between exercise duration and local post-exercise blood supply and oxygen availability. This study aimed to examine the effect of five different exercise durations, ranging from 30 to 240 s, on post-exercise muscle oxygenation and relative changes in hemoglobin concentration.

METHODS: Healthy male subjects (N = 18) performed an experimental protocol of five exercise bouts (30, 60, 90, 120, and 240 s) at 80% of peak oxygen uptake ($\dot{V}O_{2peak}$) in a randomized order, separated by 5-min recovery periods. To examine the influence of aerobic fitness, we compared subjects with gas exchange thresholds (GET) above 60% $\dot{V}O_{2peak}$ (GET60+) with subjects reaching GET below 60% $\dot{V}O_{2peak}$ (GET60−). $\dot{V}O_2$ and relative changes in concentrations of oxygenated hemoglobin, deoxygenated hemoglobin, and total hemoglobin were continuously measured with near-infrared spectroscopy of the vastus lateralis muscle.

RESULTS: Post-exercise oxygen availability and local blood supply increased significantly until the 90-s exercise duration and reached a plateau thereafter. Considering aerobic fitness, the GET60+ group reached maximum post-exercise oxygen availability earlier (60 s) than the GET60− group (90 s).

CONCLUSIONS: Our results suggest that (1) 90 s has evolved as the minimum interval duration to enhance local oxygen availability and blood supply following cycling exercise at 80% $\dot{V}O_{2peak}$; whereas (2) 60 s is sufficient to trigger the same effects in subjects with GET60+.

Keywords

muscle oxygenation; hyperemia; near-infrared spectroscopy; interval training; prior exercise

BACKGROUND

High-intensity training (HIT) and high-intensity interval training or aerobic interval training (HIIT or AIT) are commonly accepted stimuli for improving anaerobic (17, 110) and aerobic (16, 38,

43, 69, 95) functions. Although the terms HIT and HIIT are sometimes used interchangeably, HIT usually refers to near-maximal or supramaximal exercise intensities (>90% peak rate of oxygen uptake, $\dot{V}O_{2peak}$) (16), whereas HIIT or AIT is often used in the context of exercise intensities between 80% and 90% $\dot{V}O_{2peak}$ (43, 84, 95).

Acute microvascular responses to exercise (*e.g.,* enhanced blood flow and local blood supply of small vessels in active tissue (22, 57)) have also been reported as long-term adaptations following HIT (*e.g.,* by increased vasodilatory capacity (22) and augmented capillarization (38, 73)). Fu et al. showed these effects to be superior to those of moderate continuous training in patients with heart failure (43). Furthermore, several studies have suggested that aerobic adaptations are facilitated by the above-mentioned microvascular mechanisms following HIIT/AIT (43, 69, 95).

Although the literature contains substantial knowledge on the acute effects of exercise on local muscle perfusion in general (4, 40, 57, 89, 100), the acute post-exercise effects of different exercise durations on local muscle oxygen availability and blood supply have not yet been sufficiently examined. Most studies have focused on the influence of exercise intensity (6, 23, 27, 92) or examined the effect of complete training sessions with only two different work-interval durations (114).

Relative changes in local, total hemoglobin concentration (ΔTHb) is a widely used parameter for blood-volume changes in terms of capillary filling (25, 39, 58) and vasodilation (109) and can be noninvasively monitored using near-infrared spectroscopy (NIRS) (39). With continuous-wave spectrometers, relative changes in the THb and deoxygenated (HHb) and oxygenated Hb (O_2Hb) concentrations can be observed because of the distinct relative transparency of HHb and O_2Hb for specific, near-infrared light wavelengths.

The potential of exercise to prolong augmentation of the local blood supply could be an important determinant for metabolic adaptations. The aim of our study was to identify the association between durations of HIIT/AIT exercise bouts and relative changes in post-exercise concentrations of THb, O_2Hb, and HHb to examine (1) the post-exercise blood supply and (2) local oxygen availability.

Methods

Subjects

A total of 18 healthy, physically active males volunteered to participate in the present study (see Table 1). Participants exercised at least 2 times per week in various sports, whereas aerobic exercise contributed an average of 4 ± 3 h per week. The inclusion criteria included age (18–35 years) and a physically active background. Exclusions included acute or chronic diseases (such as chronic heart disease), diabetes type II, epilepsy, and relevant diseases of the liver and kidney, as well as smoking and excessive alcohol consumption. All subjects were familiar with cycling exercise and exhaustive exercise testing. They received detailed information on the purpose and procedures of the study and their written informed consent was obtained. The local Ethics Committee of the Technische Universität München approved the study protocol (#67/14).

Table 1. Subject data

Parameter	
N	18
Age (years)	28 ± 5
Weight (kg)	77.7 ± 4.9
Height (cm)	181.3 ± 5.2
Skinfold thickness (mm)	7.8 ± 2.9
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	50.4 ± 5.7
GET (%$\dot{V}O_2$peak)	58 ± 13
n GET60+	7
n GET60−	11

Values are expressed as mean ± SD.

Test design and procedures

Subjects reported to the laboratory for 2 sessions. We conducted preliminary tests (see section *preliminary maximal testing*) during the first session. During the second session, we aimed to examine the local blood supply in relation to exercise duration. Both sessions had to be completed within one week and had to be separated by at least 48 h to enable full recovery. The subjects were instructed not

to perform exhaustive activity 24 h before each session and to avoid caffeinated and high-carbohydrate beverages 2 h before each session. Special bicycle shoes and pedals were not allowed during the ergometer tests to reduce the influence of cycling technique.

Preliminary maximal testing

During the first visit, all subjects performed an incremental test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur; Lode, Groningen, The Netherlands) to determine $\dot{V}O_{2peak}$ and gas-exchange threshold (GET), according to the v-slope method (3). The 60-W load was increased by 10 W every 30 s and led to volitional exhaustion within 14.8 ± 1.5 min, which has been reported as a suitable time span for reaching maximum aerobic contribution (76). Subjects were allowed to individually choose the cadence during the cycle ergometer trials but were asked to maintain a minimum cadence of >70 revolutions/min. The test was terminated when pedaling frequency could no longer be maintained. Although all subjects were physically active, healthy, and familiar with exhaustive exercise, $\dot{V}O_2$ did not attain a plateau in 2 subjects. Thus, we measured $\dot{V}O_{2peak}$ instead of maximum oxygen uptake ($\dot{V}O_{2max}$). However, Day et al. showed that $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$ do not differ in a healthy, physically active population when maximum effort is put forth (29). To ensure maximum effort, we verbally motivated subjects at the end of the test and datasets were included only if a maximum respiratory exchange ratio >1.1 had been attained.

Experimental testing

Subjects were asked to complete a randomized protocol consisting of five exercise bouts with different durations (30, 60, 90, 120, and 240 s), separated by 5-min passive, recovery periods. Exercise intensity was determined corresponding to 80% $\dot{V}O_{2peak,}$ based on the data of the preliminary maximal test. Consequently, $\dot{V}O_2$ did not attain 80% during the 30-s and 60-s exercise bouts because of the finite $\dot{V}O_2$-kinetics during exercise on transitions (21). On the other hand, $\dot{V}O_2$ exceeded 80% slightly during the 240-s bout because of the slow component of $\dot{V}O_2$ (21) (Table 2). The exercise bouts were

randomly assigned. It is worth pointing out that Buchheit et al. used 3-min recovery periods for similar measurements (18). However, we noted that the recovery times of HHb and O_2Hb exceeded 180 s (Table 2), so we decided to use 5-min recovery periods instead. The applied exercise durations, which are commonly used in HIIT/AIT prescriptions, represent interval bouts that range from short to long duration (17). The exercise intensity of 80% $\dot{V}O_{2peak}$ is commonly used for HIIT/AIT in combination with long-duration intervals, while short-exercise bouts up to 60 s are associated with supramaximal work rates (95). In spite of that, a shorter interval (30 s) was included in this setup to obtain a detailed overview on the relationship of exercise duration and post-exercise blood supply.

A 3-min baseline measurement ($BASE_0$) was conducted prior to the first interval with the subject sitting passively on the ergometer. This measurement was followed by a warm-up, consisting

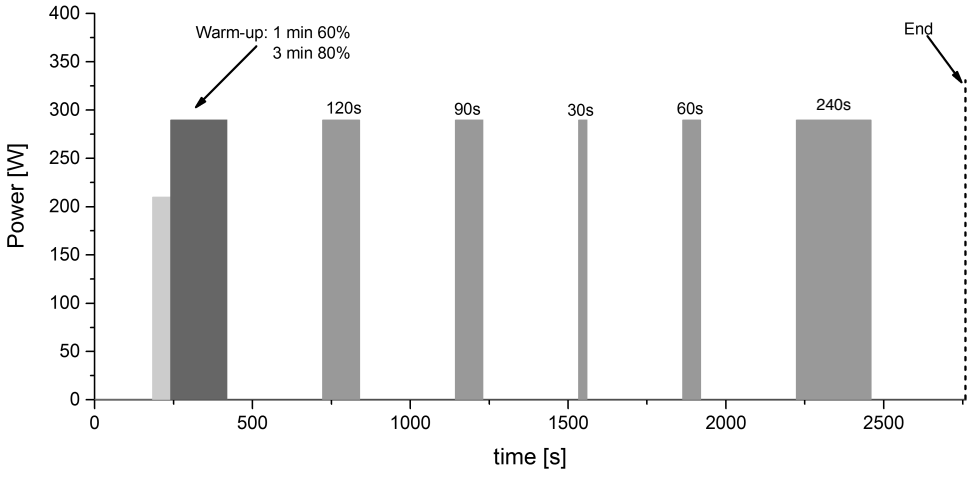

Fig. 1 Scheme of an exemplary experimental protocol. Subjects had to maintain a standardized recovery position, sitting on the ergometer during baseline measurement prior to the warm-up and recovery periods between exercise bouts.

of 1 min at 60% $\dot{V}O_{2peak}$ and 3 min at 80% $\dot{V}O_{2peak}$. Prior observations revealed that O_2Hb levels would be markedly elevated, following the first exercise bout but would attain similar values between subsequent exercise bouts, which could be due to increased skin blood flow (28). Furthermore, pre-exercise affects the speed of aerobic metabolism during subsequent exercise on-transitions (59). Hence, pre-exercise was used to activate muscle blood flow and metabolism to avoid order effects for the first experimental condition. During $BASE_0$, as well as during recovery periods, subjects had to maintain a

standardized position, keeping the right foot on a bar between the pedals to minimize movement artifacts by unwanted muscular activity on the NIRS signal.

An exemplary scheme of the experimental protocol is displayed in Figure 1.

Gas exchange and heart rate measurement

Heart rate was monitored (Polar Team^{2,} Polar Electro, Kempele, Finland) throughout all sessions and respiratory parameters were measured continuously with a breath-by-breath metabolic cart (ZAN 600USB; Nspire Health, Oberthulba, Germany), which had been used and validated previously (13, 65). The oxygen-uptake ($\dot{V}O_2$) curve was smoothed with a 30-s moving average and heart-rate data were smoothed with a 5-s moving average. $\dot{V}O_{2peak}$ was determined as the highest value reached during exercise and was expressed relative to body weight ($ml \cdot kg^{-1} \cdot min^{-1}$). GET was determined using the v-slope method (3) and was confirmed with ventilatory equivalents for O_2 and CO_2. Peak heart rate was recorded for each exercise bout.

NIRS

A wireless continuous-wave NIRS device (PortaMon, Artinis Medical Systems, Zetten, The Netherlands) was used to measure local changes in Hb concentration in the right, vastus lateralis muscle during all sessions. NIRS data were collected using the software Oxysoft (Artinis Medical Systems) and were sampled with a frequency of 10 Hz and expressed as $\Delta\mu mol \cdot L^{-1}$. The PortaMon probe consists of three light sources (wavelengths: 760 nm and 850 nm) separated by 3, 3.5, and 4 cm from the receiving optode, while only data measured with the 3.5 cm optode distance were taken into account, corresponding to a penetration depth of approximately 1.75 cm (23, 102). The probe, which was firmly attached with adhesive tape to the shaved skin above the distal part of the right vastus lateralis muscle, was covered with a lightproof cloth to prevent any influences of ambient light. The position of the probe was marked with a surgical marker to detect possible shifts (of the probe) during the test.

The PortaMon probe simultaneously uses the modified Lambert–Beer law and spatially resolved spectroscopy (39), which enables the observation of relative changes in O_2Hb, HHb, and THb. Because the exact path-length of the photons which penetrate the tissue is unknown when using continuous-wave spectroscopy, a differential path-length factor has to be used (39). In the present study, we used a differential path-length factor of 4, which has been applied by other studies in measurements of muscle tissue (18, 102). However, we are aware of the uncertainty of the differential path-length factor for muscle tissue and, hence, have reported all changes relative to individually measured baseline values (ΔTHb/O2Hb/HHb). Due to the equal absorption properties of Hb and myoglobin (Mb), it is not possible to distinguish between their contributions on the NIRS signal. However, the contribution of Mb to the NIRS signal is assumed to be minimal (39). For simplification, "Hb+Mb" are termed "Hb" in the present study.

After collection, the data were smoothed with a 1-s moving average. The data of the last 30 s of $BASE_0$ were averaged. Baseline values were measured again for each exercise bout in the last 30 s of the previous recovery period ($BASE_{30–240}$). The recovery time of ΔTHb/O2Hb/HHb was determined by assessing the time elapsed from cessation of exercise and the point where the value reached the average of the last 30 s of recovery ±5 standard deviations for the first time (Table 2). We chose five standard deviations because the behavior of the NIRS parameters was not completely stable, even in the end of each recovery period, due to subjects' involuntary movements. End-exercise values (averaged over the last 10 s of exercise) were assessed and expressed in relation to the respective baseline values for each exercise bout (EE_O2Hb/HHb/THb). The early post-exercise overcompensation appears as an overshoot (OS) of ΔO_2Hb and ΔTHb after termination of exercise and was also expressed in relation to baseline values (Figure 2), as well as the time-to-peak from cessation of exercise (Table 2). To control possible changes of BASE, we calculated the difference between the baseline before and after each exercise bout (ΔBASE40–90).

Statistical analyses

Statistical analyses were performed using the statistical software package, SPSS version 20 (SPSS Inc., Chicago, IL, USA). We used repeated measures analysis of variance (ANOVA) to detect

possible differences in the effect of the various exercise durations on OS_O_2Hb/THb, end-exercise concentrations of ΔO_2Hb/ΔHHb/ΔTHb, peak heart rate, time-to-peak for ΔO_2Hb, and ΔTHb, as well as ΔBASE for ΔO_2Hb/ΔHHb/ΔTHb. Data were tested for sphericity with Mauchly's test and were corrected using the Greenhouse–Geisser method (50) when necessary. If ANOVA showed a significant main effect, pairwise comparisons were performed with Bonferroni–Holm corrected post-hoc tests. For OS_O_2Hb and OS_THb, the relative aerobic fitness was considered a group factor, whereby subjects with GET below 60% $\dot{V}O_{2peak}$ and above 60% $\dot{V}O_{2peak}$ were assigned as "GET60−" and "GET60+", respectively. The cut-off point was chosen as 60% $\dot{V}O_{2peak}$ because this approximately corresponds to the mean GET for the entire sample (Table 1). Separate ANOVAs were performed for each level of aerobic fitness when a significant interaction of *time* × *aerobic fitness* could be observed, whereas the differences between 30, 60, 90, and 240 s of exercise were focused on. "Pre-exercise" values of ΔO_2Hb and ΔTHb were individually compared with the corresponding post-exercise peak values (which have been used to calculate OS) using *t*-tests. The purpose of the individual comparisons was to verify whether the post-exercise elevation in both parameters was significant. The same procedure was conducted to compare baseline values before and after particular exercise bouts to test for possible baseline drifts. A *P* value of ≤0.05 was considered statistically significant.

Results

Parameters during exercise

Figure 2 shows a representative time course of one subject showing post-exercise ΔHHb, ΔO_2Hb, and ΔTHb kinetics during and following 30-s to 240-s exercise bouts at 80% $\dot{V}O_2$peak. An ANOVA revealed a significant effect of the factor *time* on end-exercise ΔO_2Hb ($F_{4,68}$ = 4.1; P = 0.005), ΔHHb ($F_{3,54}$ = 22.5; P < 0.001), and ΔTHb values ($F_{3,42}$ = 24.7; P < 0.001, Figure 3). For ΔO2Hb, post hoc tests showed significantly lower values at the end of the 60-s exercise bouts than in the 120-s and 240-s exercise bouts ($P \leq 0.010$). The end-exercise ΔTHb values increased significantly with increasing exercise duration ($P \leq 0.042$). End-exercise ΔHHb showed a similar time course (*i.e.,* one

that increased significantly with increasing exercise duration [$P \leq 0.046$]), except when the value at 90 s was compared with that at 120 s.

Heart-rate values were also affected by exercise duration ($F_{3,41}$ = 116; P < 0.001) and increased significantly with this factor (P < 0.001, Figure 4A).

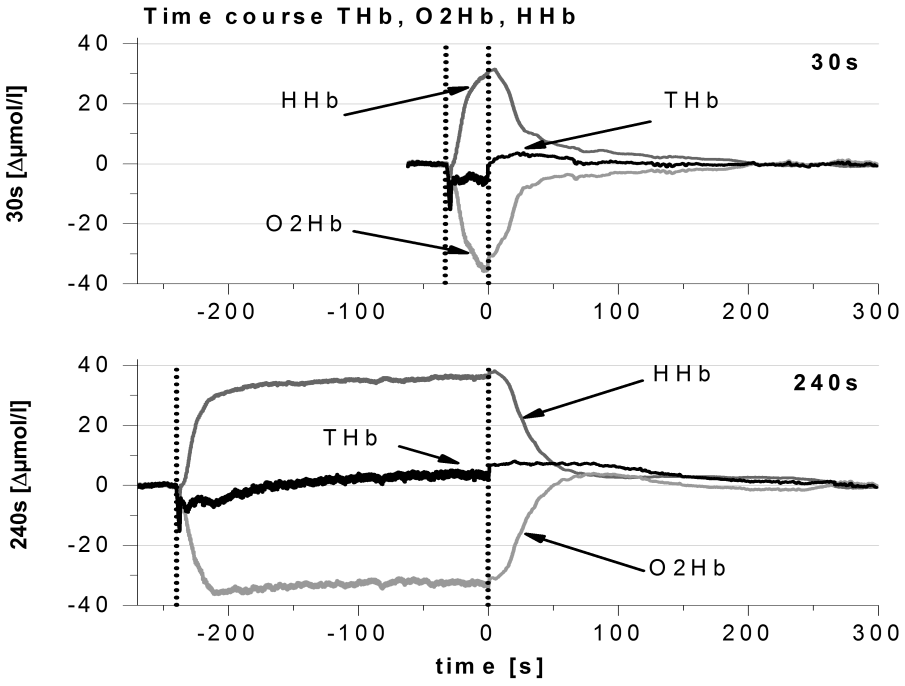

Fig. 2 Typical kinetics of ΔO_2Hb, ΔHHb, and ΔTHb during and after 30-s and 240-s cycling at 80% $\dot{V}O_{2peak}$ in relation to baseline values (set to zero). Note the marked overshoot in ΔO_2Hb following the 240-s exercise interval compared to the 30-s interval.

Post-exercise parameters

Immediately after cessation of the exercise bouts, ΔO_2Hb, ΔHHb, and (as a consequence) ΔTHb increased abruptly due to the relaxation of the working muscle. Afterward, ΔHHb decreased in an exponential manner and reached a stable level without showing any clear and systematic undershoot. ΔO_2Hb increased inversely following 30 s of exercise. Following 60, 90, 120, and 240 s, of exercising at 80% $\dot{V}O_{2peak}$, the ΔO_2Hb increased abruptly and reached an overshoot (OS_O_2Hb), relative to the pre-exercise values (P < 0.001) within 80 s. This time-to-peak was significantly longer following 30-s of exercise (ANOVA: $F_{4,68}$ = 6.0; P = 0.008; post-hoc: $P \leq 0.018$, see Table 2).

Subsequently, ΔO_2Hb decreased to baseline level. Although there was no overcompensation with a subsequent decrease in ΔO_2Hb after 30-s exercise (Figure 2), the highest post-exercise value (which actually occurred in the end of the recovery period) was determined as $OS\_O_2Hb_{30}$. Because there were small increases in baseline values throughout the trial, the overshoot following 30 s was also significantly higher ($P = 0.003$) than the pre-exercise baseline values (changes in baseline values were not related to exercise duration). The post-exercise overcompensation also occurred for ΔTHb after all exercise bouts ($P < 0.001$), whereas time-to-peak was not significantly related to exercise duration (Table 2).

ANOVA showed significant effects of the factor *time* on OS_O_2Hb ($F_{3,53} = 26.6$; $P < 0.001$) and OS_THb ($F_{2,40} = 23.2$; $P < 0.001$). Based on post-hoc tests, the OS_O_2Hb increased with increasing length of the exercise bouts up to 90-s exercise duration ($P \leq 0.005$) (Figure 4B). Also,

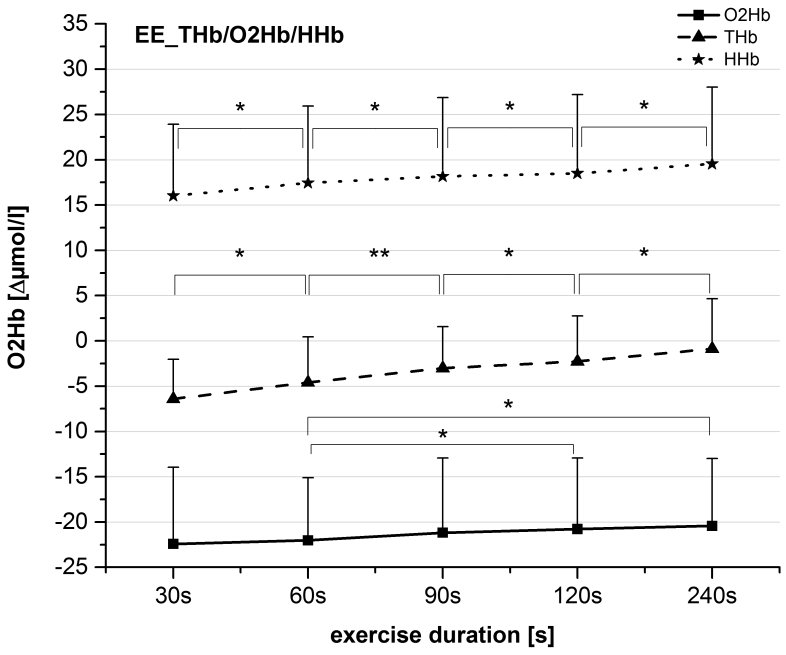

Fig. 3 End-exercise (EE) values (mean ± SD) of ΔO2Hb (solid line), ΔHHb (dotted line), and ΔTHb (broken line). Asterisks represent results from post-hoc tests in relation to values marked with double lines; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

OS_O_2Hb values following the 120-s and 240-s exercise bouts were not significantly different from those following the 60-s bout.

OS_THb had a similar time course to OS_O_2Hb. OS_THb_{60} was significantly higher ($P \leq 0.005$) than OS_THb_{30} and was significantly lower than OS_THb_{120} ($P \leq 0.008$). However, OS_THb_{90} was not different from OS_THb_{60} and OS_THb_{120} (Figure 4A). OS_THb_{240} was not different from OS_THb_{120}.

Table 2. Recovery parameters for ΔO2Hb, ΔHHb, and ΔTHb

Exercise duration	30 s	60 s	90 s	120 s	240 s
OS_THb ($\Delta\mu mol \cdot l^{-1}$)	3.05 ± 1.26	6.00 ± 2.78	6.97 ± 2.11	6.98 ± 2.30	7.25 ± 3.03
OS_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	1.09 ± 1.32	4.79 ± 2.37	6.34 ± 1.88	6.54 ± 2.90	5.98 ± 2.75
GET60+: OS_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	1.14 ± 1.60	6.00 ± 2.70	7.02 ± 2.11	--	6.08 ± 2.15
GET60−: OS_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	1.06 ± 1.19	4.02 ± 1.88	5.91 ± 1.67	--	5.91 ± 3.18
Recovery of ΔTHb (s)	198 ± 86	206 ± 82	199 ± 65	210 ± 57	234 ± 37
Recovery of ΔO_2Hb (s)	243 ± 56	222 ± 66	239 ± 23	195 ± 71	227 ± 56
Recovery of ΔHHb (s)	212 ± 88	210 ± 76	206 ± 79	185 ± 84	243 ± 31
SD_REC_ ΔTHb ($\Delta\mu mol \cdot l^{-1}$)	0.18 ± 0.21	0.36 ± 0.27	0.24 ± 0.12	0.21 ± 0.14	0.26 ± 1.23
SD_REC_ ΔO_2Hb ($\Delta\mu mol \cdot l^{-1}$)	0.19 ± 0.13	0.34 ± 0.28	0.34 ± 0.12	0.20 ± 0.09	0.28 ± 0.25
SD_REC_ ΔHHb ($\Delta\mu mol \cdot l^{-1}$)	0.11 ± 0.09	0.20 ± 0.09	0.11 ± 0.05	0.15 ± 0.09	0.12 ± 0.04
Time-to-peak ΔTHb (s)	57 ± 29	65 ± 50	79 ± 57	71 ± 37	72 ± 44
Time-to-peak ΔO_2Hb (s)	151 ± 81*	103 ± 59	81 ± 26	91 ± 48	94 ± 39
End-exercise %$\dot{V}O_2$ (%)	43 ± 8*	73 ± 5	81 ± 6	82 ± 6	86 ± 5

n = 18 (except GET60+/-); values are expressed as mean ± SD; Significant results for OS_THb/O_2Hb are presented in Figure 4; SD_REC_ΔTHb/O_2Hb/HHb represent the average standard deviation of the last 30 s of recovery; Time-to-peak values indicate the time from cessation of exercise to the highest value reached during the subsequent recovery period (asterisks indicate significant higher values compared to the other values, ** $P < 0.01$); End-exercise %$\dot{V}O_2$ values are averaged values obtained from the final 10 s of each bout.

We noted a significant interaction of *time* × *aerobic fitness* in OS_O_2Hb ($F_{3,51} = 4.1$; $P = 0.011$). In the GET60+ group, OS_O_2Hb was significantly influenced by the factor *time* ($F_{3,18} = 17.3$; $P < 0.001$). Post hoc tests showed significantly lower values for OS_O_2Hb following 30 s of exercise than those for the other exercise intensities ($P \leq 0.007$). The factor *time* also affected OS_O_2Hb in the GET60− group ($F_{3,30} = 16.5$; $P < 0.001$), but contrary to the GET60+ group, this parameter increased progressively from 30 s to 90 s in exercise duration ($P \leq 0.015$) and did not change significantly thereafter (Figure 5).

However, no significant interaction of *time* × *aerobic fitness* could be found for OS_THb ($F_{3,34} = 2.9$; $P = 0.068$).

All parameters (ΔO_2Hb, ΔHHb, and ΔTHb) reached stable values at the end of each recovery period (which was defined as the new baseline for the subsequent exercise bout). These new baseline values did not show any significant exercise "duration-related" differences; neither when sorted by exercise intensity nor by chronological order of the exercise bouts (Table 3).

Discussion

The data of the present study show that cycling exercise at 80% $\dot{V}O_{2peak}$ triggers post-exercise hyperemia, which is indicated by an overshoot in ΔTHb following exercise bouts in relation to pre-exercise values. ΔTHb has been used as an indirect measure for blood volume previously (25, 39, 58). When we considered the five different exercise durations, the 30-s and 60-s exercise bouts evoked significantly lower overshoot values than the longer exercise bouts.

Because the relationship of post-exercise overshoots of ΔTHb and ΔO_2Hb evolved similarly, we suggested that the arterial blood supply primarily accounts for the increased post-exercise ΔTHb. A reduced outflow would be expected to emerge as a concomitant increase in post-exercise ΔHHb at the point of OS. Like OS_THb, OS_O_2Hb increased stepwise from 30 to 60-s and from 60 to 90-s exercise, indicating an increasing availability of oxygen in the area of interrogation up to exercise durations ≥90 s.

To the best of our knowledge, the present study is the first to investigate post-exercise muscle reoxygenation in relation to single exercise bouts of different durations as they are used in interval-training regimens. In general, a number of studies have reported on post-exercise hyperemia following single exercise bouts of similar durations as they were applied in the present study (6, 23, 92). Furthermore, Danduran et al. (27) observed hyperemia using NIRS following a graded exercise test design in healthy children. Post-exercise reoxygenation was analyzed in various studies. A significant dependency of post-exercise Hb and muscle oxygenation recovery kinetics on exercise intensity (in terms of a forced reoxygenation following higher exercise intensities) has been reported previously (6,

23, 92). Despite this dependence, reoxygenation time is apparently not influenced by exercise intensity (18, 92). When Belfry et al. (5) compared interval-training regimens with constant-load exercise at equal intensity, they found a better matching of O_2 delivery to O_2 utilization during exercise for the interval regimens than that in constant-load exercise. O_2 delivery during interval training was enhanced when recovery was applied in the moderate intensity domain in comparison to "low-intensity" active recovery. Besides the muscle-pump effect, which was presumably increased during

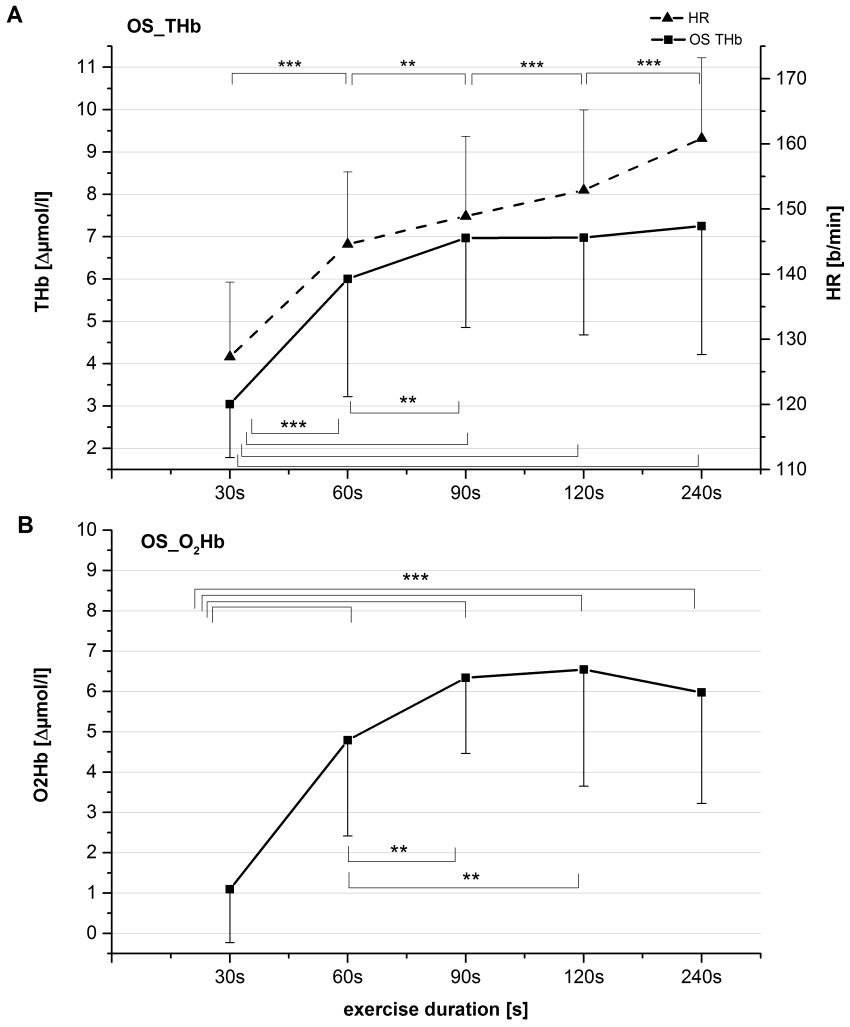

Fig. 4 (A) Overshoot values of ΔTHb (solid line) and end-exercise (EE) heart rate values (broken line) (mean ± SD) after the five exercise bouts. (B) Overshoot values of ΔO2Hb (mean ± SD) after five exercise bouts. Asterisks represent results from post-hoc tests in relation to values marked with double lines; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Heart-rate values all differed significantly from each other at $P < 0.001$

interval training with "moderate intensity" recovery, the authors suggested enhanced local vasodilation to be an important determinant for those results.

Zafeiridis et al. (114) were also able to find improved local oxygen delivery during exercise. In contrast to Belfry et al., they found no differences between constant-load exercise and two interval-training regimens with different work-interval durations. The different results in these two studies could likely be attributed to differences in the two study protocols. As we did in the present study, Zafeiridis et al. examined the effect of different work-interval durations. They used work intervals of 30 s with an intensity of 110% of the power output corresponding to $\dot{V}O_{2max}$ and 120-s intervals with an intensity of 95% $\dot{V}O_{2max}$ and did not find significantly different local-oxygen delivery during both interval-training regimens. This finding of Zafeiridis et al. is contrary to our findings, which showed significantly higher post-exercise oxygen availability and blood supply following 120 s of exercise than that following 30 s of exercise with 80% $\dot{V}O_{2peak}$. We cite two possible explanations for this difference. First, the dependency of post-exercise blood supply and oxygen availability on exercise duration, as it is shown in the present study, appears to be valid only within a certain exercise intensity range. In a previous study, we examined the effect of exercise intensity on post-exercise blood supply and oxygen availability and observed a non-linear relationship (92). We found no differences between 80 and 90% $\dot{V}O_{2peak}$, but did not include higher exercise intensities; therefore, it might be possible that our results are not valid for supramaximal exercise. Second, Zafeiridis et al. used different recovery durations between work intervals, which could have contributed to different results. It could be that 30-s work intervals cause hyperemia similar to that associated with 120-s intervals, when recovery periods are adjusted appropriately.

Although the methods used in our study do not provide sufficiently detailed information on the complex regulation of local muscle perfusion, we call attention to a few possible explanations for our results. In general, cardiac output and artery flow increase as a response to dynamic exercise (89). In exercising muscles, two antagonistic mechanisms happen: There is global sympathetic mediated vasoconstriction on the one hand whereas acute vasodilation occurs in active tissue secondary to a

promoted release of vasoactive substances on the other hand. Because vasodilation superimposes sympathetic mediated vasoconstriction in active muscles, capillary perfusion is increased (57, 67, 78). This process is supported mechanically by rhythmic contractions of the muscle during cycle exercise (muscle pump) (89).

Previous research has shown a biphasic response of muscle blood flow to exercise with an early, rapid response within the first 5–7 s and a prolonged, secondary response starting 15–20 s from the onset of exercise (100), while a steady state is reached within 1–3 min. Hence, blood-flow adjustments to exercise occur rather quickly. After termination of exercise, blood-flow recovery has been reported to be slower following heavy exercise than following moderate exercise (40). This slowing of blood-flow recovery is most likely due to the prolonged presence of vasoactive substances. In rats, it had been shown that acute, vasodilatory responses to exercise can last up to two days or more (52). Consequently, prolonged, local vasodilation seems to be dependent on the extent of the release of vasoactive substances during the previous exercise bout.

But which mechanisms are crucial for the increased post-exercise oxygen availability between 30 and 90 s of exercise at 80% $\dot{V}O_{2peak}$? We did not measure cardiac output in this study, but our data reveals some basis for speculation. The local oxygen availability was equal from 90 to 240 s of exercise duration (Figure 4B), while end-exercise heart rate increased continuously. We assumed accordingly that end-exercise cardiac output also increased continuously with exercise duration (otherwise, stroke volume would have declined, which is unlikely). Hence, if the prolonged recovery of cardiac output would have greater implications on post-exercise hyperemia, then post-exercise hyperemia would have been expected to increase continuously following exercise durations >90 s. In a previous study, we observed similar characteristics in the relationship of local blood supply and exercise intensity [19]. Among local mechanisms, the muscle pump is neglectable as an explanation of our results as it stops with cessation of exercise, which leads to an abrupt drop in muscle blood flow (40).

Local Vasodilation remains as the most plausible determinant for the hyperemia and increased post-exercise oxygen availability following cycling exercise ≥90 s at 80% $\dot{V}O_{2peak}$. Factors that trigger vasodilation are various. Among those factors, chemically and flow mediated vasodilation have to be highlighted when discussing exercise induced vasodilation (60). In particular, adenosine, acetylcholine, and shear stress are presumed to trigger exercise-induced effects on vasodilation (54, 60). It can be assumed that blood flow during exercise and prolonged vasodilation of small vessels in the post-exercise phase are positively related. It is likely that this effect was more pronounced (in our study) following exercise durations ≥90 s compared to shorter exercise bouts. And again, because we did not measure vasodilating substances or cardiac output and blood flow velocity directly, the implications of the above-mentioned mechanisms for our study results remain speculation-based.

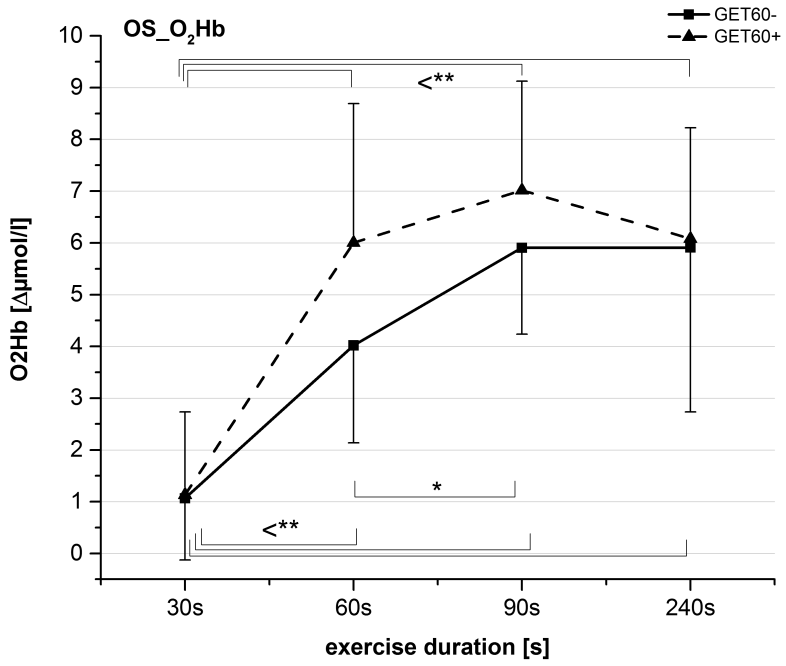

Fig. 5 Overshoot values of ΔO2Hb (mean ± SD) for GET60+ and GET60−. Asterisks represent results from post-hoc tests in relation to values marked with double lines; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

We have shown that oxygen availability following cycling exercise is dependent on exercise duration. Our results demonstrate that this dependency is influenced by the relative aerobic fitness (*i.e.,* GET), expressed as a percentage of $\dot{V}O_{2peak}$. In the GET60+ group, 60 s of exercise were sufficient to

evoke a post-exercise overshoot of ΔO_2Hb, which was equal to the overshoots following longer exercise bouts. In the GET60− group, 90 s of exercise were needed to obtain those high values. Hence, adjustments in local vasodilation are possibly faster in subjects showing high relative aerobic power. This means that in subjects with higher aerobic fitness, exercise durations of 60 s are sufficient to evoke an increased post-exercise hyperemia and increased oxygen availability.

Advantages of improved post-exercise O_2 availability

There are several advantages of improved local blood and oxygen supply, as it has been observed following exercise durations >90 s at 80% $\dot{V}O_{2peak}$. First, gas exchange is facilitated when the functional cross-sectional area of capillaries is increased (22). Moreover, muscle reoxygenation has been shown to improve with endurance training (19). A higher local muscle perfusion is associated with enhanced oxidative metabolism, such as fatty acid oxidation (56, 66, 88). Romijn et al. (88) showed reduced fatty acid mobilization during strenuous exercise (85% $\dot{V}O_{2peak}$), as well as a strongly increased post-exercise plasma fatty-acid availability following strenuous exercise (56, 88). This increased post-exercise, plasma fatty-acid availability may be one explanation for improvements in fatty-acid oxidation capacity as long-term metabolic adaptations that have been observed following HIT in women (95). Kimber et al. (63) found a significant dependency of post-exercise fatty-acid oxidation on fatty-acid availability. Exercise that increases fatty-acid availability is, therefore, likely to enhance fatty-acid oxidation after or between exercise bouts. The improved post-exercise blood supply during recovery or, when active recovery is applied, during the relief interval could therefore augment fatty-acid oxidation in particular. However, we reiterate that these suggestions are speculative because we did not measure fatty-acid oxidation.

Study Limitations

When using NIRS, the influence of adipose tissue thickness (ATT) has to be considered (102). If skin and subcutaneous tissue thickness is near to or exceeds the penetration depth of the emitted photons, muscular effects will be blunted. Skin and subcutaneous thickness was approximately 3.75 mm (7.5 ± 3.1 mm skinfold thickness, measured with a caliper). Because penetration depth has been

shown to be approximately half of the optode distance (39, 102), it can be concluded that NIRS signal was capable for measuring muscle oxidation in our study as the inter-optode distance was 3.5 cm, enabling a penetration depth of 1.75 cm.

Another point to mention is the 5-min recovery period in-between exercise bouts, which was too short to enable a full recovery of ΔO_2Hb and ΔTHb. It therefore has to be considered, that prior exercise bouts influenced the subsequent exercise bouts (59) First, this baseline drift in ΔO_2Hb and ΔTHb did not show any relationship to exercise duration. Second, we randomized the order of the exercise bouts and placed an intensive warm-up in front of the first experimental exercise bout. Consequently, this issue should not have influenced our results.

We recruited only male subjects to reduce variability in subject characteristics and because of the minor adipose tissue thickness in males relative to that in females, which apparently affects NIRS signals (102). Hence, the relevance of our results on females is limited.

Work intervals within interval-training regimens are described by interval duration and interval intensity. We investigated the influence of exercise intensity on local muscle O_2 availability and blood supply in an earlier study (92). The present study aimed to examine the effect of duration, using isolated, "interval-training-associated" exercise bouts. But besides the work interval, several other variables in interval-training prescriptions, such as intensity and duration of the recovery interval and number of repetitions, should be noted. The latter is of particular importance. Because HIT consists of repeated sets of transitions from high to low intensity, work-interval durations <90 s also are potentially effective to increase post-exercise oxygen availability due to a cumulative effect. This "repeated bout" effect could occur when low intervals are too short to provide an adequate recovery. It is therefore conceivable that effects of HIT on local oxygen availability could be similar across different exercise durations, for example when the work-to-rest ratio is kept constant. It has to be highlighted that all intervals in our study had been carried out with the same exercise intensity. Usually, work intervals are prescribed with higher intensity during "short-interval" HIT-regiments (17, 110). As a consequence, the low interval has to be extended to provide sufficient recovery to enable

the completion of the following work interval. Consequently, the relationship between interval intensity, duration and recovery has to be focused in future research.

Finally, the post-exercise O_2 availability and blood supply are two of several determinants causing aerobic adaptations. Investigation of other determinants may require other, optimal "work-interval" durations.

Table 3. Baseline drifts

A Exercise duration	ANOVA	30 s	60 s	90 s	120 s	240 s
ΔBASE_THb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.182	0.79 ± 1.18	0.76 ± 2.84	1.06 ± 1.26	1.17 ± 0.98	0.83 ± 1.14
ΔBASE_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.069	−1.54 ± 1.98	−0.62 ± 4.37	0.95 ± 2.31	1.95 ± 1.64	0.65 ± 1.00
ΔBASE_HHb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.086	0.97 ± 1.24	0.51 ± 2.01	−0.09 ± 1.22	−0.41 ± 0.75	0.72 ± 1.52
B No. of exercise bout		1	2	3	4	5
ΔBASE_THb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.564	0.87 ± 3.05	1.16 ± 1.13	0.34 ± 1.24	1.34 ± 1.20	0.17 ± 1.29
ΔBASE_O_2Hb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.271	0.18 ± 3.30	1.14 ± 1.82	0.77 ± 1.69	1.76 ± 1.75	−0.87 ± 2.43
ΔBASE_HHb ($\Delta\mu mol \cdot l^{-1}$)	P = 0.218	0.69 ± 1.40	−0.20 ± 1.23	−0.43 ± 0.63	−0.42 ± 1.54	1.04 ± 1.34

n = 18; values are expressed as mean ± SD; ΔBASE values are the differences between the baseline values before and after the exercise bouts; Panel A shows baseline drifts sorted by exercise duration while panel B shows baseline drifts sorted by chronological order of the exercise bouts.

Conclusion

Exercise duration in HIT has a significant impact on post-exercise oxygen availability following cycling exercise with 80% $\dot{V}O_{2peak}$. The highest post-exercise oxygen availability is induced with exercise durations of 90 s and longer, whereas no further increase is evoked by longer exercise durations up to 240 s. Post-exercise oxygen availability is dependent on aerobic fitness. Subjects, who reached GET below 60% $\dot{V}O_{2peak}$ needed 90 s to reach the maximum post-exercise oxygen availability, while subjects who reached GET above 60% $\dot{V}O_{2peak}$ reached the maximum post-exercise oxygen availability following 60 s exercise. To facilitate oxidative adaptations, work intervals within interval-training regimens should be prescribed with a minimum length of 60 s, depending on aerobic fitness,

whereas it has to be reiterated that the transferability of our findings to exercise intensities that are different to 80% $\dot{V}O_{2peak}$ may be limited.

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The authors thank all subjects for their cooperation in this study.

7. Conclusion and perspective

The main findings of this project are (1) that there is a significant improvement of microvascular blood supply and oxygen availability following exercise at >70% VO_{2peak} that is apparently linked to gas exchange threshold and (2) that exercise at 80% VO_{2peak} needs at least 90 s to evoke those effects in subjects with gas exchange thresholds <60% VO_{2peak} and at least 60 s in subjects with gas exchange thresholds >60% VO_{2peak}.

These results indicate, that the minimum exercise intensity to benefit from enhanced muscle perfusion is considerable low. Consequently, interval training regimens that are applied with exercise intensities ~70-80% VO_{2peak} represent an effective stimulus of the cardiovascular system. HIT is often associated with near-maximal or supramaximal loads, as it is characterized by Buchheit in his comprehensive review (16).

When anaerobic issues are not addressed in a training session, HIT with exercise intensities <100% VO_{2peak} has several advantages. Because of the low load, this kind of training is not solely to be prescribed for healthy athletes but also for older people or patients. The usage of HIT with patients with cardiac disease has been successfully applied in a growing number of studies (43, 44, 51, 77, 94) and enables new possibilities in prevention and rehabilitation. While physical activity is correlated with exercise performance, current recommendations often prefer moderate continuous training (47), maybe because the clinical stuff is afraid of risking too high intensities that may be dangerous. However, a recent study could show that the deficits of children with congenital heart disease have a diminished VO_{2max} – but their submaximal fitness status was equal to healthy controls (82). HIT could counteract deficits in the heavy or severe intensity domain. When exercise training or physical activity is settled strictly in the moderate intensity domain, there is little stimulus for adaptions that enhance performance in the heavy or severe intensity domain such as anaerobic metabolism or aerobic glycolytic metabolism. Hence, HIT could contribute to a better exercise tolerance during heavy or severe intensity exercise. For the application to patients, it is beneficial, that intensities that are considered as “low” in

context of HIT, are sufficient to enhance local muscle blood supply and O_2 availability within interval training.

There is apparently no need to exceed 90% VO_{2peak} when prescribing HIT in order to increase cardiovascular capacity. First, HIT with focus on aerobic effects should maximize the time at high intensity within one training session to maximize aerobic energy turnover (17) – this requirement can only be met when long intervals are performed with exercise intensities that are not maximal or supramaximal. Second, the results of this project show that there is no further increase in post-exercise microvascular blood supply and oxygen availability from 80-90% VO_{2peak}, which does implicate that higher intensities seem not to enhance this effect. Third, the relief interval has to be prescribed actively because one can only benefit from high postexercise substrate availability when this energy is actually utilized. Furthermore, active relief intervals contradict the application of maximal or supramaximal exercise intensities for long-interval HIT regimens for reasons of fatigue. Consequently, HIT with work interval intensities ~80% VO_{2peak} is supposed to be an ideal stimulus in order to enhance aerobic training effects.

Considering exercise duration, this project has shown that the minimum duration to evoke enhanced microvascular perfusion is dependent of the submaximal training level, i.e. ability to utilize economical metabolic pathways such as aerobic fatty acid oxidation predominantly as long as possible during incremental exercise. In detail, persons having their gas exchange threshold below 60% VO_2 need more time (≥90 s) to evoke higher post-exercise muscle blood supply and O_2 availability than persons with higher gas exchange thresholds (≥60s). It has to be noted that post-exercise blood supply and O_2 availability did not increase when exercise was maintained longer than 60 s or 90 s respectively.

Consequently, single exercise bouts evoke the best effects for local muscle perfusion when they are conducted (1) above 80% VO_{2peak} and (2) with a minimum exercise duration of 60 s or 90 s respectively. HIT should be designed accordingly.

Based on this conclusion, there remain some questions:

80% VO_{2peak} is the minimum intensity and 60 s/90 s is the minimum duration to evoke good post-exercise perfusion. But is the combination of both really the ideal training prescription or is there some interaction between intensity and duration in this issue?

The current project aimed to examine the effects of isolated exercise bouts to define the base for further research. One can speculate that more intense exercise bouts need less time to evoke enhanced post-exercise muscle blood supply and O_2 availability. However, because this project revealed that the relationship of exercise intensity and post-exercise muscle blood supply and O_2 availability is non-linear, it is likely that relationship of exercise intensity and duration on post-exercise muscle blood supply and O_2 availability is even more complex and has to be examined in further studies.

What is the influence of the relief interval? Could shorter intervals combined with shorter relief intervals have similar effects compared to 60 s/90 s intervals?

The influence of recovery-/relief-interval duration was not addressed in this project. Zafeiridis et al. used different recovery durations in their study (114) but did not standardize exercise intensity. However, two protocols with a work-to-rest ratio of 1 (30 s/30 s and 2 min/2 min) had similar effects on local muscle perfusion. This supports the hypothesis that there is a cumulative effect of interval exercise when the relief interval does not exceed the work interval.

Does the number of transitions from work to relief interval have any relevance on local muscle perfusion?

If there is a cumulative effect of exercise bouts, prolonged presence of vasodilating substances could be responsible for this. Furthermore, shear stress is an important factor for vascular compliance. The number of transitions from high to low shear stress could also have implications on muscle perfusion.

Summed up, there are several variables that have to be determined in order to design a HIT protocol that enhances local muscle perfusion most effectively. This project has done a first step to systematically examine the implication of those variables on muscle perfusion.

8. Declaration of author contribution

All parts of this study have been designed, planned and conducted independently by myself. This field of research (local muscle oxygenation assessment using NIRS) was new in this faculty. Accordingly, prior to drafting the study, a self-organized, comprehensive screening of the current state of research was necessary. I got familiar with all single methods that have been used in this project and operated the respective devices by myself. This also refers to the published studies (chapter 6.1 & 6.3) as well as the supplemental data (chapter 6.2). The manuscripts were self-drafted, including literature screening, wording, artwork and correspondence with the editors.

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This Work has been completed within a very special time of my life. My girlfriend gave birth to our wonderful children, Leonard and Maxi Laura. Both were born during data assessment and manuscript writing, providing well diversion beside working on this thesis. Remains to mention that my girlfriend Nicola Reiner is actually in the same situation and is simultaneously completing her dissertation!

Therefore, this work is dedicated to my wonderful family!